Characterisation of Vanilla Extracts Based on Sensory Properties and Chemical Composition

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Abstract

Although vanilla is one of the most commonly used flavourings in the world, there is only limited information available about its flavour and chemical composition. The aims of this research were to use sensory analysis and chemical composition analysis to characterise vanilla extracts produced from beans from different regions and to investigate correlations between sensory data and chemical composition of the vanilla extracts. Other aims were to investigate the effect of solvent extraction, concentration of extracts and the combination of vanilla and fat or sugar on the sensory profile of vanilla extracts and formulated matrices. . The vanilla extracts (ethanol or glycerol based), either commercial or laboratory extracted samples using vanilla beans sourced from India, Madagascar, Papua New Guinea, Tonga and Uganda, were characterised for aroma and flavour by a sensory panel trained. The panel found that the aroma and flavour of vanilla extracts varied depending on both the growing region and the solvent or solvent concentration used for flavour extraction. Principal component analysis (PCA) showed that extracts from Madagascar and Tonga grown beans were similar, being high in sweet type aromas and flavours such as butterscotch flavour and raisin aroma. The extracts from India and Papua New Guinea beans were higher in the woody and bourbon notes. Glycerol extracts had a reduced aroma and flavour sensory profile intensity compared to the ethanol extracts. A range of concentrated vanilla extracts concentrated using vacuum concentration, maltodextrin flavour encapsulation and supercritical carbon dioxide extraction. The vacuum concentrate extract was the most similar to the standard single fold ethanol as found from sensory analysis and gas chromatography mass spectrometry (GCMS). Concentrations up to 35mg/ml of vanillin were reached with vacuum concentration, 20 times the concentration of 1.5mg/ml typically found in a single fold extract. The trained sensory panel were also asked to evaluate solutions containing vanilla extract, with either milk fat or sugar at different concentrations. Milk fat was found to reduce the aroma and flavour intensity of the vanilla and sucrose was found to increase the perception of vanilla extract aroma due to the 'salting out' effect and reduce bitter, woody and bourbon flavours while increasing butterscotch, raisin and vanilla flavours. Using GCMS it was found that more lower boiling point volatile compounds were extracted with polar solvents, such as methanol, than non-polar solvents, such as hexane. Fifteen of these volatile compounds were identified and quantified in 16 vanilla extracts and correlated with

the sensory attributes previously used to describe the vanilla extracts. Most of the volatile compounds in the vanilla extracts had a phenolic structure. Bourbon aroma and flavour correlated with syringaldehyde. Vanillyl alcohol was correlated with raisin aroma, raisin flavour and butterscotch flavour. Vanilla aroma and flavour were associated with creosol and vanillin. Sweet flavour was correlated with p-anisic acid, maltol and 4-hydroxybenzoic acidIn model systems with milk fat, vanilla and sugar, milk fat masked vanilla flavour and aroma in solution, whereas sugar enhanced the aromas and sweet flavours and only masked bitter, woody and bourbon flavour. Using GCMS, multiple correlations between volatile chemical compounds and sensory attributes were identified. Vanilla extracts characterised by sensory and analytical methods were found to vary based on vanilla bean region, solvent extraction conditions and concentration method used.

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All sensory experiments in this thesis were registered as high risk and approved by the Massey University ethics committee (Southern A 13/040).

List of publications and presentations

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1. Introduction

Vanilla is a vine that grows in tropical regions worldwide. The fruit of this vine, the vanilla bean or pod, must be hand pollinated, harvested and cured in a labour-intensive process that results in the aromatic brown product many consumers are familiar with (Havkin-Frenkel and Belanger, 2011). This intensive process is required to fully develop the flavours of the vanilla and ensure that the vanilla is of the highest quality possible. When the flavour of the vanilla beans has been fully developed, it can be extracted with ethanol to create vanilla extract, a more versatile product than the vanilla bean for use in foods (Cameron, 2011).

Although artificial vanilla flavour is only one chemical compound – vanillin - over 500 chemical compounds have been found in vanilla beans and its products (Toth *et al.*, 2010), showing that vanilla is a much more complex flavour than many people take it to be. With this large number of compounds within the vanilla, it is important to know how these compounds are affected by the extraction conditions and growing region, how people perceive vanilla, and how the compounds relate to what people perceive. The flavour of vanilla extract can also be affected by how it is treated after the flavour extraction process, such as through concentration or evaporation, and by the different components in a food system; the effects of each of these factors needs to be understood.

Several factors that could influence the flavour and volatile content of vanilla have been investigated previously. These include improving the rate of flavour extraction (Pardio *et al.*, 2009; Van Dyk *et al.*, 2010; Naidu *et al.*, 2012), comparing bean quality (Takahashi *et al.*, 2013b) or the biosynthetic pathways within the vanilla that produce the flavour compounds (Dignum *et al.*, 2001; Pérez-Silva *et al.*, 2011; Yang *et al.* 2017).The solvent type and the vanilla bean cutting size have not been investigated, although they may be crucial to determining the final flavour and rate of flavour extraction possible for creating vanilla extracts.

The most common methods used to analyse the effects of different treatments on vanilla are chromatography and sensory analysis. The methods used for chromatography are gas chromatography, often paired with mass spectrometry (GC-MS) (Ramaroson-Raonizafinimanana *et al.*, 1998; Takahashi *et al.*, 2013a; Li *et al.*, 2014) or an olfactory detector (GC-O) (Pérez-Silva *et al.*, 2006) or high pressure liquid chromatography (HPLC) (Herrmann and Stöckli, 1982; Pyell *et al.*, 2002; Schwarz and

Hofmann, 2009). These methods will be utilised as they have been found to be reliable and accurate for use with vanilla products. Although the volatile compounds can be measured and identified using instrumentation, the characteristics of the vanilla as perceived by humans are far more complicated and require sensory analysis to determine the sensory profiles of vanilla products. Hariom *et al.* (2006) used Quantitative Descriptive Analysis (QDA), a sensory method involving training participants up until they reach consensus on the rating order of selected attributes in foods, to detect differences in four vanilla extracts from both Mexico and Madagascar. Naidu *et al.* (2012) found differences in vanilla extracts produced using tea leaf enzymes also using QDA. Takahashi *et al.* (2013a and 2013b) used the sensory spectrum method, a more structured method of training than QDA, requiring closer agreement between the panellists' ratings before being considered fully trained to analyse the aroma profile of vanilla beans. They identified a difference in vanilla beans of differing quality and species/growing region. Heymann (1994) trained two panel groups independently using descriptive analysis; comparing 3-fold, 10-fold and 20-fold vanilla extracts, the panels were able to differentiate the products using the sixteen and fourteen attributes selected by each panel group. Van Dyk *et al.* (2010*)* used an untrained panel to find a difference between vanilla beans subjected to various curing methods. All of these methods were able to determine differences within the vanilla products of interest, and should be suitable for further use in investigating vanilla for factors such as flavour extraction method and growing region. Although sensory analysis and chromatography have been used extensively to study vanilla extracts, the relationship between the two has not been investigated. With over 500 chemical compounds found in vanilla, understanding the relationship between these chemicals and the sensory perception of vanilla is important. This could lead to an understanding of the function of each compound in the final sensory profile and allow for the flavour extraction process to be tailored to ensure that the key chemical compounds are produced and retained.

An ethanol vanilla extract, while containing the flavours desired for food applications, has some limitations on its use. In particular the presence of ethanol and low concentration of flavour prevents its use in foods such as chocolate or powdered products. After understanding which volatile components in vanilla are responsible for the sensory characteristics, a range of dehydration or powdering methods could be trialled and monitored to deteremine the effects on the flavour and volatiles. Methods

that could be suitable are vacuum concentration, freeze drying and supercritical carbon dioxide extraction. Vacuum concentration has been used successfully on other ethanol containing foods such as wine or beer (Shihadeh *et al.*, 2014; Andrés-Iglesias *et al.*, 2016) and could be applied to vanilla extract. Freeze drying, with an encapsulating agent, is able to create a powder (Takada *et al.*, 2009; Hundre *et al.*, 2015) and should prove suitable for vanilla extract, although it has not been reported in this application previously. Supercritical carbon dioxide extraction has been used on vanilla beans (Fang *et al.*, 2002b; Castillo-Ruz *et al.*, 2011; Romero De La Vega *et al.*, 2016), but neither the sensory properties nor the volatile content of the extract has been studied.

One final aspect of vanilla flavour that has been neglected is the effect of food systems; the effect of varying the fat or sugar content of a food is largely unquantified for natural vanilla extract. Stampanoni Koeferli *et al.* (1996) varied the fat, sugar and solids non-fat in ice cream but found that the interactions between the components made it hard to draw definite conclusions on the effects of individual components. Other studies chose to use vanillin instead. Vanillin has been investigated in ice cream, custard and milk to determine the effects of fat (Li *et al.*, 1997; Hyvönen *et al.*, 2003; Carrapiso *et al.*, 2004; Frøst *et al.*, 2005; Tomaschunas *et al.*, 2013) and protein (Li *et al.*, 2000; Reiners *et al.*, 2000). No studies have looked at the effects of sugar on the flavour of vanilla extract or vanillin.

As there were multiple insufficiently researched areas concerning vanilla extract, the aims of this research were:

- To investigate the effects of extraction solvent and vanilla bean preparation on the volatile compound extracted from cured vanilla beans
- To determine the sensory characteristics that differentiate natural vanilla extracts using commercial vanilla extracts as a model and propose reasons for any differences observed
- To determine the volatile chemical compounds in natural vanilla responsible for key sensory attributes in natural vanilla extracts
- To investigate a range of methods for producing concentrated vanilla extract and identify the changes in the volatile compounds and sensory profile during processing
- To investigate the effects of varying milk fat and sugar concentration on the aroma and flavour profile of natural vanilla extract

2. Literature Review

2.1 Introduction

The aim of this chapter is to provide an in-depth review of literature concerning vanilla and its products. The literature review starts with an overview of the growth and production of vanilla, including methods to create a vanilla flavour extract. Information is then presented about the formation of the flavour in the vanilla beans during curing, as well as further details about the flavour compounds considered most important in vanilla in the literature. Next, there is a review of the various methods used to analyse and characterise vanilla extracts, including sensory analysis, GCMS and HPLC. This is followed by methods for creating a concentrate or a powdered form of vanilla. The final area reviewed is current information about how food components including protein, fat and carbohydrates affect the aroma and flavour of foods.

2.2 Vanilla Plant and Bean

There are three species of vanilla worldwide, with the main commercially viable species being *Vanilla planifolia*, originating in Mexico (Bory *et al.*, 2010). The second, less common species is *Vanilla tahitensis*, a hybrid that developed in Tahiti, and the third, least common species *Vanilla pompona*, from the Amazon rainforest (Cameron, 2011). Within each of these species, there is little genetic diversity, as the plants are easier to grow from cuttings than seeds (Lubinsky *et al.*, 2008).

Vanilla planifolia is a perennial vine from the orchid family that thrives in tropical climates up to an altitude of 750 metres (The Reineccius, 2006; Cameron, 2011). The vine can climb to heights of 15 to 20 metres using trees and other structures to support itself, as it is quite fleshy and weak by itself (Reineccius, 2006). The vanilla plant's leaves are oval, glossy and typically measure 15 cm by four centimetres. The flowers are greenish yellow and only bloom for one day (Cameron, 2011). The plant is able to flower two years after propagation and will fruit in its third year, reaching a maximum yield after 10 to 12 years of growth (Reineccius, 2006).

When the fruit of the vanilla vine, often called a vanilla bean, is fully mature it measures 12 to 24 cm in length and about 2.5 cm in circumference (Reineccius, 2006; Sinha *et al.*, 2008). As the fruit ripens, it changes from green to yellow and will

eventually split open from the tip and expose the thousands of tiny black seeds that are contained within (Cameron, 2011).

2.3 Cultivation of Vanilla

As the vanilla plant is a vine, a support is required to grow it commercially. These can either be trees planted at regular intervals or 'T' shaped supports made from wood, concrete or wire (Cameron, 2011). The vanilla should be planted near the base of the support and regularly pruned to a shape that promotes fruit production and allows for ease of harvest (Reineccius, 2006).

As *Vanilla planifolia* is cultivated outside of its native Mexico for the most part, the insects that would naturally pollinate the flowers are absent. A method of hand pollination using a needle to transfer pollen has been found to be highly successful (Sinha *et al.*, 2008). The vanilla plant will only produce flowers for a couple of months, each being open for only one day with a fully mature plant producing up to 1000 flowers each flowering season. Only about 50 blooms on each plant will be pollinated, with a maximum of eight flowers in each bunch being chosen to ensure that there is enough room for the beans to mature adequately. After fertilisation, the flowers take nine months to mature to the point of harvest (Reineccius, 2006).

2.3.1 Harvest

The beans should be harvested shortly before they are fully ripe. If they are left to ripen, they will split, reducing the final vanillin content of the beans, a major flavour component in the final product (Cameron, 2011). As the vanilla plants flower over a period of two months, the beans will ripen at different times (Reineccius, 2006).

2.3.2 Curing

After the beans are harvested, they need to be cured. The curing process has two main aims; to dry the beans to make them more shelf stable, and to maximise the vanillin content (Odoux, 2010). The curing process takes nine months and requires many man hours to accomplish, making it the most costly step in preparing the vanilla beans for market (Cameron, 2011).

The main steps that are involved in the curing process are killing, sweating, drying and conditioning.

a) Killing

What is commercially referred to as 'killing' is a method to prevent further ripening of the vanilla beans after they are harvested as this will decrease the vanillin that can be produced (Cameron, 2011). The killing step also results in the rupture the cells within the beans to allow the various enzymes in the cells to come in contact with their substrates, specifically the conversion of glucovanillin to vanillin (Setyaningsih *et al.*, 2005). To accomplish the 'killing' step, the beans can be submerged in hot water, put in an oven or exposed to strong sunlight (Cameron, 2011).

b) Sweating

For the sweating step, the beans are transferred from the killing stage to an insulated crate, or similar, in order to maintain as much of the heat as possible. Sweating aims to begin the drying process of the beans and to allow enzyme catalysed reactions to occur, starting vanillin production and takes 2-3 days (Lepers-Andrzejewski *et al.*, 2010; Odoux, 2010).

c) Drying

Drying aims to keep the beans warm to decrease the moisture content, to make the beans more stable against microbial deterioration and to increase the rate of reaction of the enzyme catalysed flavour production within the cells (Ashurt, 1999). Typically drying is accomplished by laying the beans out in the sun for a couple of hours a day, then moving them to be covered by sacking in the shade. When this step is completed, the beans are instead laid out on racks in a building and turned at regular intervals. This step can take up to three months, until the beans are deemed to be adequately dried (Odoux, 2010). The moisture content of the vanilla beans starts at 85% and is decreased to 25-38% (Ashurt, 1999).

d) Conditioning

The conditioning step involves leaving the dried beans in an insulated container for a couple of months with the aim of developing the final vanilla flavour (Lepers-Andrzejewski *et al.*, 2010). The beans are wrapped into bunches and put into the containers and monitored to ensure that no mould has developed. After this stage they are considered ready for the market (Odoux, 2010).

As many of these curing stages are unique to the area the beans are harvested in, it is difficult to separate out the effect of the growing region compared to the curing technique when comparing different vanilla beans. However, as the curing methods are standard within each growing region, these two factors can be combined for analysis.

2.4 Vanilla Extract

2.4.1 Definition

The United States of America's Federal Drug Association (FDA) describes vanilla extract as "the solution in aqueous ethyl alcohol of the sapid and odorous principles extractible from vanilla beans. Ethyl alcohol content of such an extract is not less than 35% by volume, and the extractible matter of one or more units of vanilla constituent." (FDA, 1993). The FDA defines a unit of vanilla to be 378g of vanilla beans, with no more than 25% moisture content, per 3.78L of final extract. This equates to no less than 287g of beans on a moisture free basis (FDA, 1993). This 'unit' is also called a 'single fold'. Other additives that vanilla extract may contain are glycerine, propylene glycol, sugar, dextrose and corn syrup.

2.4.2 Production

Commercial production of vanilla extract is typically carried out using one of two methods: percolation or oleoresin. Percolation requires circulating a mixture of ethanol and water containing 35-50% ethanol for two to three days through chopped vanilla beans, resulting in a four-fold extract. The oleoresin method requires the pulverisation of the pods, which then have ethanol circulated amongst the beans in a vacuum at 45^{o}C. Excess ethanol is removed by evaporation, and the whole process takes eight to nine days. The product of this is up to 10-fold extract (Reineccius, 2006; Sinha *et al.*, 2008). Very little information is available about the production of vanilla extract, and the effect that the various conditions have on the extract produced.

2.5 Biosynthesis of Vanillin in Vanilla Beans

There have been several studies completed with the aim of identifying the biosynthetic pathway that creates vanillin, the flavour compound present at the highest concentration within vanilla beans, but there is some contradiction in the results

(Ranadive, 1992; Knorr *et al.*, 1993; Funk and Brodelius, 1994; Kanisawa *et al.*, 1994; Gallage *et al.*, 2014; Kundu, 2017; Yang *et al.*, 2017). Figures 2.1 to 2.6 show the various proposed biosynthetic pathways for producing vanillin during curing. All proposed pathways produce vanillin, although the details of the pathway differ. It should also be noted that these studies were either on cell cultures or on enzyme activity, rather than the vanilla beans themselves.

Figure 2.1 shows the biosynthetic pathway proposed by Ranadive (1992). This pathway starts with coniferyl alcohol and ends with glucovanillin converting to vanillin. This pathway was proposed based upon experiments that extracted compounds from green and cured vanilla beans and looked at the differences between the two. Kanisawa *et al.* (1994) and Dignum *et al.* (2001) proposed the biosynthetic pathway seen in Figure 2.2. This was based on research into the glucosides present in green beans and the products in the cured beans. The pathway was proposed as a possible method for the glucosides to become the final products including vanillin. The pathway seen in Figure 2.3 was proposed by Funk and Brodelius (1994). This was based upon experiments conducted on cell cultures taken from green vanilla beans. This biosynthetic pathway was based on the enzymes contained within the vanilla beans and show how these could be involved in the formation of vanillin and vanillic acid. The pathway proposed, in Figure 2.4, was by Knorr *et al.* (1993). This was also based upon cell cultures from green vanilla beans. The focus of this pathway was benzoic acid derivatives, and how they could be altered in the cell to become vanillin.

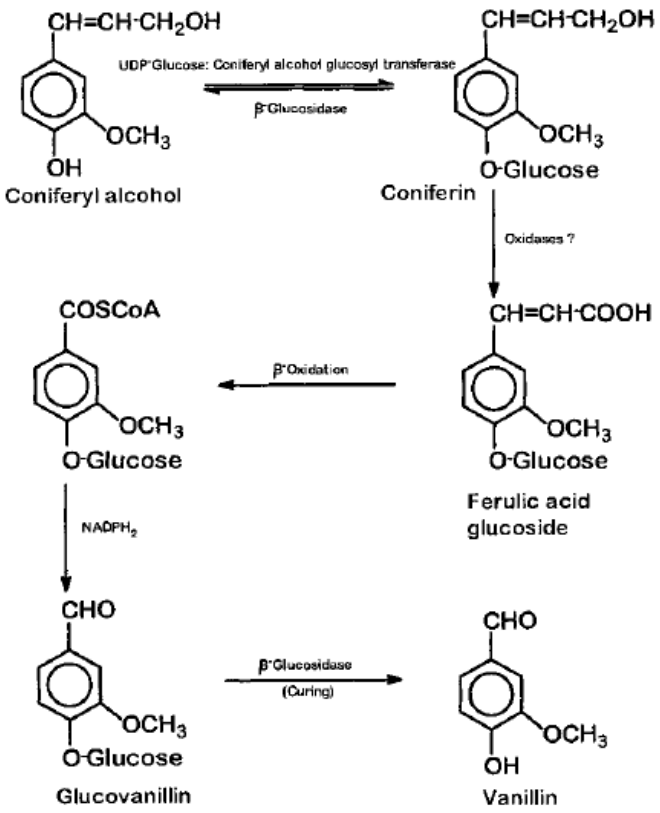


Figure 2.1: Hypothetical pathway for glucovanillin synthesis in green vanilla beans. (Ranadive, 1992).

In recent years, a process was proposed by Kundu (2017) (Figure 2.5), whereby the final step in the biosynthesis of vanillin is an enzymatic conversion from ferulic acid to vanillin, however a rebuttal was quickly published by Yang *et al.* (2017) (Figure 2.6), which showed that the markers for this enzyme were not present in the vanilla beans, therefore the enzyme (vanillin synthase) could not exist. This highlights the fact that the biosynthetic pathway for the production of vanillin is highly complex and not well understood, therefore further research is needed in this field before any conclusions can be drawn.

Figure 2.2: Proposed pathway for the formation of vanillin and other phenolic compounds in vanilla beans. The thick arrows indicate the most likely pathway. From Dignum *et al.* (2001) and Kanisawa *et al.* (1994).

Figure 2.3: Outline of the phenylpropanoid pathway in cell cultures of *Vanilla planifolia*. (Enzymes: PAL: phenylalanine ammonia lyase, CA4H: cinnamic acid 4-hydroxylase, CA3H: cinnamic acid 3-hydroxylase, 3-COMT: caffeic acid 3-O-methyltransferase, FA5H: ferulic acid 5-hydroxylase, 50MT: 5-0-methyltransferase, 4COMT: caffeic acid 4-0-methyltransferase, GT: glucosyltransferase, β-Glu: β-glucosidase, 4CL: 4-coumarate CoA ligase, CCR: cinnamoyl-CoA reductase, CAD: coniferylalcohol dehydrogenase, CCR: Cinnamoyl-CoA reductase, DMCA: N,N-dimethylcarbamic acid and DMBA: 7,12-dimethylbenzanthracene . From Dignum *et al.* (2001) and Funk and Brodelius (1994).

Figure 2.4: Proposed biosynthesis of benzoic acid derivatives from *Vanilla planifolia* cell cultures. The most likely pathway is indicated in bold arrows. (Enzymes: BAD: benzylalcohol dehydrogenase, BAR: benzoic acid reductase, OMT: 0-methyltransferase, PPO: polyphenoloxidase). From Dignum *et al.* (2001) and Knorr *et al.* (1993).

Figure 2.5: Vanillin biosynthetic pathway as proposed by Kundu (2017). Abbreviations are as follows: PAL phenylalanine ammonia lyase; C4H cinnamic acid- 4-hydroxylase; 4CL 4-hydroxycinnamoyl-CoA ligase; HCT hydroxycinnamoyl transferase; C30 H coumaroyl ester 30 -hydroxylase; COMT caffeic acid/5-hydroxyferulic acid O-methyltransferase; HBS hydroxybenzaldehyde synthase; OMT O-methyltransferase

Figure 2.6: Potential pathways for vanillin biosynthesis as proposed by Yang *et al.* (2017). Abbreviations are as follows: 4HBS, 4-hydroxybenzaldehyde synthase; C4H, cinnamate 4-hydroxylase; 4CL, hydroxycinnamic acid CoA ligase; HCT, hydroxycinnamoyl CoA shikimate hydroxycinnamoyl transferase; C3H, coumaroyl shikimate 30 -hydroxylase; CSE, caffeoyl shikimate esterase; P450, cytochrome P450 enzyme; CCoAOMT, caffeoyl CoA 3-O-methyltransferase; CCR, cinnamoyl CoA reductase; AldDH, aldehyde dehydrogenase; OMT, O-methyltransferase.

2.6 Main Flavour Compounds in Vanilla

There are a number of different compounds that are deemed to be important to the flavour and aroma of vanilla products. The compounds that have been focussed on the most in past studies are 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, guaiacol, vanillic acid, vanillin and vanillyl alcohol (Archer, 1989; Ranadive, 1992; Negishi and Ozawa, 1996; Scharrer and Mosandl, 2001; Boyce *et al.*, 2003; de Jager *et al.*, 2007; Waliszewski *et al.*, 2007; Cicchetti and Chaintreau, 2009b; Pardio *et al.*, 2009; Zhang and Mueller, 2012; Maruenda *et al.*, 2013). The following is a detailed description of each compound, with names, structure, flavours and aromas, and how they fit into the biosynthetic pathways proposed in ***2.5 Biosynthesis of vanillin in vanilla beans***.

2.6.1 4-hydroxybenzaldehyde

Other names for 4-hydroxybenzaldehyde include p-formylphenol, p-hydroxybenzaldehyde and para-hydroxybenzaldehyde (Figure 2.7) and it is primarily an aldehyde, also containing a phenol group. The melting point of 4-hydroxybenzaldehyde is 116^{o}C and it is slightly soluble in water, soluble in organic solvents and freely soluble in alcohol (Burdock, 2009).

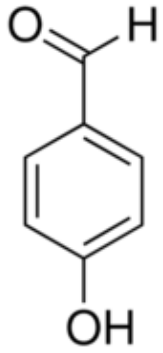


Figure 2.7: Molecular structure of 4-hydroxybenzaldehyde.

The aroma has been described as vanillic or nutty, as well as faintly woody/balsamic with the taste being sweet with no real flavour impression (Burdock, 2009). It has been found in vanilla extracts at concentrations from 55 to 1265ppm, with concentrations of between 288 and 467ppm in Tongan vanilla products (Toth *et al.*, 2010). Within the biosynthetic pathway in Figure 2.4, 4-hydroxybenzaldehyde has been found to be able to form 4-hydroxybenzoic acid, 3,4-dimethoxybenaldehyde and 4-hydroxybenzyl alcohol directly, thus allowing for the formation of vanillin and most

other main flavour compounds indirectly (Knorr *et al.*, 1993). Another proposed route, shown in Figure 2.2, has 4-hydroxybenzaldehyde being produced from benzaldehyde and 4-coumaric acid, and the 4-hydroxybenzaldehyde is the precursor of 4-hydroxybenzaldehyde glucoside. This pathway eventually leads to glucovanillin, and thus vanillin and other phenolic compounds formed during the curing process (Kanisawa *et al.*, 1994).

2.6.2 4-hydroxybenzoic acid

4-hydroxybenzoic acid (Figure 2.8) is a phenolic derivative of benzoic acid. Other possible names include p-carboxyphenol and p-hydroxybenzoic acid. The melting point of 4-hydroxybenzoic acid is 214^{o}C and it is easily soluble in hot water, alcohol, ether and acetone and slightly soluble in cold water and benzene (Burdock, 2009).

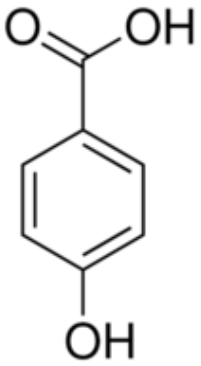


Figure 2.8: Molecular structure of 4-hydroxybenzoic acid.

4-hydroxybenzoic acid gives a slight sweet taste, which rapidly becomes acidic (Burdock, 2009). It has been found in vanilla extracts at concentrations between six and 2478 ppm, with concentrations of 439 to 472 ppm found in Tongan vanilla (Toth *et al.*, 2010). Within the biosynthetic pathway of Figure 2.4, 4-hydroxybenzoic acid has been found to be produced from coumaric acid and is the precursor to the production of 3,4-dihydroxybenzoic acid, which in turn leads to vanillic acid and thus vanillin (Knorr *et al.*, 1993).

2.6.3 Guaiacol

Guaiacol (Figure 2.9) is a phenolic compound with a methoxy group and is the monomethyl ether of catechol. Other names for guaiacol include 2-methoxyphenol, 2-

hydroxyanisole and methyl catechol. The melting point of guaiacol is 28°C and the boiling point is 203°C. It is slightly soluble in water and alcohol (Burdock, 2009).

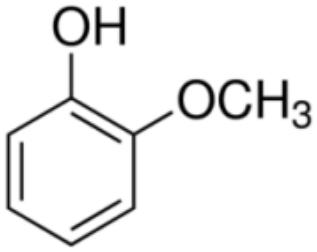


Figure 2.9: Molecular structure of guaiacol.

Guaiacol aroma can be detected at between three and 31 ppb, with the aroma at 1% concentration being described as phenolic, smoky, spicy, medicinal, vanilla, savoury, meaty, and woody with a hint of bourbon whiskey cask. The taste at 2 ppm is described to be woody, phenolic, bacon, savoury, smoky and medicinal (Burdock, 2009). It has been found in concentrations ranging from 3-332 ppm with concentrations of 77 ppm found in Tongan vanilla (Toth *et al.*, 2010). It has not been proposed in any biosynthetic pathways to date.

2.6.4 Vanillic acid

The systematic name for vanillic acid is 4-hydroxy-3-methoxybenzoic acid (Figure 2.10). The melting point of vanillic acid is 210°C, and it is slightly soluble in water, more soluble in ethanol (Burdock, 2009).

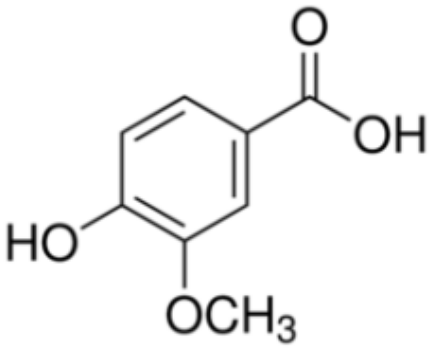


Figure 2.10: Molecular structure of vanillic acid.

Vanillic acid is a phenolic acid described to have a weak vanilla aroma and taste (Burdock, 2009). Vanillic acid has been found to range in concentration from 112 to 1963 ppm in vanilla beans and its extracts, with concentrations between 439 and 472

ppm found in Tongan vanilla (Toth *et al.*, 2010). Within the biosynthetic pathway proposed in Figure 2.4, vanillic acid is produced from ferulic acid, which then leads directly to vanillin (Knorr *et al.*, 1993). Figure 2.3 outlines a process in which vanillic acid is created from 3,4-dimethoxybenzoic acid, leading to vanillin (Funk and Brodelius, 1994).

2.6.5 Vanillin

The systematic name for vanillin is 4-hydroxy-3-methoxybenzaldehyde, a phenolic aldehyde (Figure 2.11). Vanillin is considered the most important of the vanilla flavour compounds, as this is the most characteristic of the vanilla flavour (Walton *et al.*, 2003). The melting point of vanillin is 80^{o}C, and it is slightly soluble in water, soluble in organic solvents and oils and freely soluble in ethanol (Burdock, 2009).

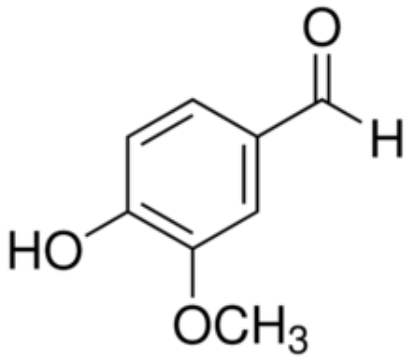


Figure 2.11: Molecular structure of vanillin.

Vanillin is described to have a creamy, vanilla aroma with a sweet taste. Its aroma can be detected at between 29 ppb and 1.6 ppm, with recognition at 4 ppm. The taste at 10 ppm can be described as sweet, vanilla, marshmallow, creamy, coumarin and caramel-like (Burdock, 2009). The concentrations that vanillin has been found in vanilla products ranges from 1439 to 28593 ppm, with concentrations of between 10429 and 12193 ppm found in Tongan vanilla (Toth *et al.*, 2010). Within the biosynthetic pathways, vanillin is considered to be the end product by all, as can be seen in Figures 2.1 to 2.6 (Ranadive, 1992; Knorr *et al.*, 1993; Funk and Brodelius, 1994; Kanisawa *et al.*, 1994).

2.6.6 *Vanillyl alcohol*

Vanillyl alcohol is also called 4-(hydroxymethyl)-2-methoxyphenol (Figure 2.12). The melting point of vanillyl alcohol is 115°C, and it is slightly soluble in hot water, soluble in oils and also other organic solvents (Burdock, 2009).

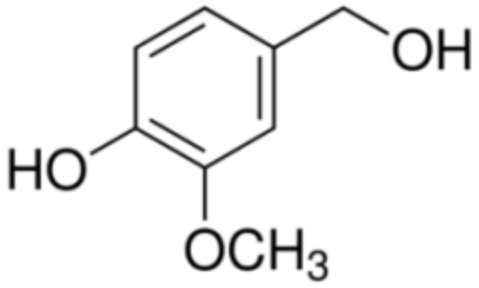


Figure 2.12: Molecular structure of vanillyl alcohol.

Vanillyl alcohol is described to have a mild sweet, balsamic and vanilla aroma, with a sweet, creamy and milky taste (Burdock, 2009). The concentration of vanillyl alcohol has been found to be between 11 and 2676 ppm, with the concentrations in Tongan vanilla of 12 ppm (Toth *et al.*, 2010). It has not been found to be an important compound in the vanillin biosynthetic pathway.

Over 500 compounds have been identified in vanilla and vanilla products in the past (Toth *et al.*, 2010). A full list of these compounds can be found in Appendix 1. Although not all of the compounds identified in vanilla provide flavour or aroma, the large number of compounds indicates that there are more compounds responsible for the flavour of vanilla extract than previously thought.

2.7 *Sensory Analysis of Vanilla Extract*

There are a variety of different methods that can be used to analyse the sensory properties of vanilla and vanilla products. These include gas chromatography-olfactory, electronic nose analysis and various forms of human sensory evaluation.

2.7.1 *Gas Chromatography-Olfactory*

Gas chromatography-olfactory (GC-O) is a method by which the aromas in complex mixtures can be separated and characterised by GC and assessed by a trained human assessor. The principle of the method is that the compounds are separated out via a gas chromatogram, and then directed to a 'sniffing' port (McNair and Miller, 2009). A

trained assessor is then responsible for identifying the aroma or class of aroma as the compound is released from the end of the column (Sparkman *et al.*, 2011).

The method that is used for GC-O is fairly consistent within the previous studies. The gas chromatography column type might vary, which will affect the order that the compounds elute from the column, but for the olfactory part, the common method is to have two or three people taking turns of about 20 minutes long on the sniffing port (Pérez-Silva *et al.*, 2006; Brunschwig *et al.*, 2012; Zhang and Mueller, 2012; Takahashi *et al.*, 2013a; Takahashi *et al.*, 2013b). Duplicates or triplicates are run to ensure that the results are consistent, and in the study by Pérez-Silva *et al.* (2006) the aromas were compared to those of reference compounds to confirm that the smells were the same. The compounds and aromas previously identified are summarised in Table 2.1. It can be seen that there are a wide range of compounds within vanilla that contribute to the aroma, varying in both aroma characteristics and chemical properties.

Table 2.1: Summary of the compounds identified, and aromas described in the studies by Pérez-Silva *et al.* (2006), Brunschwig *et al.* (2012), Takahashi *et al.* (2013a), Takahashi *et al.* (2013b) and Zhang and Mueller (2012).

Compound	Aroma Description
Acid	
2-hydroxybutyric acid	suffocating odour
2-methylbutanoic acid	cheese, fruity, animal, buttery, cheese-like, acidic, sweaty
3-methylbutanoic acid	buttery, cheese-like
3-phenylpropanoic acid	metallic, buttery
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	sweet aromatic, somewhat vanilla, creamy, milky
acetic acid	acidic, sour, vinegar, vinegar-like
butanoic acid	penetrating, reminiscent of rancid butter
butyric acid	buttery, oily
cinnamic acid	sweet aromatic, balsamic, somewhat cinnamic-like
dodecanoic acid (lauric acid)	mild fatty
heptanoic acid	sour, fatty
isobutyric acid	buttery
isovaleric acid	cheese, unpleasant, buttery, oily, acidic, cheese-like
nonanoic acid	oily, fatty, caprylic, cheesy
octanoic acid (caprylic acid)	caprylic, fatty, oily
pentanoic acid	strong acidic, caprylic
phenylacetic acid	buttery, honey-like
valeric acid	cheese
Alcohol	

Compound	Aroma Description
(Z)-1,5-octadien-3-ol	mushroom, metallic
2,3-butanediol	floral, oily, soft ethereal
2-methylfuran-3-thiol	meat, bacon
3-methyl-2-buten-1-ol	glue
3-methyl-2-butene-1-thiol	meat, burnt
3-phenol-2-propen-1-ol (cinnamyl alcohol)	sweet-warm balsamic, slightly cinnamon
4-methoxybenzylalcohol (anisyl alcohol)	sweet aromatic, balsamic, somewhat strawberry-like, anise-like, floral, herbal
benzyl alcohol	chemical, fruity with balsamic nuances
hexan-1-ol	roast, nutty, pleasant cheesy
isoamyl alcohol	fresh, ethereal, fusel-like, fermented and yeasty
isobutanol	chocolate
nonan-1-ol	orange, floral, oily, citronella-like
octan-1-ol	roast
phenylethanol	honey, fruity, sweet floral-rose, floral, rose
Aldehyde	
(2E,4E)-deca-2,4-dienal	oily
(E)-2-decenal	aldehyde, olive, herb-like, floral
(E)-2-nonenal	aldehyde, leather
(E,E)-2,4-decadienal	fatty, wood, cooking fat, fat, wax, herb-like, fresh
(E,Z)-2,6-nonadienal	melon
(Z)-6-nonenal	melon
2,4-heptienal	oily, green, aldehyde
2,5-dihydroxybenzaldehyde	mild aromatic, somewhat spicy, medicinal
2-furaldehyde (2-furfural)	sweet, caramel-like, nutty, baked bread, almonds
2-heptenal	green, oily
2-hydroxybenzaldehyde	spicy, medicinal, astringent
3,5-dimethoxy-4-hydroxybenzaldehyde	sweet aromatic, slightly floral
3-methylbutanol (isovaleraldehyde)	acrid, fruity, peach, cocoa-like, chocolate
4-methoxybenzaldehyde	sweet, herbaceous, spicy, creamy, powdery, vanilla
anisaldehyde	anise-like, almond, raspberry-like
benzaldehyde	sweet, aromatic, spicy, bitter almond and dark cherry-like
cinnamaldehyde	sweet aromatic spicy, cinnamic and cassia-like, balsamic
hexanal	green, grass, fruity, aldehyde, green-apple-like
methional	cooked potato
nonanal	fat, green, orange, aldehyde, peel, floral
octanal	fat, green, orange
phenylacetaldehyde	honey
p-menthenal	fat, floral
valeraldehyde	aldehyde
Ester	

Compound	Aroma Description
(E) methyl cinnamate	fruity
anisyl acetate	fresh, anise-like, floral, raisin-like
ethyl (E)-cinnamate	cinnamon-like, fruity
ethyl linolenate	sweet
methyl (E)-cinnamate	fruity, cinnamon-like
methyl 4-hydroxybenzoate (methyl paraben)	sweet aromatic, phenolic, fruity
methyl anisate	anise-like
methyl cinnamate	Sweet, fruity, balsamic, somewhat strawberry-like
methyl decanoate	winey, slightly sweet, honey-like
methyl nonanoate	oily, fatty, slightly fruity
methyl octanoate	fruity, fatty
methyl salicylate	medicinal, phenolic, sweet, characteristic wintergreen, chalk
methyl vanillate	sweet aromatic, spicy, slightly vanilla
p-cresol methyl ester	plastic, ether
phenethyl acetate	sweet, floral, fruity, green, rose, dried fruit
Ether	
1,2-dimethoxy-4-methylbenzene (methyl creosol)	candy sweet
1,2-dimethoxybenzene (veratrole)	aromatic, phenolic, medicinal, slightly spicy
vanillyl methyl ether	sweetish, fruity
Ketone	
1-octen-3-one	mushroom
2,3-butanedione (diacetyl)	sweet, buttery, creamy, milky, butter
2,3-pentanedione	butter
3,5-octadien-2-one	fruity green grassy
3-methyl-5-propyl-2-cyclohexen-1-one	slightly sweet, warm, celery-like
3-methylnonane-2,4-dione	floral, medicinal
5-ethyl-2(5H)-furanone	rice, fruity
acetol (hydroxyacetone)	aromatic, caramel
acetophenone	sweet aromatic, almond-like, nutty, benzaldehyde, with musty fruity nuances
gamma-nonalactone	creamy-fatty, coconut and apricot-like
gamma-octalactone	sweet creamy with coconut character
hydroxymaltol	honey, toasty caramel
maltol	sweet aromatic, caramel
methyl vanillyl ketone	sweet, powdery, vanilla, creamy, balsamic
Phenol	
2-acetylfuran	balsamic
2-furfurol	burnt, sweet, caramel, brown
2-methoxy-4-methylphenol (creosol)	powerful cresylic, smoky
2-methoxy-4-vinylphenol	aromatic, spicy, somewhat phenolic
2-methoxyphenol (guaiacol)	smoky, vanilla bean-like, phenolic, medicinal, chemical, sweet, spicy, aromatic, burnt
4-(2-propenyl)-phenol	aromatic spicy, medicinal, phenolic

Compound	Aroma Description
4-allyl-2-methoxyphenol (eugenol)	strongly warm spicy, clove-like
4-methylguaiacol	sweet, woody
4-methylphenol (p-cresol)	phenolic
4-vinylguaiacol	phenolic, spicy, chemical
4-vinylphenol	sweet, woody
5-isopropyl-2-methyl phenol	spicy, somewhat herbal phenolic
acetovanillone	vanilla, sweet, honey, aromatic, somewhat vanilla-like
isovanillin	phenolic, medicinal
pantolactone	burnt sugar
p-cresol	Faecal, balsamic, woody, spicy, animal, leather
phenol	strongly phenolic, medicinal
p-hydroxybenzaldehyde	vanilla-like, biscuit
p-hydroxybenzoic acid	vanilla-like, sweet
p-vinylguaiacol	smoky, phenolic
vanillin	sweet, vanilla-like, vanilla, intensive sweet, tenacious creamy, characteristic vanilla
vanillyl alcohol	vanilla-like

2.7.2 Electronic Nose

The electronic nose (E-nose) is a device that can perform simple analysis of the aroma of samples, approximating human perception but limited in range and flexibility. Any aromas to be analysed must be determined by a key aroma compound which is detected by the apparatus and measured. The E-nose analyses a gaseous environment above a sample or in the headspace of an enclosed container, producing information about the quality of the sample. It is able to recognise simple and complex aromas, based on reaction kinetics and volatility (Hariom *et al.*, 2006). The E-nose has been used to determine differences between samples of vanilla, such as with varying methods of curing (Hariom *et al.*, 2006) and the use of enzymes to decrease the time required for curing (Naidu *et al.*, 2012). It is typically used in combination with human based sensory evaluations, to confirm the results.

2.7.3 Human Sensory Evaluation

The most applicable method to analyse food samples for sensory properties is the use of people. Using people, there is a wide variety of information that can be gathered, from liking to rating of attributes to detection of differences. With all human based sensory trials, there is the chance of variation due to outside factors, such as concentration, ability to smell and distractions in the surrounding environment. All

trials with people should be run under controlled conditions, within a designed facility that eliminates as many of these potentially confounding factors as possible (Kemp *et al.*, 2009).

The following are details of the methods used by previous studies on vanilla and vanilla products, which include sensory panels, hedonic ratings and triangle tests.

2.7.3.1 Descriptive Analysis

Descriptive analysis is carried out by a trained sensory panel and can rate food products on a range of sensory attributes on a scale. Liking is not determined by the panel, unlike the hedonic scale, as for consumer testing, only the objective rating of the sensory attributes is determined. The sensory panel is typically a group of between six and sixteen people that have been screened for ability to taste and smell the compounds of interest in the food products. After screening, they are trained to analyse the food products of interest.

There are a few different methods by which a panel can be trained; mainly qualitative descriptive analysis (QDA) and the sensory spectrum method (Kemp *et al.*, 2009). For QDA, training on the attributes is received by the members of the panel, with the main aim for the participants to be consistent with their own results rather than those of the other panel members although the order of the ratings for the samples should be the same for all panellists. For the sensory spectrum method, all of the members of the panel are expected to be able to rate attributes within the sample food as being the same intensity on the scale. This method requires a lot more time for training to ensure that all the members of the panel are working as one (Kemp *et al.*, 2009). Using a combination of these two methods is called generic descriptive analysis, and features from each method can be used to suit the requirements of the study (Kemp *et al.*, 2009).

Previously, work has been carried out on vanilla using a range of these different trained panel methods. Hariom *et al.* (2006) and Naidu *et al.* (2012) used a panel with limited training to rate vanilla samples on a set of pre-determined attributes using the QDA method, with the aim of identifying differences in the aroma profiles of vanilla samples depending on the method that was used to cure the beans. Table 2.2 shows the attributes and references that were chosen to analyse the vanilla by Hariom *et al.* (2006).

Table 2.2: List of the attributes selected for use in sensory analysis of vanilla by Hariom *et al.*, (2006), with reference compound.

Attribute	Reference compound
Vanilla	Vanillin
Alcoholic	3-hexanol
Sweet	Aldehyde/Vanillin
Fruity	Citral
Floral	Phenyl ethyl alcohol
Woody	Wood-like
Beany	Isopropyl quinoline

Takahashi *et al.* (2013a) and Takahashi *et al.* (2013b) used a trained panel of 13 people to analyse vanilla samples using generic descriptive analysis. The panellists were presented with a list of 44 descriptors and asked to agree on a list of seven that described the majority of the attributes in the vanilla. The descriptors chosen were floral, dried fruit-like, resinous, hay-like, metallic, phenolic and sweet and a seven-point scale was used to rate the attributes. Heymann (1994) compared two independently trained panels, also trained using descriptive analysis on vanilla extract, comparing vanilla extract at 3-fold, 10-fold and 20-fold concentration. The descriptors they chose are in Table 2.3. It was found that both groups were able to differentiate between the vanilla extract samples using the descriptors chosen.

Table 2.3: List of descriptors for vanilla extracts chosen by two independently trained panels. Adapted from Heymann (1994).

	Panel 1	Panel 2
Aroma	Marshmallow	White Chocolate
	Butterscotch	Butterscotch
	Nutty	Vanillin
	Tea	Fruity
	Raisin	Chocolate
	Prune	Rum
	Woody	Kahlua Chocolate
	Almond	Bourbon
	Rum	Yeasty
	Smoky	Earthy
	Kahlua Chocolate	Tobacco
		Smoky
		Caramel
		Musty
Flavour	Coffee	White Chocolate
	Sweet Milk	Bourbon

Kwak *et al.* (2016) used the sensory spectrum method to compare vanilla ice creams on a range of 17 sensory attributes using a 16 point numerical scale, combined with consumer acceptability tests. The panel of 16 compared 10 vanilla ice creams and found 14 of the sensory attributes were able to differentiate between the products. Eighty consumers were also recruited to analyse the acceptability of the ice creams and found differences in the acceptability of the products.

Van Dyk *et al.* (2010) in contrast used a group of 64 untrained people to rate vanilla on a set of attributes that were predetermined by the researchers; sweet, fruity, floral, beany, straw, spicy, fermented and acidic. With a large group, they were able to find differences in the samples being rated.

All of these sensory methods have been used to successfully characterise and differentiate vanilla extracts, therefore future studies should also be able to differentiate vanilla extracts using any one of the methods or a combination of methods.

2.7.3.2 Hedonic Scale

The hedonic scale determines the degree of 'liking' of a sample. The scale usually ranges from strongly dislike to strongly like, with the number of intervals ranging from 5 to 9 points, being decided as suitable for the research being conducted (Kemp *et al.*, 2009).

Pardio *et al.* (2010) used a hedonic scale to determine the differences of changing vanilla bean extraction methods on the likeability of vanilla flavoured ice creams. They found that the use of enzymes to create vanilla extract reduced the curing? time required and there was no change in the flavour and aroma profile, as determined by the consumers, however, the new extract was paler in colour and less shelf stable. Van Dyk *et al.* (2010) used a hedonic scale to rate the changes in vanilla likeability depending on the method of curing that was used. They found that beans sweated for longer had superior aroma compared to beans that were blanched in hot water and then sweated however the beans that were not blanched were not as appealing in physical appearance.

2.7.3.3 Discrimination Tests

A triangle test is a discrimination test, with the purpose of comparing two or more samples in order to determine if there is a significant difference between the samples

(Kemp *et al.*, 2009). Pérez-Silva *et al.* (2006) used a triangle test to determine if there was a noticeable difference between vanilla extracts depending on the solvent that was used to extract them. It was found that the solvent combination that produced a vanilla extract closest to that of ethanol extract was a 1:1 (v/v) mixture of pentane and diethyl ether.

2.8 Instrumental Analysis of Vanilla

There are many methods that have been previously used to analyse vanilla, using instruments. The common methods are gas chromatography, possibly with mass spectrometry coupled to it, and high-performance liquid chromatography. Other methods include x-ray fluorescence spectroscopy, micellar electrokinetic chromatography and thin layer chromatography.

2.8.1 Gas Chromatography

Gas chromatography (GC) is a method for separating and identifying components of a mixture. As a mixture passes through the chromatograph, the components are "absorbed or impeded to different extents" and thus separate (Hutchinson, 1990). To force the analyte to move through the column, a carrier gas or mobile phase is passed through, typically an inert gas such as helium (McNair and Miller, 2009). The counter point to this carrier gas is the stationary phase, which is part of the column, impeding the forward progress of the analyte at different rates depending on the specific compound as a result of differing interactions between the various analytes, and thus separating out the components of a mixture (Sparkman *et al.*, 2011). This separation is based on a number of factors, including molecular size, charge and boiling point. Separation is also affected by the column selected, and the affinity of the analytes for the stationary phase in the column (Sparkman *et al.*, 2011).

2.8.2 Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry (GC-MS) is the coupling of a gas chromatograph to a mass spectrometer allowing for the pure, separated compounds produced in the GC to be analysed by MS. Mass spectrometry is a method that can provide information about the likely identity of a compound, based on how it interacts with an electric field after being fragmented and ionised (Downard, 2004).

2.8.2.1 Applications of GC

There are many different applications of GC for analysis of vanilla and its products. Previous studies have used GC to determine the origin of vanilla, to identify any adulteration of vanilla samples, to measure concentrations of key volatiles, to identify volatiles and to determine the aroma of the individual compounds. A summary of the methods used for analysis of vanilla products can be found in Table 2.4.

a) Determination of Growing Region of Vanilla

The origin of vanilla can be determined by using GCMS. Sostaric *et al.* (2000) was able to use GCMS in combination with solid phase micro-extraction (SPME) to determine the country of origin of vanilla extracts by looking at the concentration of key compounds and the presence of compounds unique to certain locations. Schipilliti *et al.* (2016) was also able to determine the nature of the vanilla extract, whether it was from vanilla beans or from bioconversion from ferulic acid, eugenol, turmeric acid, lignin or guaiacol using GCMS.

b) Detection of Adulteration

Adulteration of vanilla extract samples can be due to the addition of compounds to enhance the flavour, such as coumarin and ethyl vanillin. Coumarin has been banned in the United States of America due to toxicity (FDA, 1993) but with its vanilla-like aroma it has been added to vanilla extract samples to reduce cost (Marles *et al.*, 1987).

de Jager *et al.* (2007) was able to use LC-MS to determine both the identity and concentration of coumarin, ethyl vanillin and vanillin in vanilla products and Marles *et al.* (1987) created a separation method for GCMS that would allow for the identification of vanillin and coumarin in vanilla extracts within five minutes, with the compounds identified by mass spectra.

Table 2.4: Summary of the conditions used in gas chromatography from various studies on vanilla products. The abbreviations in the table are as follows; EI: Electron Ionisation, FID: Flame Ionisation Detector, GC: Gas Chromatography, i.s.: Ion Source, i.v.: Ionisation Voltage, MS: Mass Spectrometer, O: Olfactory, DTD: Direct Thermal Desorption.

Method: Material	Sample Preparation	Injection mode	Column	Carrier gas/Temperature Profile	Detector type/conditions	Reference
GC-MS: Extract	Methylene Chloride	1µL of sample splitless for 1 min at 170°C	Fused silica capillary column; DB-1, 300mm x 0.32mm i.d., 0.25 µm film thickness	Helium: 2ml/min. 120°C for 2min, 120-150°C at 4°C/min, 150-200°C at 15°C/min.	MS: EI, i.v. 70eV, emission energy 0.25mA, electron multiplier voltage 1200V, i.s. 140°C, scan rate 1/s, scan to scan settling time 0.05s, m/z 50-300amu.	(Marles *et al.*, 1987)
GC-MS: Pods	DTD	Direct Thermal Desorption: directly onto injection port of GC for 5 mins at 220°C	DB-1 capillary column: 60m x 0.32mm i.d. x 0.25 µm film thickness	Helium: 1.0ml/min. -20°C for 5min, -20-40°C at 10°C/min, 40-280°C at 4°C/min, 280°C for 30min.	FID MS: EI, m/z 35-350 each second with 0.8s interscan time, i.s. 280°C	(Adedeji *et al.*, 1993)
GC: Pods	Pentane + water, diisopropyl ether, hexane.	Temp 295°C	OV-I glass capillary column, 25m x 0.31mm i.d. x 0.15mm film thickness	Hydrogen: 3 ml/min. 70-220°C at 3°C/min.	FID: 300°C	(Ramaroson-Raonizafini manana *et al.*, 1997)
GC-MS: Pods	-	SPME: 1µL sample size	OV-1701 fused capillary column: 50m x 0.32mm i.d. x 0.30 µm film thickness	Helium: 4ml/min, split 80ml/min. 100-280°C at 3°C/min.	MS: quadrupole, i.s.270°C, i.v. 70eV	Ramaroson-Raonizafini manana *et al.*, 1997)
GC: Pods GC-O: Pods GC-MS: Pods	Diethylether, pentane/ diethylether (1:1 v/v) or pentane/dichloro methane (2:1, v/v).	Automatic injector: 2µL, heated from 20-245°C at 180°C/min and held for 90 minutes. Injector temperature was 250°C	DB-Wax fused silica column: 30m x 0.32mm i.d., 0.25 µm film thickness preceded by 2m x 0.32mm uncoated precolumn.	Hydrogen: 2ml/min. 40°C for 3min, 40-245°C at 3°C/min.	FID: 250°C Sniffing port: 245°C MS: Quadrupole	(Pérez-Silva *et al.*, 2006)
GC-MS: Pods	Diethylether,	Automatic injector: 2µL, heated from 20-245°C at	DB-Wax fused silica column: 30m x 0.32mm	Helium: 1.1ml/min	MS: Quadrupole, EI, i.v.70eV, i.s. 230°C, quadrupole temp	(Pérez-Silva *et al.*, 2006

Method: Material	Sample Preparation	Injection mode	Column	Carrier gas/Temperature Profile	Detector type/conditions	Reference
	pentane/ diethylether (1:1 v/v) or pentane/dichloro methane (2:1, v/v).	180°C/min and held for 90 minutes. Injector temperature was 250°C	i.d., 0.25 µm film thickness preceded by 2m x 0.32mm uncoated precolumn.		150°C. m/z 40-600amu at 1s intervals	
GC-MS: Extract	Diluted 1:10 with water	SPME: PDMS, PA and CW/DVB fibres. Exposed to headspace for 40min then inserted to GC.	DB-5 glass capillary column, 30m x 0.2mm i.d x 0.25 µm film thickness	Helium: 1.0ml/min. 40°C for 2min, 40-200°C at 8°C/min, 200-250°C at 50°C/min.	MS	(Sostaric *et al.*, 2000)
GC:MS: Extract	Diluted with water	SPME: PA fibre exposed to headspace for 40min.	DB-5 column: 30m x 0.2mm x 0.25µm film thickness.	Helium: 1.0ml/min. 40°C for 2min, 40-200°C at 8°C/min, 200-250°C at 50°C/min	MS	(Boyce *et al.*, 2003)
GC-MS: Extract	-	Autosampler with SPME: incubated at 75°C for 10min, PA fibre exposed to headspace for 30min, fibre desorbed for 5 mins at 250°C	5% phenyl methyl polysiloxane copolymer capillary column: 30m x 0.25mm i.d. x 0.25 µm film thickness	Helium: 1.2ml/min, filtered. 100°C for 1min, 100-153°C at 10°C/min, 153-154°C at 0.2°C/min, 154-250°C at 40°C/min.	MS: single quadrupole, m/z 50-300	(de Jager *et al.*, 2007)
GC: Pods	Ethanol extraction	Programmed temperature split/splitless injector operated in the splitless mode at 280°C	Elite 5 column; 30m x 0.32mm i.d., 0.25mm film	Nitrogen: 2.4mL/min. 60-300°C at 10°C/min, 300°C for 10min	FID	(Pardio *et al.*, 2009)
GC-O: Pods	Simultaneous Distillation Extraction	Auto-injector: 1µL sample in splitless mode	HP-1 polymethyl siloxane column: 50m x 0.32mm i.d. x 0.52µm film thickness	Helium: 1.5ml/min. 40-130°C at 2°C/min, 130-250°C at 4°C/min, held at 250°C for 50min.	FID: 250°C. Sniffing port: 250°C.	(Brunschwig *et al.*, 2012)

Method: Material	Sample Preparation	Injection mode	Column	Carrier gas/Temperature Profile	Detector type/conditions	Reference
GC-MS-O: Pods	Dichloromethane	-	Non-polar fused silica capillary column: 60m x 0.32mm i.d. x 1.0µm film thickness	Helium: 3.8ml/min. 40°C for 5min, 40-300°C at 4°C/min, 300°C for 20min.	MS: EI, i.v.70eV, m/z 35-425 at 3 spectra/min. i.s. 230°C, quadrupole 150°C.	(Zhang and Mueller, 2012)
GC-MS-O: Pods	Dichloromethane		Polar fused silica capillary column: 30m x 0.32mm i.d. x 1µm film thickness	Helium: 4.9ml/min. 40°C for 5min, 40-240°C at 4°C/min, 240°C for 20min.	Sniffing port FPD MSD	Zhang and Mueller, 2012)
GCMS: Pods	Dichloromethane Simultaneous Distillation Extraction	Auto-injector: 1µL sample in splitless mode at 250°C	Apolar: HP-1 polymethyl siloxane column: 50m x 0.32mm i.d. x 0.50µm film thickness Polar: HP-Innowax polyethyleneglycol: 60m x 0.25mm i.d. x 0.50µm	Helium: 1.5ml/min. 40-130°C at 2°C/min, 130-250°C at 4°C/min, held at 250°C for 50min.	MS: EI, i.v. 70 eV, m/z 35-450, i.s. 230°C, quadrupole 150°C,	Brunschwig *et al.* (2015)
GC-MS: Extract	-	Splitless injection at 260°C.	SLB-5 ms (5% diphenyl/95% methylsiloxane): 30m x 0.25mm i.d. x 0.25µm film thickness	Helium: 1.5ml/min. 50°C for 3min, 50-260°C at 3°C/min, 260-280°C at 10°C/min	MS: m/z 40-400 at 4/sec.	Schipilliti *et al.* (2016)

c) Concentration of Compounds in Vanilla using GC

It is possible to use GC to determine the concentrations of various compounds of interest within vanilla extracts. Pardio *et al.* (2009) used GC to determine the concentration of compounds of interest in vanilla extracts, looking at the effects of different 'killing' methods in the curing process. The compounds of interest were vanillin, 4-hydroxybenzaldehyde, vanillyl alcohol, vanillic acid, ethyl vanillin and veratraldehyde and it was found that the concentrations of some of these compounds within Mexican beans on a dry weight basis were: 2.96% vanillin, 0.2% 4-hydroxybenzaldehyde, 0.57% vanillyl alcohol and 0.19% vanillic acid.

d) Identification of Volatiles

Using the combination of gas chromatography and mass spectra, it is possible to identify volatile compounds within vanilla extracts. Using the methods described in Table 2.4, Adedeji *et al.* (1993) was able to identify 19 compounds that were found in all of the vanilla beans, regardless of the origin and 30 compounds that could be found in most of the vanilla samples. Pérez-Silva *et al.* (2006) was able to identify 65 compounds in Mexican beans, and Zhang and Mueller (2012) were able to identify 246 compounds within vanilla extracts from various growing regions. Brunschwig *et al.* (2012) identified 120 compounds within Tahitian vanilla. These are all identified within Table A1 in Appendix 1, a complete list of all compounds found and identified within vanilla samples to date. Ramaroson-Raonizafinimanana *et al.* (1997) used GCMS to determine the hydrocarbon content of three species of vanilla; *V. fragrans, V. madagascariensis*, and *V. tahitensis*. They found 25 n-alkanes, 17 branched alkanes, and 12 alkenes in the various vanilla samples, with the major constituents being odd numbered hydrocarbons. They also managed to determine the composition of the vanilla samples based on the number of hydrocarbons.

e) Aroma Identification

Aroma identification is carried out using gas chromatography olfactory (GC-O), with a person sniffing the compounds as they are eluted from the column. Previous studies that have used this and key results are presented in section *2.7.1 Gas Chromatography-Olfactory*.

With the use of GC and related analytical techniques, there are many options for the type of data that can be gathered. Almost all aspects of the composition, concentration

and aroma profile can be determined making this a powerful technique in the analysis of vanilla.

2.8.3 High Pressure Liquid Chromatography

High pressure liquid chromatography or HPLC is a high-pressure form of liquid chromatography; chromatography with a liquid mobile phase (Larson *et al.*, 1997). HPLC can be applied to anything that is soluble in a suitable liquid solvent (Rounds and Gregory III, 2003). The principle of HPLC is that there is a stationary phase attached to the column, and a liquid mobile phase that moves through the column. When a sample is introduced to the system, its components are separated based on relative affinities for the stationary and mobile phases, eluting from the end of the column at different times. The affinity of the compound for the stationary phase is dependent on a number of factors including charge and particle size. HPLC uses high pressure to push the liquid phase through faster, decreasing the time required for the analysis (Rounds and Gregory III, 2003). Typically, the output from HPLC is measured by either absorbance or refractive index. The absorbance is measured by passing light at different wavelengths through the solution as it is eluted and recording the response. Refractive index works similarly, in that the refraction of light entering the solution increases when there is a compound being eluted. Both methods produce a graph of intensity against retention time (Larson *et al.*, 1997).

2.8.3.1 Applications of HPLC

High performance liquid chromatography has several different applications for analysis of vanilla samples. These include determining the concentration of key compounds, checking for adulteration and determining the origins of the vanilla. A summary of the methods used can be found in Table 2.5.

Table 2.5: Summary of the conditions used in high pressure liquid chromatography from various studies on vanilla products. The abbreviations in the table are as follows; PDA: Photo diode Array, DAD: Diode Array Detection, HPLC: High Performance Liquid Chromatography, MPLC: Medium Pressure Liquid Chromatography, i.v.: Ionisation Voltage, MS: Mass Spectrogram, i.s.: Ion Source, UV-Vis: Ultraviolet-Visible light. It should also be noted that within the column dimensions, the first is length, the second is the inner diameter and the third is the particle size.

Method/Material	Preparation	Column/Stationary phase	Mobile Phase/Conditions	Detector	Reference
Reverse phase HPLC: Essence	-	Microsorb C18: 150mm x 4.6mm x 5µm with Brownlee RP-18 30mm guard column	50ml methanol, 100ml acetonitrile, 10ml acetic acid made up to 1L with water. 1ml/min	UV-VIS at 275nm, 0.08 absorbance units	(Archer, 1989)
MPLC: Extract	Ethanol extraction	MPLC RP-8 spheri-5 column, 100mm x 4.6mm x 5µm	Methanol:acidified water (10:90), 1.5ml/min	UV at 254nm	(Ranadive, 1992)
HPLC: Extract	Diluted with water	LiChroCart Superspher 100 RP-18, 50mm x 4mm x 4µm with guard column RP:18, 35mm x 4mm x 5µm	Aqueous solution 0.01M CH_3COONa with A = HCl, B = methanol: 15-85% B in 0-25min, 100% B 25-30min, 0.9ml/min	DAD at 340nm	(Lamprecht *et al.*, 1994)
HPLC: Pods	Methanol:water, pentane:ether	C18 TSK-ge, ODS 80Ts, 150mm x 4.6mm x 5µm	water:methanol:acetic acid:triethylamine	UV at 280nm	(Negishi and Ozawa, 1996)
HPLC: Essence	-	Lichrospher RP	H_3PO_4:water (1:10,000)/ Acetonitrile (14:86), 1ml/min	UV at 278nm	(Ehlers, 1999)
HPLC: Essence	-	Nova-Pak C18	Acetic acid:methanol:tetrahydrofuran (70:30:0.2), 1ml/min	UV at 275nm	(Jagerdeo *et al.*, 2000)
HPLC: Pods	Ethanol:water, Diethyl ether	Lichrospher 60	Aqueous H_3PO4 (1%): Acetonitrile:methanol (95:2:3), 1ml/min	UV at 275nm	(Scharrer and Mosandl, 2001)
HPLC: Extract	Diluted with water	RP-18-15, 250mm x 4mm	Aqueous methanoic acid with A = conc. HCl and B = methanol: A:B 85:15 to 15:85 in 35min, 15:85 to 85:15 in 5min, 0.8ml/min	DAD at 210-360nm	(Pyell *et al.*, 2002)
HPLC: Extract	Diluted with water	Altima C18, 250mm x 4.6mm x 5µm	A = methanol, B = 95:5 water:acetic acid. 0-1min 18% A in B, 1-8min 18-50% A in B, 8-20min 50-75% A in B, 20-30min 75% A in B	UV-VIS at 280nm	(Boyce *et al.*, 2003)
HPLC: Pods	Ethanol extraction	RP-C18 ODS, 250mm x 4.6mm x 5µm	A = acetonitrile, B = water:H_3PO_4 (99.999:0.001). 0-10min 10-40% A, 10-20min 40-80% A, 20-25min 80-100% A, 1.4ml/min	PDA at 254nm	(Sharma *et al.*, 2006)

Method/Material	Preparation	Column/Stationary phase	Mobile Phase/Conditions	Detector	Reference
HPLC: Extract	Diluted with water 100-500 times	Nucleosil C18	water:methanol, acidified water with phosphoric acid: methanol, water:acetontrile, acidified water with phosphoric acid: acetonitrile	UV at 231nm	(Waliszewski *et al.*, 2007)
LC-MS: Extract	Dilution (1:100) with 25% ethanol:75% aqueous (1% v/v) formic acid	Luna ODS C18, 250mm x 2mm x 5µm	Isocratic solution = 35% acetonitrile : 65% aqueous (0.1% v/v) formic acid.	UV at 254nm. MS: 125-250 m/z	(De Jager *et al.*, 2008)
HPLC: Pods	Ethanol extraction	Hypersil C18 RP: 250mm x 4.6mm x 5µm	Acetonitrile: acidified water with H_3PO_4 (10^{-2} M)	DAD at 240-360nm	(Cicchetti and Chaintreau, 2009a)
UHPLC: Pods	Ethanol extraction	Acquity C18 RP 50mm x 2.1mm x 1.7µm	"	"	(Cicchetti and Chaintreau, 2009b)
LC-MS: Pods	Extracted with phosphate buffer	LiChrospher 100 RP-18, 250mm x 4.6mm x 5µm	A = 0.1% acetic acid in water, B = acetonitrile: 0-55min 100-15% A, 55-60min 15-100%, 1.0ml/min	DAD at 200-400nm. MS: negative ion mode, i.e. 70eV, i.v. 4000V, 50-1000 m/z.	(Palama *et al.*, 2009)
HPLC: Pods	Methanol:water, 50:50 v/v	QSLichrospher ODS2: 250mm x 4.6mm x 5µm	A = water:formic acid (98:2 v/v), B = water:acetonitrile:formic acid (18:80:2, v/v/v). 8-13% B for 0-10min, 13-20% B for 10-30min, 20-8% B for 30-35min.	UV at 280nm	(Brillouet *et al.*, 2010)
Reverse phase HPLC: Vanillin	n-pentane: dichloromethane (1:1)	Reverse Phase C18: 250mm x 4.6mm	10% water, 10% acetonitrile, 80% methanol	UV at 271nm	(Van Dyk *et al.*, 2010)
HPLC: Pods	Methanol/H_3PO_4 (10^{-2} M; 28/72 v/v	Licrospher 100 RP18: 250mm x 4mm	30% methanol, 70% phosphoric acid ($1x10^{-2}$ M), 0.7ml/min	UV at 254nm	(Pérez-Silva *et al.*, 2011)
Reverse phase HPLC: Extract	Methanol, water, acetic acid	BDS-Hypersil C18, C8 or Cyanopropyl: 100mm x 4.6mm x 5µm	methanol, methanol:water, water, KNO_3, 1.0ml/min	Diode array	(Lavine *et al.*, 2012)

Method/Material	Preparation	Column/Stationary phase	Mobile Phase/Conditions	Detector	Reference
HPLC: Extract	Diluted with water	Waters u-Bondapak C-18 column: 300mm x 3.9mm	acetonitrile:water - 10:90 v/v, 1ml/min	UV-VIS at 278nm	(Naidu *et al.*, 2012)
HPLC: Pods	Ethanol extraction	Cromolith RP-18e: 100mm x 4.6mm	A = 1 x 10^{-3} M KPO_4, B = Methanol: 30°C, 3-7% B in 2min 1.0ml/min, 7-9% B in 10min 1.0-2.0ml/min, 9-19% B in 7min 2.0ml/min	UV-VIS DAD at 230nm, 254nm and 280nm	(Maruenda *et al.*, 2013)

a) Concentration of Compounds in Vanilla using HPLC

There have been many previous studies that used HPLC to determine the concentration of key compounds within vanilla and vanilla products. HPLC is well suited for samples such as vanilla extract, being able to quickly separate out the components and with a standard curve from external standards, the concentrations can be easily determined.

Archer (1989) used HPLC to determine the concentration of key compounds in vanilla essences, extracts and oleoresins, both natural and artificial. The compounds of interest were vanillin, p-hydroxybenzaldehyde, vanillic acid and p-hydroxybenzoic acid. The concentrations of these compounds as determined by Archer (1989) can be seen in Table 2.6.

Table 2.6: Range of values for compounds of interest in various vanilla products. Adapted from Archer (1989)

Vanilla type	4-hydroxybenzoic acid	Vanillic acid	4-hydroxy-benzaldehyde	Vanillin
Vanilla Essence (mg/100ml)	0.14-2.57	0.50-8.62	2.09-8.34	79.3-128.2
Bourbon Vanilla Extract (mg/100ml)	0.90-1.85	3.49-9.30	5.90-11.20	85.4-163.2
Vanilla Oleoresin (mg/100g)	9-28	17-192	93-305	1580-3980

Ranadive (1992) used HPLC to determine whether the precursors to various flavour compounds were present in green vanilla beans, focussing on vanillin, p-hydroxybenzoic acid, p-hydroxybenzaldehyde and vanillic acid. They found that all of these compounds were present in the green vanilla beans in the form of glycosides, which were released upon curing.

Brillouet *et al.* (2010) used HPLC to determine the distribution of key phenolics and enzymes in vanilla pods. They found that the highest concentration of the aroma forming phenolics and enzymes could be found at the stem end of the pod and decreased with distance from the stem.

Pérez-Silva *et al.* (2011) used HPLC to determine the change in concentration of odour-active compounds in vanilla beans during traditional curing processes. They were able to find a range of different pathways that their chosen compounds of interest

took during the curing process and proposed a biosynthetic pathway from this information.

A number of studies were completed that aimed to measure the concentration of compounds of note within vanilla extract, in order to assess the suitability of a new method of analysis of vanilla (Lamprecht *et al.*, 1994; Negishi and Ozawa, 1996; Pyell *et al.*, 2002; Boyce *et al.*, 2003; Waliszewski *et al.*, 2007; Cicchetti and Chaintreau, 2009b; Palama *et al.*, 2009; Lavine *et al.*, 2012).

The other methods that were compared were stable isotope ratio analysis, which was found suitable for determining concentrations (Lamprecht *et al.*, 1994), octadecyl silica gel columns, which were found to be suitable for vanilla (Negishi and Ozawa, 1996), micellar electrokinetic chromatography (MEKC), which was found to require a much shorter time than HPLC and was more sensitive (Pyell *et al.*, 2002) and ultra-high performance liquid chromatography (UHPLC), which was found to be faster, give better peak resolutions and be more sensitive than HPLC (Cicchetti and Chaintreau, 2009b).

Another method that was compared was a rapid HPLC method that would allow for accurate determination of vanillin content (Waliszewski *et al.*, 2007) and nuclear magnetic resonance was compared to HPLC and found to be accurate (Palama *et al.*, 2009).

A final method that was compared using HPLC was the use of water rich mobile phases in HPLC to determine the concentration of key compounds in synthetic vanilla samples. The target molecules were vanillic acid, isovanillin, o-vanillin, ethyl vanillin, vanillin and coumarin (Lavine *et al.*, 2012).

There were some studies that used HPLC as a method to determine the changes in concentration based on different treatments of vanilla beans before extraction. Sharma *et al.* (2006) compared microwave assisted extraction and ultrasound assisted extraction to the traditional use of ethanol as an extraction agent, finding that although the new methods were adequate, the highest concentration of vanillin was extracted using the ethanol.

Cicchetti and Chaintreau (2009a) determined a method by which accelerated solvent extraction could allow for determination of the vanillin content during curing and processing. Van Dyk *et al.* (2010) tried to find a method that could reduce the curing

time of vanilla beans, while still maintaining the same concentrations of compounds in the final extract. The conclusion was that mild blanching in hot water, followed by sweating at 35-45^{o}C, and rapid drying produced the beans with the best appearance and aroma.

Naidu *et al.* (2012) aimed to find a method using tea leaf enzymes to cure green vanilla beans, using HPLC to measure the concentrations of the main flavour compounds; 4-hydroxy-3-methoxybenzyl alcohol, vanillin, 4-hydroxybenzyl alcohol, vanillic acid and 4-hydroxybenzaldehyde.

Maruenda *et al.* (2013) determined the flavour profile of *Vanilla pompona* ssp. *Grandiflora*, a subspecies of vanilla recently found in the Amazon rainforest. The compounds studied were 4-hydroxybenzyl alcohol, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, glucovanillin, vanillin, vanillyl alcohol, vanillic acid and anisyl alcohol. They found all of the compounds in the mature beans, and concluded that this product would be suitable for further commercialisation as a specialty crop.

b) Adulteration

HPLC has been used to check for adulteration, or the addition of other flavour compounds. Ehlers (1999) and Jagerdeo *et al.* (2000) both used HPLC as a method to check for adulteration in vanilla products, with common adulterants being coumarin and ethyl vanillin.

c) Origins

Another use that HPLC can be used for in relation to vanilla is to determine the origin of the vanilla in question. Scharrer and Mosandl (2001) used concentrations of vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid to successfully determine the origin and year of harvest of various samples of vanilla extracts.

HPLC can be applied to vanilla to determine the concentrations of compounds of interest. HPLC is highly accurate at this, and one of the most suited methods available. In determining the concentration, it is also possible to identify any potential adulteration of vanilla products and the origin of the vanilla in question.

2.8.4 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is based on the principle that hydrogen and carbon atoms absorb electromagnetic radiation at a frequency characteristic to the isotope under investigation. Different chemical groups absorb the radiation differently, allowing for information about the structure of a compound of interest to be determined (Ault and Dudek, 1976). Palama *et al.* (2009) was able to use NMR to determine changes in vanilla beans with maturity. NMR and LC-MS were used to measure the concentration of the compounds, and principal component analysis was used to visualise the relationship of compounds in the methanol:water extracts made from the beans. They found that younger pods had more glucosides, glucose, malic acid and homocitric acid and the older pods had more glucovanillin, vanillin, p-hydroxybenzaldehyde and sucrose. NMR would be a good supporting method to combine with GCMS or LCMS, in order to more accurately identify unknown compounds in vanilla.

2.8.5 Micellar Electrokinetic Chromatography

One method that has been trialled for determining flavour components in vanilla and screening for adulteration is micellar electrokinetic chromatography. The basis for this method is the same as liquid chromatography, but the stationary phase is comprised of micelles instead of a solid matrix. Both Boyce *et al.* (2003) and Bütehorn and Pyell (1996) found the method to be highly effective for screening and identifying vanilla flavours, however there was no significant improvement in precision or speed using this technique rather than HPLC or GC-MS (Boyce *et al.*, 2003).

2.8.6 X-Ray Fluorescence Spectroscopy

Another method that has been applied to identifying adulteration in vanilla extracts is wavelength dispersive X-ray fluorescence. This method serves to determine the elemental concentrations within vanilla extract samples to allow for differentiation of the source and possible contamination. Hondrogiannis *et al.* (2013) found that this method was less time consuming than more commonly used methods, such as mass spectrometry, and the method can be fully automated, enabling more efficient analysis as well. This method would be suitable to use in combination with GC-MS or HPLC-MS to provide more information about the vanilla under investigation.

2.8.7 Correlation of Sensory Analysis and Instrumental Analysis

Although both sensory analysis and instrumental analysis have been applied extensively to the analysis of vanilla and its products, there is limited research on the correlations between the two data sets. Brunschwig *et al.* (2015) combined QDA sensory analysis with GCMS volatile analysis and determined the volatile compounds likely responsible for some of the sensory characteristics of Tahitian vanilla extracts. The conditions used for the GCMS analysis are in Table 2.4. The compounds in the dichloromethane simultaneous distillation vanilla extracts were identified using the mass spectra library (NIST 2008) combined with Kovat's retention indices using C5-C28 n-alkanes. Compounds were then quantified using relative ratios to vanillin based on peak area. They found that the volatile composition of the vanilla extracts varied depending on the growing region with French Polynesian vanilla mainly anisyl compounds (70%); the Parahurahu region had more aldehydes and ketones (2-5%), less phenolics (5%) and less vanillin (<1%). Papua New Guinea vanilla extracts were higher in esters (2%), vanillin (10%) and lower in anisyl compounds (64%) compared to the French Polynesian samples. Madagascar vanilla was much higher in vanillin (30%), phenolics (44%), esters (1%) and aldehydes (2%), and lower in anisyl compounds (7%) compared to the French Polynesian samples. Using sensory analysis (QDA) on ethanol vanilla extracts, they found using nine aroma attributes that *Vanilla planifolia* was most represented by phenolic, woody, smoky, fruity and spicy notes. The *Vanilla tahitensis* samples were more characterised by anise and caramel notes. The Papua New Guinean vanilla, of the *Vanilla tahitensis* variety differed from the other extracts of the same variety, being stronger in fruity, spicy and brown rum notes.

PLSR (partial least squares regression) was able to explain 72% of the variation in the sensory analysis and 81% of the variation in the GCMS analysis and produce the plot in Figure 2.13.

Figure 2.13: Biplot of PLSR of vanilla samples and relationships between sensory attributes and GCMS volatile quantification as determined by Brunschwig *et al.* (2015).

They were not able to draw any definite conclusions about which compound was responsible for each attribute, citing that phenolic compounds had high OAV (odour activation values) and overpowered other attributes and sensory analysis is influenced by interactions between compounds whereas GCMS separates all the compounds out individually. They did manage to draw similarities between the currently reported correlations and the previously reported aromas of the compounds using GC-O (Brunschwig *et al.* 2012). For example, 4-vinylguaiacol was correlated with brown rum notes, and helped differentiate PNG vanilla from the other extracts.

Although this research did explore the relationships between the sensory attributes and the volatiles in the vanilla extract, the exact identification of the volatiles was never confirmed, neither was the concentration. Another limitation of the study was the use of a dichloromethane extract for volatile analysis in GCMS and an ethanol extract for the sensory analysis rather than the same extract for both studies.

2.8.8 Summary of Sensory and Analytical Instrumental Methods for Analysis of Vanilla

For the complete analysis of vanilla and its products, a range of methods would be required. For analysis of the sensory properties of the vanilla, the most suitable method would be a trained sensory panel using descriptive analysis, as instrumental methods are still not able to provide the same information as human participants, and an untrained panel requires a much larger sample size and is not suitable for a multiphase study.

For the analysis of the volatiles within the vanilla extract, GCMS would be the most suitable method, as it is able to separate the compounds, and also provide identification for the compounds produced. GCMS is more suitable than HPLC or HPLC-MS, as it can separate the compounds based on their volatilities, therefore the information obtained is more relatable to the sensory properties of the vanilla, which are largely provided by the aroma compounds volatilising upon consumption.

2.9 Dehydration Methods for Vanilla Extract

Multiple methods could be used to dehydrate vanilla extract to either a powder or an oleoresin (highly concentrated flavour, typically a liquid). The possible drying methods that could be applied to vanilla are spray drying, freeze drying, spray-freeze drying, supercritical fluid extraction, vacuum distillation and encapsulation of flavours (Fang *et al.*, 2002b; Shihadeh *et al.*, 2014; Hundre *et al.*, 2015). Each of these methods will be discussed in more detail.

With the ethanol extract, any methods that use heat are in danger of fire/explosions as well as the loss of volatile aroma/flavour compounds and any methods that use freezing need to consider the freezing temperature of ethanol. The flash point of pure ethanol is 14^{o}C and the autoignition temperature is 363^{o}C. The freezing point of pure ethanol is -115^{o}C (Li *et al.*, 2005). For 35% ethanol, as is found in standard vanilla extract, the flash point is 27.5^{o}C and the freezing point is -19^{o}C.

2.9.1 Spray Drying

Spray drying is one of the more common methods of drying food products. The principal method behind spray drying is the atomisation of the feed solution by a nozzle into a stream of hot air. These small particles dry very quickly, trapping

volatiles inside and the powder is recovered through air circulation through the spray drier unit. Optimal conditions typically occur when the infeed has high solids content, inlet temperatures have been optimised, exit temperatures are high (>100^{o}C) and the flavour molecules are high molecular weight (Masters, 1985).

Several studies have looked at spray drying of ethanol extracts, although none have focussed on vanilla extract (Table 2.7). Lee *et al.* (1999) aimed to encapsulate ethanol soluble pharmaceuticals. The ethanol concentrations investigated ranged up to 30%. It was found that the addition of dextrin was able to encapsulate better but made for a bulky product. Sodium lauryl sulphate (SLS) was able to increase the amount of ethanol encapsulated to 35% with 67.6% efficiency using 1% SLS and a dextrin/water/ethanol ratio of 1.2/1/1.

Fernandes *et al.* (2012) looked at 67% ethanol (m/m) extracts from *Lippia sidoides* (pepper rosmarin) with a target compound of the extraction was thymol, for medicinal purposes. The encapsulating agents used were maltodextrin and gum arabic in varying ratios; 4:1, 3:2, 2:3 and 0:1 (maltodextrin:gum arabic). To prepare the mixtures for spray drying, the carbohydrates were hydrated in 50^{o}C water for two hours then cooled to room temperature. The extract was concentrated using a rotary evaporator before it was spray dried, to remove the ethanol. They were able to retain 70.2 – 84.2% of the target compound (thymol) using higher ratios of gum arabic. Under electron microscope, they found that with more gum arabic, the particles tended to be more spherical with many dents in the surface. With more maltodextrin, the particles were more broken or incomplete.

Table 2.7: Summary of conditions used in spray drying of ethanol plant extracts from various studies.

Extract Key Compound	Ethanol Content	Spray Drier Type	Spray Drier Conditions	Encapsulating aids	Reference
Pharmaceuticals in ethanol	Up to 30% w/w.	Büchi 190 nozzle type mini spray drier.	68.75% w/w solids feed. Inlet 98°C. Outlet 68°C. Feed flow 5 ml/min. Spray pressure 3 kg/cm^2.	Dextrin, Sodium Lauryl Sulphate.	Lee *et al.* (1999)
Lippia sidoides (Pepper Rosmarin)	67% w/w. Concentrated with vacuum evaporator.	Bench-top Lab-Plant SD-05 spray drier. Air cyclone separator to collect dried product.	20% w/w solids feed. Inlet 50°C. Drying air 60m^3/h.	Gum arabic, maltodextrin.	Fernandes *et al.* (2012)
Gingko Leaves	Contains ethanol, concentration unknown	Niro Minor drier with rotating disk for atomisation at the top of the chamber	50% w/w solids feed. Inlet 120-220°C. Outlet 100 - 130°C. Feed flow 22 - 68ml/min.	Gum arabic, maltodextrin, soybean protein.	Haidong *et al.* (2012)
Vanillin	None	Tall form co-current lab-scale dryer (Spray Mate)	27% w/w solids feed. Inlet 110°C. Outlet 60°C. Feed flow 20 ml/min.	B-cyclodextrin, whey protein concentrate	Hundre *et al.* (2015)
Vanillin	None	Two-flow nozzle, counter current, lab scale.	Inlet 180°C-200°C.	Soy protein isolate, maltodextrin.	Noshad *et al.* (2015)
Lippia sidoides (Pepper Rosmarin)	67% w/w. Concentrated with vacuum evaporator.	Bench-top Lab-Plant SD-05 spray drier. Air cyclone separator to collect dried product.	20% w/w solids feed. Inlet 50°C. Drying air 60m^3/h.	Gum arabic, maltodextrin.	Fernandes *et al.* (2012)
Star Fruit	65% w/w. Concentrated with vacuum evaporator.	Lab Plant system spray drier.	Inlet 185°C. Outlet 88°C. Feed flow 6 ml/min.	Maltodextrin	Saikia *et al.* (2015)

Haidong *et al.* (2012) looked at spray drying ethanol extracts of ginkgo leaves, to create a product that could be used for its medicinal properties. The coating materials used were maltodextrin, gum arabic and a soluble soybean protein, homogenised with the ethanol extract before spray drying. It was also found that the more viscous the mixture was after homogenisation, the better the encapsulation.

Saikia *et al.* (2015) compared spray drying with freeze drying for ethanol extracts of star fruit. The ethanol extract was first vacuum concentrated then freeze dried before spray drying and freeze drying. The samples were combined with the encapsulating aid and homogenised before the final drying step. They were able to achieve encapsulation efficiencies up to 97%.

Although these studies all managed to produce a viable end product from an ethanol-based product, all required an encapsulating agent to assist in the processing. Therefore, it seems unlikely that an ethanol extract would be able to be concentrated into a pure powder without an encapsulation aid such as maltodextrin.

2.9.2 Freeze Drying

Freeze drying is a method by which water, or another solvent, is removed as vapour through sublimation from a frozen product under vacuum (Tang and Pikal, 2004). Once the solvent has sublimated into a vapour, it is removed from the drying chamber, and can be condensed on a coil set at a temperature much lower than the freezing point. This method is particularly good for products that are damaged by traditional heated drying methods, and only causes minimal losses of flavour and aroma (Liapis and Bruttini, 2006). There have not been any published studies on freeze dried vanilla extracts, but there have been a few that looked at other ethanol products such as mannitol, vanillin and star fruit (Table 2.8).

Takada *et al.* (2009) looked at freeze drying mannitol in up to 40% v/v ethanol. A 5ml sample was subjected to freeze drying under the conditions shown in Table 2.9. The first stage of freeze drying at the lower temperature was targeted to remove ethanol, and the later drying was targeted to remove water. It was found that the mannitol dissolved in ethanol resulted in a looser dry powder, while when the mannitol was dissolved in water, the product was more a cake-like powder. All ethanol concentrations evaluated were able to achieve a moisture content of 0.37% w/w or less after the freeze drying was completed.

Table 2.8: Summary of conditions used in freeze drying of ethanol plant extracts from various studies.

Extract Key Compound	Ethanol Content	Freeze Drier Conditions	Encapsulating aids	Reference
Mannitol	Up to 40% v/v	Shelf temperature -50°C for 10 hours then -10°C for 20 hours. Second stage drying shelf 30°C for 20 hours. Chamber pressure 10Pa.	N/A	Takada *et al*. (2009)
Vanillin	None	Operating temperature -24°C for 16 hours.	B-cyclodextrin, whey protein concentrate	Hundre *et al.* (2015)
Star Fruit	65% w/w	Samples frozen at -40°C overnight. Freeze dried at -55°C for 24 hours.	Maltodextrin	Saikia *et al.* (2015)

Saikia *et al.* (2015) looked at the microencapsulation of ethanol extract from star fruit. They looked at different ratios of maltodextrin as well as comparing spray drying to freeze drying. To start with, the 65% ethanol extract was concentrated using a vacuum evaporator at 50°C. This was then freeze dried at -55°C and the powder was mixed with maltodextrin at different concentrations (1:10, 1:15 and 1:20 – core:coating material ratio). These mixtures were homogenised at 12,000 rpm. From this, two portions of mixture were separated, one for spray drying, one for freeze drying. The conditions for each are shown in Table 2.7 and Table 2.8. It was found that the freeze-dried powders were more soluble and had a higher encapsulating efficiency while the spray dried powder was paler in colour.

2.9.3 Spray Freeze Drying

Spray freeze drying is a method that combines spray drying with freeze drying to retain the benefits of the gentler process in freeze drying (Hundre *et al.*, 2015). Hundre *et al.* (2015) compared spray drying, freeze drying and spray freeze drying on a model system composed of 10 g β-cyclodextrin, 10 g vanillin, 10 g whey protein concentrate

and 80 g water. The conditions used in the spray drier and freeze drier are shown in Table 2.7 and Table 2.8 respectively.

For the spray freeze drying, they first sprayed the liquid over liquid nitrogen to freeze the particles into spheres, and then used a freeze drier to remove the water from the particles. The conditions used in the spray drier were an inlet temperature of 110°C and an outlet temperature of 60°C. The feed was atomised using compressed air at 24 psi and the feed flow rate was 20 ml/min. The freeze drier was set at -24°C for four hours.

It was found that the spray freeze drier was the best method, as the particles were spherical with small pores which would allow for better hydration later. The spray drier alone created spherical particles, but there were no pores, which would reduce the solubility of the product, and the freeze drier created particles that were very irregularly shaped and larger than the other two methods, as can be seen in Figure 2.14.

Figure 2.14: Comparison of particle shape as obtained in spray drying, freeze drying and spray freeze drying of vanillin using whey protein isolate (WPI) and/or β-cyclodextrin (β-cyd). From Hundre *et al.* (2015)

2.9.4 Supercritical Fluid Extraction

Supercritical fluid extraction is a method by which compounds are extracted from a material using a supercritical fluid as the solvent (Sinha *et al.*, 2008). Carbon dioxide (CO_2) is the most commonly used supercritical fluid solvent, with a triple point of 304 K and 7.4 MPa (Huang *et al.*, 1984). The issue with CO_2 is that it is poor at extracting polar components - the addition of a polar solvent such as water or ethanol can be used to remedy this (Mukhopadhyay, 2007).

Some studies have been published that investigated the use of supercritical carbon dioxide to create a vanilla extract. A summary of the conditions used by each study is presented in Table 2.9.

Table 2.9: Summary of conditions used in supercritical carbon dioxide extraction of vanilla from various studies.

Extract Key Compound	Temperature (K)	Pressure (MPa)	Time (mins)	Reference
Vanilla Beans (freeze dried)	306 - 309	10-13		Nyugen *et al.* (1991)
Vanilla Beans	730	35	140	Fang *et al.* (2002a). Retrieved from Sinha *et al.* (2008)
Vanilla Beans	730	35	150	Fu *et al.* (2002). Retrieved from Sinha *et al.* (2008)
Vanilla Beans (freeze dried)	309	11		Mukhopadhyay (2007)
Vanilla Beans	313	40.8	40	Castillo-Ruz *et al.* (2011)

A number of studies which used supercritical extraction to extract vanilla beans will be described. Firstly, Nyugen *et al.* (1991) was able to extract up to 95% of the available vanillin from freeze dried, ground vanilla beans, using the conditions in Table 2.9. They found that the final flavour extract was 74-97% vanillin, compared to 61% for an ethanol extract, as determined by liquid chromatography. Castillo-Ruz *et al.* (2011) was able to extract 5.82% oleoresin from vanilla beans, with 97.25% of the available vanillin being removed. They found that a smaller particle size, higher pressure and higher temperature were all able to increase the yield of oleoresin. Mukhopadhyay (2007) reported that they were able to extract 10.6% yields of oleoresin using the conditions in Table 2.9. The vanillin content of the oleoresin was 16 to 36% in the oleoresin - between 74% and 97% of the total vanillin content. The other compounds in the oleoresin were 4-hydroxybenzaldehyde, vanillic acid and 4-hydroxybenzoic acid. Mukhopadhyay

(2007) claimed that the extract produced from this process was superior to that of the ethanol extract, having compared the extracts based on vanillin extraction efficiency and colour.

Although these studies were positive about the quality of the extract produced using supercritical carbon dioxide, the sensory properties of the extracts were not investigated, so the true potential for this method to produce a commercially viable product is unknown and should be investigated further.

2.9.5 Vacuum Concentration

Vacuum concentration uses reduced pressure to decrease the boiling point of solvents in a mixture, allowing for a gentler evaporation process than would occur at atmospheric pressures and thus reducing thermal damage to sensitive compounds within the mixture (Pouliot *et al.*, 2014). Vacuum concentration is able to remove the ethanol from ethanol-water mixtures and is commonly used in industry for processes such as distillation of spirits (Brennan, 2011).

Although there is no published work regarding the application of vacuum concentration to vanilla beans or vanilla extract, there have been numerous studies on the use of vacuum concentration on wine and beer. As these both contain alcohol and characteristic flavours that are to be preserved, the findings may be able to be applied to vanilla extract.

Shihadeh *et al.* (2014) used vacuum distillation to reduce the ethanol content during corn fermentation, allowing for a higher yield from the fermentation. The ethanol was reduced from 10% to 6% at multiple stages through the fermentation. The effect on the volatiles and sensory properties were not monitored.

Andrés-Iglesias *et al.* (2015) used vacuum distillation at 102 mbar and 50^{o}C as well as 200 mbar and 67^{o}C to reduce the ethanol content of beer. Analysing the results of seven flavour compounds, it was found that the alcohol content was able to be reduced from 4.7% ethanol to 1% ethanol, when 15% of the total volume had been removed. They found that as the process continued, more water was removed with the ethanol, as well as the more volatile flavour compounds. At 102 mbar, 97% of the esters and 88% of the alcohols were lost, and at 200 mbar, 76% of the esters and 95% of the alcohols were removed. They concluded that it was the higher temperature that was causing more of the volatiles to be removed.

Andrés-Iglesias *et al.* (2016) analysed the vapour fraction removed from beer during vacuum distillation using the same conditions as the previous study. They found that the compounds retained in the beer were amyl alcohols and 2-phenylethanol, which produced a sweet, fruity and flowery flavour in the beer.

All of these studies prove that it was possible to remove ethanol from a mixture of ethanol and water, with volatiles present, but there will be losses in the more volatile aroma compounds, which increase as more ethanol is removed and with higher temperatures, which could affect the final flavour profile of the product.

2.9.6 Encapsulation of Flavours

Encapsulation is a process that allows for the entrapment of the material of interest within a secondary layer made of the encapsulating agent (Gharsallaoui *et al.*, 2007). This allows the material of interest to be stabilised and protected from the environment, while still maintaining its original physical properties (Fang and Bhandari, 2012). This mixture is dried using a range of methods, which are specific to the end product that is desired, with the most common methods being spray drying (*section 2.10.1*) and freeze drying (*section 2.10.2*) due to their simple set up and wide availability (Jafari *et al.*, 2008).

A range of different materials are used as the encapsulating agent. These are summarised in Table 2.10. There are different properties of the encapsulating agent that can affect the material chosen for a specific application. There have been no previously published works that looked at vanilla extract, however there are some studies that have used microencapsulation which will be reported here.

Lee *et al.* (1999) investigated the application of sodium lauryl sulphate (SLS) in the spray drying of ethanol for the purposes of microencapsulating poorly water-soluble medical drugs. The ethanol content of the samples was from 0 to 30% (w/w), and the encapsulating agents used were dextrin and SLS. They found that the best encapsulation efficiency was found with a dextrin:ethanol:water ratio of 0.4:1:1 and the best concentration of SLS to use was 1%, which allowed for an encapsulation efficiency of 67.6%.

Table 2.10: Summary of various types of encapsulating agents, with general physical properties. Adapted from Fang and Bhandari (2012) and Jafari *et al.* (2008)

Wall Material	Type	Example	Encapsulation related properties	Previous Applications
Carbohydrates	Hydrolysed starches	Corn syrup solids Maltodextrins	Very good oxygen barrier, low viscosity at high solids, no/limited emulsion stabilisation, low cost	Citral and linalyl acetate, ethyl caprylate, cheese aroma, linoleic acid, orange peel oil, lemon oil
	Modified starches	Acetylated starch Monostarch phosphate	Good emulsion stabilisation, varying quality, usage restricted based on regulations, low cost	Meat flavour, fish oil, orange oil, d-limonene, l-menthol, butter oil, cream, black pepper oleoresin, vitamin E
	Cyclodextrins	α-cyclodextrin β-cyclodextrin γ-cyclodextrin	Good inclusion of volatiles, excellent oxygen barrier, relatively expensive	Pine flavour, shiitake flavour, d-limonene, ethyl hexanoate, caraway fruity oil, lemon oil
	Gums	Agar Arabic Xanthan Alginates	Good emulsions, very good retention of volatiles, varying quality, price depends on availability, sometimes impurities	Essential oils, monoterpens, orange peel oil, cardamom oil, vegetable oils, cardamom oleoresin, linoleic acid, bixin, short-chain fatty acids, lipids, acetyl pyrroline, soy oil, d-limonene, ethyl butyrate
Proteins	Milk Proteins	Whey Protein	Good emulsions, properties dependant on other factors such as pH and ionic strength, allergenic potential, relatively expensive	Milk fat, linoleic acid, soy oil, ethyl butyrate, ethyl caprylate
		Caseinates Skim milk powders		Fish oil, soy oil
	Other proteins	soy proteins egg proteins gelatine		Orange oil

Fernandes *et al.* (2012) looked at the spray drying of various carbohydrate blends in order to microencapsulate *Lippia sidoides*, an aromatic herb with antimicrobial properties. The herb was made into an ethanol extract, and then concentrated to a dry powder using rotary vacuum concentration. The carbohydrates used were maltodextrin (DE10) and gum arabic, in ratios of 4:1, 3:2, 2:3 and 0:1, with a 1:4 ratio of sample powder to encapsulating agent. They found that the higher concentrations of gum arabic resulted in better retention of the microbially active components of the extract.

Haidong *et al.* (2012) studied the use of combinations of gum arabic, maltodextrin (DE unknown) and soybean protein in the microencapsulation of gingko leaf extracts via spray drying. The gingko leaf extracts were ethanolic, but the preparation of these is not mentioned. The ratio of encapsulating agent that was found to have the highest encapsulation efficiency was 6.1:2.87:11.75:4.28 (core material:gum arabic:maltodextrin:soybean protein), with an efficiency of 82.4%.

Hundre *et al.* (2015) used whey protein isolate and β-cyclodextrin to microencapsulate vanillin in spray drying, freeze drying and spray freeze drying. To prepare the vanillin for the encapsulation, either 10g of β-cyclodextrin, 10g of whey protein isolate or 10g of a 50:50 mixture of the two were mixed with 80g of water and 10g of vanillin. The process with the highest encapsulating efficiency, as determined by available vanillin, was the spray drying with the whey protein isolate alone, with an efficiency of about 86% (exact value not reported).

Noshad *et al.* (2015) looked at maltodextrin (DE 15-20) and soy protein isolate to encapsulate vanillin using spray drying. They found that the optimum encapsulating efficiency (58.3%) was with 8.5% maltodextrin, 1% soy protein isolate and 0.36% vanillin. The remainder of the weight of the prepared solutions was made up with either sunflower oil, which was used to dissolve the vanillin or water, which was used to dissolve the soy protein isolate.

Saikia *et al.* (2015) investigated the application of spray drying and freeze drying in the microencapsulation of star fruit pomace. They used star fruit juice, dried in a tray drier at 50^{o}C for 12 hours, then ground to a powder. This pomace was then extracted with 50% ethanol, acidified to pH 3 and concentrated to a powder in a vacuum concentrator. They encapsulated this with maltodextrin (DE20) at concentrations of 1:10, 1:15 and 1:20 parts extract to maltodextrin. They found that the freeze-dried

samples had lower moisture contents, higher solubility, were less hygroscopic and had a higher encapsulating efficiency, based on retention of phenolics, as analysed by HPLC. The best ratio of extract to maltodextrin, in regards to encapsulating efficiency was 1:20, for both freeze drying and spray drying.

Turchiuli *et al.* (2015) investigated the encapsulation of an aroma in maltodextrin (DE 12) and acacia gum at ratios of 3:2. This was made up with 60% (w/w) water, 32% encapsulation agents and 8% aroma, and compared using spray drying, agglomerated spray dried powders and coated agglomerates. It was found that the spray drying resulted in smaller particle sizes, less than 15% of the aroma was lost during the processing and the flavour was comparable to the original aroma compound mixture.

Krasaekoopt and Jongyin (2017) were able to encapsulate vanilla extract using β-cyclodextrin achieving a 94.5% encapsulation efficiency with 9% vanilla extract. A tray drier was used to dry the mixtures, with kneading used to combine the vanilla extract into the β-cyclodextrin.

Based on literature, it is likely that vanilla extract would be able to be encapsulated. An encapsulating material such as maltodextrin, β-cyclodextrin or gum arabic, or combination thereof, could be used, as they have been found to be successful in a range of similar products (Noshad *et al.*, 2015; Saikia *et al.*, 2015; Turchiuli *et al.*, 2015).

2.9.7 Conclusions on Dehydration

A wide range of processes are available for creating a concentrated form of vanilla extract. The most suitable is likely to be freeze drying, or supercritical carbon dioxide extraction, as both occur at lower temperatures. Vacuum concentration or membrane concentration could increase the range of available methods, as less ethanol would allow for more vigorous heating methods to be applied to the vanilla without danger of fire, although it should be noted that the volatile aroma compounds in vanilla extract could be taken off by the more intensive processes like spray drying.

2.10 Effects of Food Components on Vanilla Aroma and Flavour Perception

The three main components in a food matrix, excluding water are protein, fat and carbohydrates. A combination of these constitutes the majority of food systems and can affect the perception of the flavour of the food (Guichard, 2002).

There have been very limited studies published investigating the effects of these different food components on the aroma and flavour of natural vanilla extracts. Some studies have looked at specific foods in particular, such as ice cream (Stampanoni Koeferli *et al.*, 1996), and others have looked at individual food components in isolation. There have also been some studies that have looked at similar, simpler flavours, such as vanillin, both in full food matrices and looking at individual food components.

2.10.1 Whole Food Matrices

Stampanoni Koeferli *et al.* (1996) investigated the effect of fat, sugar and not-fat milk solids on various attributes of vanilla ice cream. The flavouring used was a natural vanilla extract, sourced from Givaudan-Roure Flavors Ltd., Switzerland. The attributes investigated were sweetness, vanillin, phenolic, caramel, buttery, creamy, milky and whey-like for the flavour, and manual firmness, coldness, ice crystal perception and melting rate for texture. Stampanoni Koeferli *et al.* (1996) found that most of the attributes were affected by the non-fat milk solids and sugar, with two of the attributes significantly affected by the fat (Table 2.11).

Table 2.11: Significantly affected flavour attributes by variable changed in sensory analysis of vanilla ice cream (Stampanoni Koeferli *et al.*, 1996). The values shown are the p-values obtained through ANOVA. Those without numbers were not significantly affected. SNF refers to milk solids non-fat.

Effects	Sweet	Vanillin	Phenolic	Caramel	Buttery	Creamy	Milky	Whey
Fat					<5%	<5%		
SNF	<0.1%	<0.1%	<0.1%	<0.1%	<1%	<0.1%		
Sugar	<0.1%	<0.1%		<0.1%		<0.1%	<0.1%	

They found that the fat increased the buttery and creamy notes, sugar increased the sweetness, caramel and vanillin notes, and decreased milkiness and milk solids-non-fat (SNF) was found to increase creaminess. There were also a range of significant

interactions between the components. The effects seen were concentration dependent, so components could not be considered flavour enhancers or suppressors alone, rather they were flavour modifiers.

There have been a number of studies that looked at the effects of individual components on food flavour and aroma. These components were protein, fat and sugar, which first need to be understood in terms of their individual physical and chemical properties before any understanding of the effect that they might have on a food system can be investigated.

2.10.2 Milk Protein

The most common type of protein used in conjunction with vanilla flavourings is milk protein, in products such as milk, ice cream and custard. Milk contains two main types of protein: casein and whey. Casein is the protein that is precipitated out when the milk is adjusted to a pH of 4.6, and whey is the protein that remains soluble at this pH (McKenzie, 1970; Singh and Flanagan, 2005; Smith *et al.*, 2016).

2.10.2.1 Casein

Casein is a globular protein, with distinct areas of positive and negative charge, resulting in an amphiphilic protein. There are several different types of casein, each with slightly varying properties, but overall, they follow the same trends. They have to associate with themselves and with other casein molecules in order to remove the hydrophobic areas from contact with the water, leading to the formation of micelles (Thompson *et al.*, 2009; Smith *et al.*, 2016).

2.10.2.2 Whey

Whey proteins make up 20% of the protein in dairy milk. There are more different types of whey protein than there are types of casein, but they follow similar patterns in their behaviour. Whey proteins are typical globular proteins, with a structure highly dependent on the conditions – pH, temperature, protein concentration and ionic concentration (Thompson *et al.*, 2009; McSweeney and Fox, 2013).

2.10.2.3 Interactions between Aromas and Proteins

A wide range of different interactions can be formed between a flavour compound and protein including hydrophobic interactions, ionic effects and covalent bonds

(between the NH_2 and SH groups on the protein and the flavour compound). The interactions are affected by the type of protein (amino acid make-up), flavour compound, ionic strength, pH, temperature and time (Guichard, 2002; Van Ruth and Roozen, 2002).

For flavour compounds that have primarily hydrophobic interactions, binding between flavour compounds and the protein increases in strength with increasing carbon chain. Flavour compounds that can react with protein functional groups, such as –OH, -NH_2, or –SH, may be lost to a large extent and no longer able to be perceived when tasted (Guichard, 2006). pH also has an effect on the interaction between flavour and protein, as the changes in protein structure can expose different functional groups. Denaturation of the protein tends to open up hydrophobic parts of the protein, making them more accessible to binding. Therefore, denatured proteins will bind more flavour compounds than native proteins and hence reduce the flavour perception (Guichard, 2002; Van Ruth and Roozen, 2002; Guichard, 2006; Reineccius, 2006).

Hansen and Heinis (1991) investigated the effects of sodium caseinate and whey protein concentrate on vanillin flavour intensity. A trained panel was asked to rate the intensity of the vanillin flavour, sodium caseinate flavour and the whey protein flavour, as compared to references. The protein contents ranged from 0% to 0.5%, and each contained 2.5% sucrose. It was found that for both the sodium caseinate and the whey protein concentrate, the vanillin flavour intensity was less when the protein concentration was increased. It was hypothesised that this was caused by cysteine-aldehyde condensation or Schiff base formation, which reduced the amount of vanillin available for detection in the solutions as it was tightly bound to the proteins.

The same experiment was also conducted using benzaldehyde, d-limonene and citral flavours by Hansen and Heinis (1992). Benzaldehyde flavour intensity decreased with the addition of whey protein, but there was no change with increasing concentration of sodium caseinate. d-limonene flavour intensity decreased with increasing protein concentration, with either type of protein and citral flavour intensity did not change with the addition of protein. It was proposed that the decreased benzaldehyde and d-limonene flavour may be due to non-polar interactions in the casein, interactions with non-polar binding sites, cysteine-aldehdye condensation or Schiff base formation in the whey protein concentrate, reducing the amount of flavour compound freely available in the solution, and reducing perception.

Li *et al.* (2000) found with HPLC that the interaction between vanillin and whey protein isolate was strong as the free vanillin present was reduced, indicating that it would not be released as readily to be detected during tasting. They also found that sodium caseinate interacted with the vanillin, decreasing the free vanillin as the sodium caseinate concentration was increased.

Reiners *et al.* (2000) looked at the interactions between beta-lactoglobulin and 35 flavour compounds in a water-protein solution. The flavour compounds had a wide variety of properties and included vanillin. It was found that longer hydrophobic chain length increased the affinity between the flavour compounds and the protein for esters, pyrazines and phenolic compounds. However, the addition of beta-lactoglobulin did not affect the flavour of the vanillin, as determined by a trained sensory panel.

Saint-Eve *et al.* (2006) looked at varying the caseinate to total protein content in strawberry yoghurts. The ratios investigated were 60%, 81% and 86% caseinate and the yoghurt was sweetened with sucrose. They found that the flavour intensity and the fruity notes were less intense in the yoghurts with the higher caseinate ratio, most likely due to changes in the texture of the yoghurt.

Overall, the chemical structure of the aroma compound determines how it is affected by proteins within foods, with most studies (Hansen and Heinis, 1992; Li *et al.*, 2000; Reiners *et al.*, 2000) agreeing that cysteine-aldehyde condensation or Schiff base formation accounted for the differences observed in the various protein-based food systems.

2.10.3 Fat

Fat is defined as a triglyceride, which is comprised of three fatty acid chains attached to a central glycerol type molecule (Figure 2.15). The $(CH_2)_XCH_3$ group can be made up from any of the fatty acids. Milk fats contain a wide range of different fatty acids, ranging from butyric acid (C4:0), a short chain saturated fatty acid, through to alpha-linolenic acid (C18:3), a long chain polyunsaturated fatty acid (Månsson, 2008). These fats can affect the flavour and aroma that is perceived in foods.

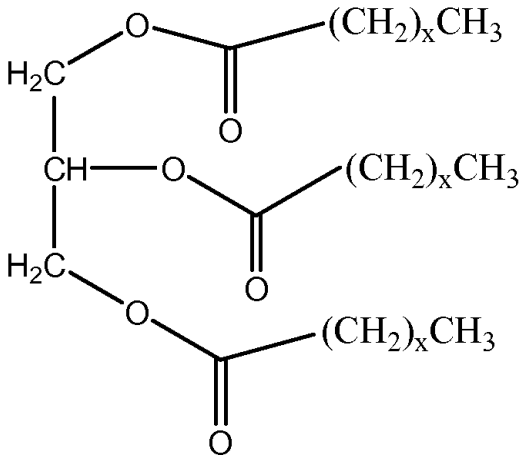


Figure 2.15: Molecular structure of a generic fat.

The amount of aroma detected is determined by the concentration of aroma compounds released into the headspace above the food (Guichard, 2012). Therefore, if a component in the food causes a reduction of the aroma released into the headspace, the overall aroma of the food will be affected.

The presence of fat in a food system can influence the release of lipophilic (fat-loving) flavour/aroma compounds. In Figure 2.16a, the food system contains hydrophilic and lipophilic flavour compounds in water. The lipophilic compound is released more readily into the headspace than the hydrophilic compounds, as it is not bound as tightly to the water (Reineccius, 2006). In Figure 2.16b, an oil has been added to the food system. The lipophilic flavour compound is attracted to the oil and is largely contained within it. Hence, less lipophilic flavour is released into the headspace. The concentration of the hydrophilic compound in the headspace does not change as water is still present to bind to this component (Van Ruth and Roozen, 2002; Reineccius, 2006; Guichard, 2012).

Most aroma compounds are easily dissolved by lipids (Voilley and Etievant, 2006). When the fat is melted, the aroma compounds are released into the headspace and perceived - the fatty acid profile of the fat affects the temporal release of aroma from food (Relkin *et al.*, 2004).

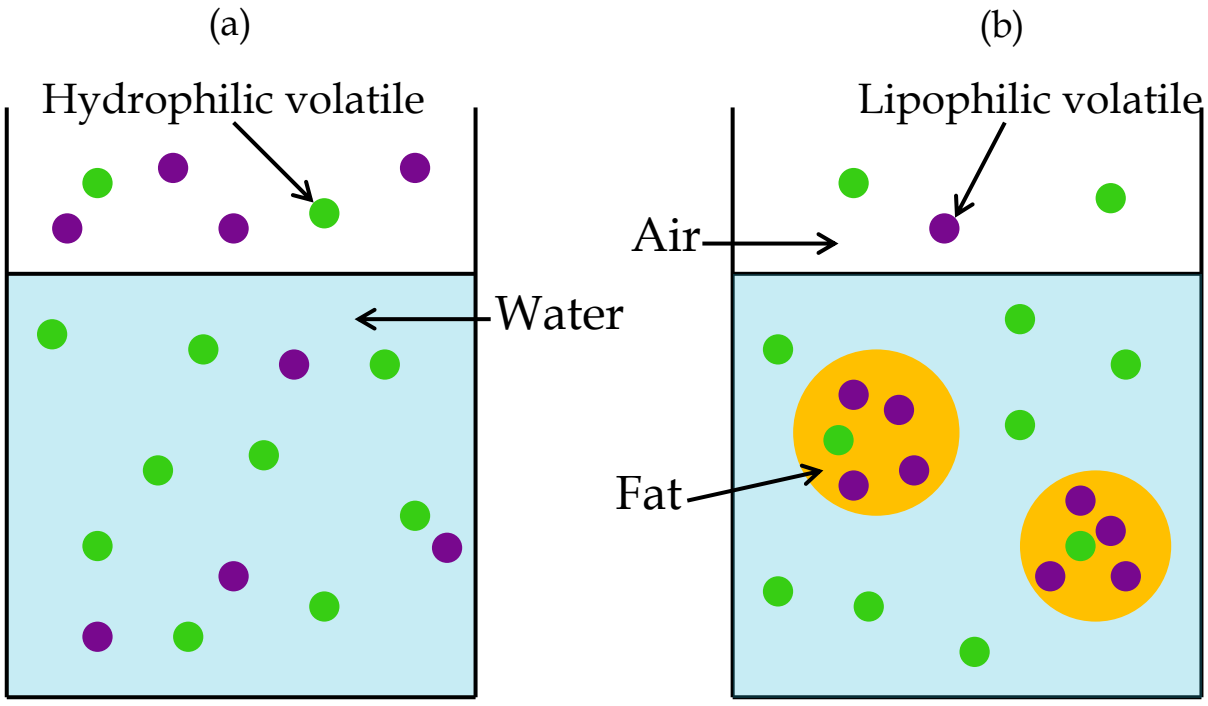


Figure 2.16: Effect of fat on the headspace concentration of hydrophilic and lipophilic flavour compounds. Adapted from Reineccius (2006).

Most taste compounds that give the sweet, sour, bitter, salty, umami tastes are water soluble, they will not dissolve into the fat phase. This means that fat has a limited effect on the five basic tastes. However, as the fat is displacing water in the system, it will increase the concentration of the taste stimuli in the water, although it is also possible that the fat will coat the taste receptors in the mouth and reduce the perceived strength (Reineccius, 2006).

The main types of dairy foods that have been investigated in regard to the effect of the changing fat content are ice cream and milk. These have been investigated with a range of different flavours, mostly vanillin and strawberry flavours.

Li *et al.* (1997) looked at vanilla ice cream, flavoured with vanillin. The fat content ranged from 0.5% to 10%. The mixtures that they made used sucrose for sweetening and cream for the fat addition. They looked at the effects as perceived by a trained panel, looking at the vanilla and sweetness by time intensity. No significant difference in the sweetness perception was found. For the vanilla, it was found that there was a significant difference in the time taken to reach the maximum flavour intensity, taking longer to reach maximum intensity when the fat content was increased.

Ice creams with fat content ranging from 0% to 18% were investigated for the effect on the time intensity of strawberry flavour (Hyvönen *et al.*, 2003). The fat used was both dairy and vegetable. The vegetable fat had a slightly faster flavour release than

the dairy fat and the intensity of the strawberry flavour and aroma was higher in the non-fat samples. No specific flavour compound was used for the strawberry flavour, just a blend from Danisco Ingredients.

Carrapiso *et al.* (2004) investigated vanilla milk with fat contents of 0.1%, 3.5% and 7%. Vanillin was used as the flavouring. It was found that there was no significant difference in the perceived vanilla aroma, but the sweet flavour increased significantly with fat content. It was mentioned that there were insufficient replicates to be able to draw robust conclusions.

Frøst *et al.* (2005) investigated ice creams flavoured with vanillin, as well as other common flavourings: b-ionone (berry), d-nonalactone (coconut) and isopentyl acetate (banana). They made ice creams with fat contents of 3%, 6% and 12% milk fat, and looked at the effects that these had on the physicochemical properties. They found that the effect of changing the fat content depended on the flavour that was being investigated, but overall, increasing fat content increased the perception of the flavour, as determined by trained panellists.

Liou and Grün (2007) used different types of fat, and a range of fat contents to look at strawberry flavours in ice cream. The fats used were cream and fat replacers: Litesse, a polydextrose powder, and Simplesse 100, a microparticulated whey protein concentrate. The fat contents investigated were 4% and 10%. Furaneol and ethyl-3-methyl-3-phenylclycidate are both readily fat soluble and were perceived more strongly in the higher fat ice cream. Cis-3-hexen-1-ol is slightly soluble in water, readily soluble in fats, alpha ionone is water soluble, and gamma-undecalactone is insoluble in water (Burdock, 2009) and were perceived more strongly in the low-fat ice cream. As there is no apparent relationship between the solubility of the compounds and the perception, the effects of the fat were more complex than just solubilities.

Tomaschunas *et al.* (2013) looked at the effects of varying fat content and fat type on the sensory properties of starch based vanilla custard. The fat contents ranged from 0.1% to 15.8% and the fat used was either dairy cream or vegetable fat. It was found that there was a significant difference in the perceived vanilla flavour between the 0.1% fat samples and the other, higher fat content samples. There was no significant effect on the vanilla aroma or the sweet flavour with the changing fat content.

In 2016, Mostafavi *et al.* investigated the rheological and sensory properties of reduced fat vanilla ice cream containing milk protein concentrate, with the protein used to replace the reduced fat. They found that increasing the fat content of the ice cream caused a significant increase in viscosity, smoothness, firmness and overall acceptability. The increase in viscosity was attributed to the increased protein concentration, rather than changes in fat content.

Overall, there were conflicting results in the effect of fat on the flavour of foods, with Liou and Grün (2007) seeming to summarise the effects best – the effects of fats are more complex than just solubilities. For example, Carrapiso *et al.* (2004) found that fat increased the perception of sweetness, however Tomaschunas *et al.* (2013) found that there was no difference in the perceived sweetness with changing fat content.

2.10.4 Sugar

Sugar is a name given to a range of different saccharides, which are often found in plants. The most common sugar used in foods is sucrose (Figure 2.17), a disaccharide made of a combination of glucose and fructose. The chemical structure of sucrose is fairly stable, offering no significant groups that could interact with flavour compounds in foods (Reineccius, 2006).

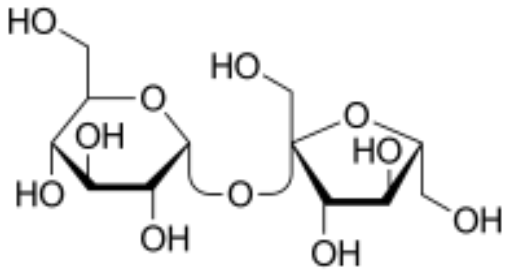


Figure 2.17: Molecular structure of sucrose.

The main interaction that has been found between food flavours and sucrose is a "salting out" effect. "Salting out" is caused by a high concentration of salt, which increases the volatility of flavour compounds in food, increasing the headspace concentration of the aroma/flavour compound. "Salting out" has been found to apply to high concentrations of polysaccharides as well as sodium chloride and MSG (Ventanas *et al.*, 2010). This "salting out" effect has been found to be most active at sucrose concentrations over 20% (Guichard, 2012).

The second interaction between flavours and sugar is that the addition of sugars to sweet foods tends to enhance the flavour perceived. Whether this is due to the "salting out" effect, or an association between sweetness and an increased flavour intensity however is not clear (Van Ruth and Roozen, 2002; Reineccius, 2006; Guichard, 2012).

In regards to the effects of sugars on the five basic tastes, increased sugar in the solution will compete for the receptors on the tongue, and either bind preferentially, or saturate the receptors (Reineccius, 2006). It is through this method that bitter taste is able to be masked by sweet taste.

Marsh *et al.* (2006) looked at the effects of adding sugars and acids to kiwifruit pulps. Through the use of a trained sensory panel, they found that the addition of sugar increased the sweetness and banana flavour perceived, and decreased the acidity, lemon flavour and astringency. It was proposed that the effect on the banana and lemon flavour was more due to panellist associations rather than an actual effect. They also found that the addition of sugar or acid to the pulps affected the headspace volatiles, as determined by GCMS. The effects were different depending on the nature of the compound. For example, hexanal, (E)-3-hexen-1-ol and (Z)-3-hexenol increased significantly when acid was added to the pulps, possibly due to better release of the alcohols in acidic conditions.

Baldwin *et al.* (2008) looked at the effect of sugars and acidity on the perception of the aroma volatiles earthy, medicinal, musty, green, viney, fruity and floral in a deodorised tomato puree. They found that the addition of sugars increased the green and musty aromas and decreased the floral aroma, as well as the sour, citrus and bitter flavours. Adding acid decreased green and floral aroma as well as the sweet taste.

Niimi *et al.* (2014) looked at the effects of the five basic tastes on cheese flavour and aroma. They found that the cheese flavour was enhanced by sugar and salt and suppressed by lactic acid. MSG enhanced the flavour at low concentrations but suppressed it at high concentrations. Interactions between the taste compounds meant that the greatest enhancement of the cheese flavour and aroma was found with a combination of all five taste compounds.

Overall, sugar tended to enhance sweet type flavours, such as fruit flavours, and decrease sour and bitter flavours, with most studies concurring on this point (Marsh *et al.*, 2006; Niimi *et al.*, 2014).

All components in a food matrix have been found to affect flavour and aroma compounds, with varying degrees and effects. As there are a wide range of chemical compounds in vanilla extract, it is impossible to predict the possible outcome of varying these components on the sensory profile.

2.11 Conclusion

Vanilla is a complex food, containing a large number of volatile compounds. Previous research has thoroughly identified many of these compounds, but there is very little information about how the volatile compounds in the vanilla extract are related to the flavour. A range of methods can be used to detect, identify and/or quantify the volatile compounds in the vanilla, including HPLC and GCMS. This combined statistically with any of a number of sensory analysis methods, including QDA, the sensory spectrum method or simply generic sensory analysis, could allow for a better understanding of the connections between the volatiles and the flavour.

After identification of key aroma and flavour volatiles, it will be possible to investigate other aspects of vanilla processing in greater detail, such as the dehydration of vanilla extract into a more concentrated form. Methods that could be used for the dehydration of the vanilla are vacuum concentration, freeze drying, spray drying and supercritical carbon dioxide extraction. The fate of the volatiles could be monitored using GCMS, and when combined with sensory analysis, the relationship between the volatiles and the sensory profile could be better understood along with the effects of the processing on the sensory and volatile profile of vanilla extracts.

Furthermore, once the sensory profile of the vanilla extract has been fully studied, an investigation into the effects of fat and sugar on the vanilla extract sensory profile would also be of benefit, as there is little information available about natural vanilla and how its sensory profile is affected by foods. An understanding of the chemical and physical properties of the volatiles in the vanilla extract would allow for a better understanding of the causes of any sensory differences occurring with the changing fat and sugar levels.

3. General Materials and Methods

Some methods were used throughout the research, to investigate various aspects of vanilla. These methods were a trained sensory panel, chromatography, specifically GCMS, HPLC and GC, and moisture content analysis. The details for these methods are presented in this section, with additional details as to how the methods were adapted to meet each chapter's aims presented in the relevant sections.

3.1 Sensory Analysis of Vanilla extracts

The method chosen for sensory analysis of vanilla extracts was generic descriptive analysis. To conduct descriptive analysis, a group of 8-12 participants were trained on sensory analysis until they were able to reliably rate the attributes of the products. The main stages in the training were screening, training and validation, after which product testing began.

3.1.1 Screening of Potential Trained Participants

Twenty two people were screened for their suitability for the sensory panel. The screening tests used were a basic taste identification test, aroma identification and ranking of flavours and aromas. All samples were presented with a random three-digit code for identification to prevent any bias associated with a name or the order of presentation.

3.1.1.1 Taste Identification

The basic tastes test was based on the standard method ISO 3972:1991 (E), Sensory analysis – Methodology – Method of investigating sensitivity of taste (ISO, 1991), using the solution and concentrations in Table 3.1. All samples were dissolved in reverse osmosis (RO) water (Merck Millipore, Darmstadt, Germany), chosen for its very low level of flavour taints and impurities. Samples of 30 ml were presented in a 60 ml clear disposable plastic (PET) cup at 20±2 ^{o}C.

A selection of 10 samples was presented to each participant, including at least one of each taste with some in duplicate, and some water as blanks. Samples were balanced, randomised and presented monadically. Participants were instructed to taste each sample then record their response on the sheet provided and to rinse their mouths with

RO water between samples. No re-tasting was allowed, the participants were to rely on the first decision made.

Table 3.1: Reference compounds and concentrations used for screening basic tastes of participants.

Taste	Compound	Source	Concentration (g/L)
Bitter	Pure caffeine	Invita, NZ	0.195
Salty	Sodium chloride	Homebrand, Non-iodised, Woolworth's, Australia	1.19
Sour	Citric acid	Hansell's, NZ	0.43
Sweet	Sucrose	Chelsea White Sugar, NZ Sugar, NZ	5.76
Umami	Monosodium glutamate	Shanghai Totole Food Ltd, China	0.595
None/blank	Reverse osmosis water	-	-

3.1.1.2 Aroma Identification and Description

Using ISO 8586-1:1993, Sensory analysis – General guidance for the selection, training and monitoring of assessors – Part 1: Selected assessors (ISO, 1993) as a guide, the participants were tested on their ability to identify and describe aromas, listed in Table 3.2. All dilutions were made using reverse osmosis (RO) water.

Table 3.2: List of flavourings used for aroma identification and description during participant screening.

Aroma	Reference	Source	Concentration (% v/v)
Vanilla	Heilala Single Fold Vanilla Extract	Heilala Vanilla Ltd, NZ	20
Caramel	Natural Caramel Flavour N3522	Sensient Technologies, NZ	5
Strawberry	Natural Strawberry flavour	Sensient Technologies, NZ	30
Clove	Natural Clove #14310	Formula Foods, NZ	1
Chocolate	Natural Chocolate flavour	Sensient Technologies, NZ	20
Bourbon	Jack Daniel's Bourbon Whiskey	Jack Daniel Distillery, USA	100
Almond	Natural N4324	Sensient Technologies, NZ	5
Coconut	Natural essence	Hansell's, NZ	50
Vodka	42 Below Vodka	Lion Nathan, NZ	100

The samples were presented at 20±2 °C in 5 ml amber glass jars with plastic screw lids, with plastic liner. The order of presentation was randomised and samples presented monadically. The participants were asked to remove the cap of the jar, gently sniff the headspace and either identify or describe the aroma. To evaluate the results, correct identification of the aroma was allocated three marks, a close association two marks and a vague association one mark.

3.1.1.3 Aroma and Flavour Intensity Ranking

A ranking test was conducted based on ISO 3972:1991 (E), Sensory analysis – Methodology – Method of investigating sensitivity of taste (ISO, 1991). Both aroma and flavour samples were chosen; the concentrations are in Table 3.3.

All four concentrations for each flavour type were presented at the same time, and participants were asked to rank the intensity of the samples from one as the least intense, to four as the most intense. Each participant received 30 ml of sample at 20±2 °C in a 60 ml disposable clear plastic PET cup.

Table 3.3: Reference flavours and concentrations used for screening potential participants on ability to rank flavours and aromas.

Aroma/ Flavour	Reference	Source	1	2	3	4	Preparation
Vanilla Flavour	Natural Vanilla N97 DG	Sensient Technologies, NZ	0.1	0.2	0.5	1	% v/v in 2% milk
Caramel Flavour	Natural Caramel flavour	Formula Foods, NZ	0.05	0.1	0.2	0.5	% v/v in 2% milk
Almond Aroma	Natural Almond essence	Hansell's NZ	0.1	0.2	0.3	0.4	% v/v in RO water
Strawberry Aroma	Natural Strawberry essence	Hansell's NZ	0.1	0.2	0.3	0.4	% v/v in RO water

Flavour samples were mixed with Anchor light blue 2% fat milk (Fonterra Brands, Auckland, NZ) and aroma samples were mixed with RO water. Hariom *et al.* (2006) used vanilla extract diluted in milk solutions to evaluate the flavour of vanilla extract and Takahashi *et al.* (2013a) used water for aroma analysis of vanilla beans, so both bases were used during screening. Participant scores were only marked correct if they got three or more of the samples in the correct order.

3.1.2 Panel Selection

The final, screened participants were chosen based on their overall score for the three tests (Identification, Description and Ranking). Any participant who scored over 60% was asked to participate in the training and twelve participants were selected.

3.1.3 Panel Training

The panellists attended weekly one hour training sessions. The steps in the training were; to develop a testing procedure as well as to determine a list of attributes to describe natural vanilla extracts and then to reach agreement on the rating of a range of natural vanilla extracts.

3.1.3.1 Sample Presentation

The presentation method for the vanilla extract samples was guided by panellist feedback. Initially, milk was trialled as the presentation medium, but it was found to introduce its own aroma and flavour, making analysis of the vanilla extract aroma/flavour more difficult.

For flavour, a range of concentrations of Heilala single fold extract (details in Table 3.4) were presented to the panellists, from 0.5% v/v to 5% v/v in RO water with concentrations of sucrose ranging from 1% w/v to 5% w/v. It was determined that a concentration of 1.5% v/v of vanilla extract (0.0225 mg/ml vanillin) in 3% w/v sucrose solution was most suitable. Higher concentrations of vanilla extract led to an overpowering alcohol flavour and at lower concentrations attributes could not be detected. The sucrose concentration allowed for a wider range of attributes to be detected, without excess sweetness or bitterness.

Flavour samples were presented in blue glass jars, typically used for olive oil analysis, with 50 ml of sample in the 200 ml capacity glass (Figure 3.1). A watchglass was used as a lid to prevent the escape of any volatiles and the blue of the glass would prevent any bias based on the colour of the sample. Between flavour samples, participants were provided with 20±2 ^{o}C RO water to rinse their mouths.

Figure 3.1: Photographs of blue glasses used for flavour analysis, and brown glass jars used for aroma analysis of vanilla extract samples.

For aroma, a range of presentation methods as well as concentrations were trialled. The presentation methods included smelling strips, with one drop of the pure vanilla extract on the paper strips, and brown glass jars with sealed lids containing dilute vanilla extract. It was determined that a concentration of 10% v/v vanilla extract (0.19 mg/ml vanillin) diluted with RO water in a jar was most suitable. This concentration of vanilla did not allow the alcohol to overpower the other attributes in the extracts but was still strong enough to provide the full aroma profile.

The aroma samples were presented in 100 ml brown glass jars with plastic lids (Figure 3.1) with 50 ml of sample in each. The empty space above the sample in the jar allowed for the development of aromas in the headspace and the colour of the jar prevented any bias based on the colour of the sample. Between aroma samples, the panellists were asked to smell a 100 ml brown glass jar containing 1g of ground coffee (Gregg's granulated rich roast instant coffee), which would act as a cleanser for the nose (Kemp *et al.*, 2009).

The temperature of the samples, for both aroma and flavour was 20±2 ^{o}C, as per ISO 3972:1991 (E), Sensory analysis – Methodology – Method of investigating sensitivity of taste (ISO, 1991). The temperature was controlled by using an incubator set at 20±2 ^{o}C (Polar 1000C, Contherm, NZ) and the samples tested in an air-conditioned room at 20±2 ^{o}C. The samples were periodically checked to ensure they remained within the required temperature range throughout testing.

3.1.3.2 Attribute Generation

After determining the testing conditions, the panellists were asked to generate a list of descriptors to describe the characteristics of natural vanilla extracts. The descriptors had to be understandable to all members of the panel and a reference material found to

support the description of each attribute. Fourteen natural vanilla extracts were used during the training (Table 3.4).

Table 3.4: List of vanilla extracts, with supplier details, used for attribute generation and training of panel members.

Natural Vanilla Extract	Source
Equagold Pure Vanilla Extract – Pacific Tahitian Variety	Equagold, NZ
Heilala 5-Fold Extract	Heilala Vanilla Ltd, NZ
Heilala Glycerol Extract	Heilala Vanilla Ltd, NZ
Heilala Infusion 2	Heilala Vanilla Ltd, NZ
Heilala Infusion 3	Heilala Vanilla Ltd, NZ
Heilala Single Fold Extract	Heilala Vanilla Ltd, NZ
Natural Vanilla N97 DG	Sensient Technologies, NZ
Nielson Massey Madagascar Bourbon Vanilla Extract	Nielson Massey Vanillas Inc., IL, USA
Queen Finest Vanilla Extract with Seeds – Vava'u	Queen Fine Foods, Australia
Queen Natural Organic Vanilla Essence – Extract	Queen Fine Foods, Australia
Queen Natural Organic Vanilla Madagascan Extract – Certified Organic	Queen Fine Foods, Australia
Simply Organic Madagascar Pure Vanilla Extract	Frontier Co-op, Norway
Vanilla Flavour N7609-NAT	Sensient Technologies, NZ
Vanilla Natural #15300	Formula Foods, NZ

The panellists initially defined 37 descriptors for the aroma and flavour, which were eventually narrowed down to 14 attributes; overall aroma, artificial fruity aroma, sweet aroma, raisin aroma, spicy aroma, vanilla aroma, overall flavour, bitter flavour, butterscotch flavour, raisin flavour, straw flavour, sweet flavour, vanilla flavour and woody flavour. A full description of each attribute is given in Table 3.5 and the reference materials and concentrations are in Table 3.6. Two attributes, bourbon aroma and bourbon flavour were introduced later, after further training identified the need for these attributes.

Table 3.5: Lexicon of the attributes chosen for aroma and flavour analysis of natural vanilla extracts.

Attribute	Description
Overall Aroma	The overall intensity of the aroma.
Artificial Fruity Aroma	An artificial type banana/fruity smell. Also includes terms such as tropical fruit and plastic.
Bourbon Aroma (Introduced later)	The characteristic smoky, woody bourbon smell. Includes an alcohol smell.
Sweet Aroma (renamed as Caramel Aroma)	A brown, sweet smell. The caramel type aroma from brown sugar, but not including the mineral, black or molasses type notes.
Raisin Aroma	The aroma of raisins. Dried fruit.
Spicy Aroma	The aroma of a combination of sweet spices. "Hot-cross buns" or spiced buns. Spice shop.
Vanilla Aroma	Aroma associated with pure vanillin.
Overall Flavour	The overall intensity of the flavour.
Bitter Flavour	The bitter taste associated with pure caffeine. Also astringent/drying in the mouth.
Bourbon Flavour (Introduced later)	The smoky, woody alcohol flavour of bourbon.
Butterscotch Flavour	The caramel, buttery and brown sugar flavour associated with butterscotch sweets.
Raisin Flavour	The characteristic flavour of raisins. Also dried fruit flavour.
Straw Flavour	An oaty, straw flavour. Also hay and dried grass.
Sweet Flavour	The sweet taste from sucrose.
Vanilla Flavour	The flavour of vanillin.
Woody Flavour	A smoky, wine-like wood.

Table 3.6: Details of references selected for each attribute, with preparation methods. The value in brackets is the rating for each reference on the nine-point scale.

Attribute	Reference	Low	Medium	High	Preparation
Overall Aroma	Heilala single-fold vanilla extract	5% v/v (2)	10% v/v (4)	15% v/v (7)	Diluted with RO water at 20°C.
Artificial Fruity Aroma	Sensient artificial banana flavour No. N26	0.001 g/L (2)	0.005g/L (5)	0.01g/L (9)	Diluted with RO water at 20°C.
Bourbon Aroma	Jim Beam bourbon whiskey	0.5% v/v (3)	1.5% v/v (6)	3% v/v (9)	Diluted with RO water at 20°C.
Caramel Aroma	Chelsea brown sugar		10 g/kg (4)	50g/kg (8)	Mix given weight of Chelsea brown sugar with Chelsea white sugar. (g brown sugar/kg white sugar)
Raisin Aroma	Sunmaid Californian raisins		50 g/L (4)	100g/L (8)	Soak raisins in 20°C RO water overnight (12-15 hours), then strain. Use strained liquid as reference.
Spicy Aroma	Gregg's ground spices: 5g cinnamon, 9.4g allspice, 1.1g nutmeg		2 g/kg (4)	8 g/kg (8)	Mix spices together. Mix this combination with Chelsea white sugar to strength specified. (g spices/kg white sugar)
Vanilla Aroma	Brenntag Rhovanil® Vanillin		25 g/L (4)	50 g/L (8)	Dissolve into fresh sunflower oil at 20°C.
Overall Flavour	Heilala single-fold vanilla extract	1% v/v (3)	1.5% v/v (6)	2% v/v (8)	Diluted with RO water at 20°C. Add 3% w/v Chelsea white sugar.
Vanilla Flavour	Brenntag Rhovanil® Vanillin	0.16 g/L (4)	0.24 g/L (6)	0.32 g/L (8)	Diluted with RO water at 20°C. Add 3% w/v Chelsea white sugar.
Sweet Flavour	Chelsea white sugar	1.5% w/v (2)	3% w/v (5)	4.5% w/v (8)	Diluted with RO water at 20°C.
Butterscotch Flavour	Kiwiland butterscotch sweets	5 g/L (3)	7.5 g/L (5)	10 g /L (7)	Diluted with RO water at 20°C until the sweets were fully dissolved.
Raisin Flavour	Sunmaid Californian raisins	30 g/L (3)	45 g/L (5)	60g/L (7)	Diluted with RO water at 20°C with 3% (w/v) Chelsea white sugar. Leave overnight at 20°C (12-15 hours). Strain out raisins and use liquid as reference.
Bitter Flavour	Caffeine (Invita, pure caffeine)	0.25g/L (3)	0.40g/L (5)	0.54 g/L (8)	Diluted with RO water at 20°C.
Straw Flavour	Morlife oat straw tea leaves	0.8g/L (3)	1.25g/L (5)	1.6 g/L (8)	Soak tea leaves in boiling RO water for 5 minutes, then strain. Add 1.5% w/v Chelsea white sugar.
Woody Flavour	Vintner's Harvest French toasted oak chips	1:20 dilution of stock solution (3)	1:15 dilution of stock solution (5)	1:10 dilution of stock solution (8)	Stock solution is 1g of wood chips into 250 mL of boiling RO water for 5 minutes, then strain.
Bourbon Flavour	Jim Beam bourbon whiskey	0.1% v/v (3)	0.3% v/v (5)	0.5% (7)	Diluted with RO water at 20°C.

3.1.3.3 Training

After selection of the attributes the panellists were trained in the use of a nine-point interval scale, with half marks allowed for greater precision. Training was continued until panellists were able to use the scales consistently, standard deviations within the groups were less than one or within 10% of the scale used, and they were consistent from one session to the next. The total training time to reach this point was 30 hours.

3.1.3.4 Training of Additional Panellists

At the end of the training only six participants remained. Others of the original 12 had left due to lack of motivation, missing too many sessions or other unavoidable commitments. As a result, a second round of screening and training was carried out to increase the number of panellists. The methods in *3.1.2 Panel Selection and 3.1.3.3 Training* were used. Sample presentation and attribute generation were not required, as attributes had been set by the original group. Sixteen participants were chosen from screening and 10 remained after 24 hours of training. This new group was then combined with the previously trained panellists and validated using the vanilla extracts and concentrations in Table 3.7 to make a total of 16 participants. To improve their performance, the attribute sweet aroma was renamed as caramel aroma, to reduce confusion with sweet flavour (a white sweet instead of a brown sweet). The attributes bourbon aroma and bourbon flavour were introduced to include the alcohol notes that were being detected. A further eight hours of training was required before beginning the first set of sample testing.

Table 3.7: Details of vanilla extract dilutions used for validation of aroma and flavour

	Concentration (ml/L)	
Vanilla Extract	**Aroma**	**Flavour**
Heilala I1 Vanilla extract	18	2.32
Heilala I3 Vanilla Extract	150	15.52
Heilala Glycerol	32	4.13
Queens Organic Vanilla Extract	130	15
Sensient N97-DG Natural Vanilla Extract	20	2
Equagold Vanilla Extract	130	15

3.1.4 Sensory Testing

3.1.4.1 Sample Organisation

Samples were separated into blocks of no more than seven samples for testing in one session, to reduce sensory fatigue. Within each block, samples were presented in a randomised order, with one random duplicate sample presented at the end of each session as a check of performance.

Panellists attended three sessions in one week to complete triplicates for each sample. The specific samples used varied depending on the experiment and are stated within their corresponding chapters within this thesis.

3.1.4.2 Testing Conditions

Testing was conducted in temperature controlled individual sensory booths at 20±2 ^{o}C under white light. The temperature of the samples was determined by the type of sample – samples in water were presented at 20±2 ^{o}C, as per ISO 3972:1991 Sensory analysis - Methodology - Method of investigating sensitivity of taste (ISO, 1991) and samples in milk (*Chapter 8*) were presented at 16±2 ^{o}C, as per ISO 22935-2:2012, Milk and milk products – Sensory analysis – Part 2: Recommended methods for sensory evaluation (ISO, 2012).

All references were presented to the panellists at the start of each session, which they kept and referred to during the testing. Aroma references were presented in 100 ml brown glass jars with lids. Flavour references were presented on a tray with 20 ml of each reference sample in a 30 ml glass shot glass. To remove lingering after tastes and aromas between samples, panellists were given RO water for flavour samples in water, 1% lemon juice (100% Squeezed Lemon Juice, Lemon Fresh, New Zealand) in RO water for flavour samples in milk and 1 g of coffee powder in a 100 ml brown glass jar, with sealed plastic lid, for all aroma samples.

As a check of panel performance, one sample was randomly chosen to be presented to each panellist in duplicate at the end of each session, comparing the results of the sample in the session with the duplicate using a Student's t-test.

3.1.5 Sensory Results Analysis

Results of the sensory testing were analysed using Minitab (Version 16.1.0, Minitab, USA), XLStat (Version 2015.4.01.20270, Microsoft, USA), Statistica (Version 13, Dell Inc., USA) and SPSS (Version 21, IBM, USA).

To analyse panel performance, ANOVA was carried out in SPSS, with panellist as a random factor and product and session as main effects. Statistical significance was set at $\alpha = 0.05$. As a confirmation of the panel performance, a Student's t-test was conducted to compare the duplicate samples at the end of each session with the same sample within the session.

3.2 Quantification of Chemical Properties

To be able to fully understand the vanilla extracts, a range of different methods were used to analyse the physical properties, including gas chromatography-mass spectrometry, high pressure liquid chromatography, gas chromatography and moisture content.

3.2.1 Gas Chromatography – Mass Spectrometry to Identify and Quantify Volatiles

Gas Chromatography- Mass Spectrometry (GCMS) was used to investigate the presence, identification and the concentration of volatile chemical compounds present in the natural vanilla extracts.

A Shimadzu GCMS-2010 gas chromatogram with a GCMS-QP2010 mass spectrometer was used (Shimadzu Corporation, Japan) with a Restek (Restek, USA) Rtx-5 column, with fused silica, low polarity phase, crossbond diphenyl dimethyl polysiloxane (60 m x 0.25 mm with 0.25 µm stationary phase). The temperature programme was as follows: 40 ^{o}C for two minutes, ramping up at 5 ^{o}C/min to 250^{o}C and holding for five minutes - 49 minutes in total. The carrier gas was Helium (Zero Grade, >99.995%, BOC, New Zealand) at a flow rate of 0.96 ml/min, under pressure control, and a total flow rate of 20 ml/min. The sample size was 1 µl with splitless direct liquid injection into a 200^{o}C injector port. The interface temperature was 200^{o}C, with the detector voltage set to 1kV and the scanning range was 40-350m/z with electron ionisation. The solvent cut time was 13 minutes, after which input to the detector was recorded.

The mass spectra of the samples were analysed using the NIST 2008 library (Scientific Instrument Services, Inc., NJ, USA), and compared to the reference standards in Table 3.8 to confirm the identification of the compounds, using both retention times and mass spectrograms. If no reference compound was available to purchase, identity was only tentative based on the NIST library. The identification was based on the most similar mass spectrum, with a similarity over 85%.

To determine the concentration of each reference standard within the natural vanilla extracts, the reference compounds were diluted in water or ethanol, depending on solubility properties, to a concentration expected within natural vanilla extracts (<1mg/ml for most) based on values reported by Toth *et al.* (2010). A minimum of five concentrations was used to create each standard curve. The standard curves can be found in Appendix 2. The vanilla extract samples were not diluted before injection to ensure that the smaller peaks were resolved sufficiently.

Table 3.8: List of reference standards used for GCMS and HPLC analysis of natural vanilla extracts, with supplier and purity. All suppliers were based in New Zealand.

Compound	Purity	Supplier
2-methoxy-4-methyl phenol	≥98%	Sigma-Aldrich
3,4-dimethoxybenzaldehyde	99%	Aldrich
3-methyl-2-furoic acid	97%	Aldrich
4-hydroxy-3-benzyl alcohol	98%	Aldrich
4-hydroxybenzaldehyde	>98%	Aldrich
4-hydroxybenzoic acid	>99%	Aldrich
5-(hydroxymethyl)furfural	>99%	Aldrich
Acetovanillone	≥98%	Sigma-Aldrich
Benzaldehyde	≥99.5%	Sigma-Aldrich
Benzoic acid	≥99.5%	Sigma-Aldrich
Ethyl homovanillate	97%	Aldrich
Guaiacol	>99%	Acrōs organics
Hexanoic acid	≥99.5%	Aldrich
Isovanillin	≥95%	Aldrich
Maltol	≥99%	Sigma-Aldrich
Methyl benzoate	≥99.5%	Sigma-Aldrich
p-cresol	>99%	Sigma-aldrich
Syringaldehyde	98%	Aldrich
Valeraldehyde	≥97.5%	Sigma-Aldrich
Vanillic acid	≥97.0%	Fluka
Vanillic alcohol	>98%	Fluka
Vanillin	>97%	Sigma

3.2.2 High Pressure Liquid Chromatography to Quantify Four Phenolic Compounds

The vanilla extract samples were analysed by High Pressure Liquid Chromatography (HPLC) to determine the concentration of four phenolic compounds at the greatest concentration in the extracts. The phenolics were 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillic acid and vanillin.

The system used was a Shimadzu HPLC 10AVP (Shimadzu Corporation, Japan) fitted with a Gemini 5u C18 110A column (150mm x 4.6mm i.d. x 5µm) (Phenomenex, USA) and a diode array at 254 nm as the detector. The mobile phase was run at 0.8 ml/min, at 30 ^{o}C and was made up with a gradient of solvent A: 2.5% acetic acid (100%, Fisher Brand, New Zealand) and solvent B: 100% acetonitrile (HPLC Grade, Fisher Brand, New Zealand). A linear gradient starting at 95% (v/v) A was used. At 20 mins the gradient was 75% solvent A, at 40 minutes the gradient was 50% solvent A and held for 10 minutes, before being returned to 95% solvent A at 55 minutes. A 0.1 ml sample was injected for each run, and all samples run in triplicate. Samples were filtered using a Nalgene 13 mm Nylon syringe filter (Thermoscientific, New Zealand) into a 2 ml glass sample vial.

To determine the concentration of the phenolics, a standard curve was prepared using six concentrations of each compound. The chemicals were dissolved in 40% ethanol (Absolute, Fisher Scientific, NZ) in water and the samples filtered. A 10ml glass syringe, fitted with a Nylon membrane filter (pore size 0.45 µm, diameter 13mm, GRACE, New Zealand) was used to filter the samples into the 2ml glass sample vials. The area under the peak was used to create the standard curve. The standard curves created can be found in Appendix 3.

3.2.3 Gas Chromatography for Quantification of Ethanol Concentration

Gas chromatography (GC) was used to measure the ethanol concentration in the vanilla extracts. A GC-17A Shimadzu unit (Shimadzu Corporation, Japan). The column was a Phenomex DBwax column 30 m x 0.32 mm I.D. with 0.25 µm stationary phase (Phenomenex, USA). Detection on the GC was with FID (flame ionising detector) with nitrogen (Oxygen Free, BOC, New Zealand) as the carrier gas, with a flow rate of 76 ml/min at 142 kPa under pressure control. Samples were analysed using a temperature programme, holding at 40 ^{o}C for 10 minutes, then increasing the temperature at 10

°C/min up to 250 °C for a total time of 31 minutes. The temperature of the injector port was 150 °C and 0.2 µl of the liquid sample was injected with a 10:1 split.

To determine the concentration of ethanol in the samples, a standard curve with seven different concentrations from 2.5% v/v to 15% v/v was created. The dilutions were made using absolute ethanol (Fisher Scientific, NZ) and ultra-pure deionised water (Millipore, USA). The standard curve can be found in Appendix 3.

3.2.4 Moisture Content

3.2.4.1 Moisture Content of Vanilla Beans

Aluminium moisture dishes were placed into a Contherm incubator (Polar 1000C, Contherm, NZ) for a minimum of eight hours at 70±1 °C. The dishes were transferred to a desiccator with desiccant, left for 45 minutes to cool to room temperature then weighed (CP4202S, D=0.0001g, Sartorius, Germany).

Fifteen to twenty grams of vanilla bean was cut into pieces 3-5 mm in length and weighed out into the moisture dishes with 5-7 g being portioned into each dish. The dishes were placed into the incubator set at 70±1 °C and left for 48 hours until constant weight was achieved. The dishes and samples were left at 20±2 °C for 45 minutes in a desiccator before being weighed. The final weight of the beans was recorded, and the moisture content of the beans calculated as per Equation (3.1).

$$Moisture\ content\ (\%) = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Initial\ weight\ (g)} \times 100 \qquad (3.1)$$

3.2.4.2 Moisture Content of Other Materials

For other materials, such as freeze-dried powders, a similar method to *3.2.4.1* was used. The temperature of the incubator was set at 105±1 °C, and ~5 g of sample used with all other procedures the same.

4. Vanilla Bean Extraction Conditions

4.1 Introduction

Vanilla beans contain over 500 different volatile compounds (Toth *et al.*, 2010). These volatiles have a range of different properties, such as boiling point, polarity and solubility, all of which affect how they are extracted during the flavour extraction process in vanilla production. Therefore, the extraction of these volatiles could be affected by a number of factors including ethanol concentration, solvent type and size of vanilla bean pieces.

There is little information available about these factors, with the most relevant study by Pérez-Silva *et al.* (2006). They produced an extract using pentane/ether, finding 65 compounds with GCMS, which were then analysed using GC-O to determine the aroma of these compounds. Only three solvents were investigated, and no other factors which could have affected the extraction efficiency, leaving much room for further research into other factors that affect the extraction of volatiles from vanilla beans.

With the limited information available about vanilla volatile extractions, further investigation was required. Thus, the aims of this chapter were:

- To investigate the effect of varying ethanol concentration on the extraction of volatiles in cut vanilla beans using GCMS
- To compare the volatile compounds extracted from cut vanilla beans using six different solvents
- To compare the volatiles extracted with ethanol and water on hand-cut, blended and finely ground, freeze-dried vanilla beans.

4.2 Materials and Methods

Tongan vanilla beans (2014, Heilala Ltd., New Zealand) were used for all extractions in this chapter.

4.2.1 Solvents for extractions

Six different solvents were used for the extractions (Table 4.1), with ethanol used at a range of concentrations to match with standard flavour extraction in industry. Glycerol was not able to be used as its viscosity was too high at 20^{o}C and the

extractions were not successful through lack of migration of flavour compounds during the extraction process, although it is used in commercial extractions at lower concentrations (Cameron, 2011). The solvents were chosen as they represented a range of properties for boiling point and polarity (Table 4.10).

Table 4.1: Details of different solvents used for extraction of Tongan vanilla beans.

Solvent	Grade/Supplier	Concentration
Acetonitrile	HPLC Grade, >99%, Fisher Scientific Ltd., New Zealand	100%
Ethanol	Absolute, Lab Serv , New Zealand	100%
		75% (v/v with RO water)
		50% (v/v with RO water)
		25% (v/v with RO water)
n-Hexane	98%, Fisher Scientific Ltd., New Zealand	100%
Methanol	HPLC Grade 99.99%, Fisher Scientific Ltd., New Zealand	100%
n-Pentane	Analytical Grade 99%, Univar, New Zealand	100%
Water	Reverse Osmosis (RO), Millipore, USA.	100%

4.2.2 Preparation of Vanilla Beans

Three different preparations were used for the vanilla bean extractions – cut, blended and ground. The cut beans were hand cut to a length of 3-5 mm. The blended beans were first hand cut, then blended 30 seconds (Waring 7011HS, Waring Commercial, USA) until a homogeneous paste was formed. The ground beans were freeze dried as follows; the hand cut beans were frozen at -20^{o}C ± 2 ^{o}C for 12 hours then freeze dried for 24 hours on trays using a Labconco FreeZone6 Freeze Drier (Labconco, USA). The vacuum averaged 0.1 mbar and the trays started at -20^{o}C and were increased to 20^{o}C gradually as the vanilla dried and the collector coil was set to -50^{o}C ± 1^{o}C. After the beans had dried, they were removed from the freeze drier and ground to a fine powder using a coffee grinder (Sunbeam EM0405 Multigrinder, Sunbeam New Zealand), pulsing for 20 s until a consistent texture was attained.

4.2.3 Vanilla Bean Extraction

Each extraction used 5 ± 0.05 g of vanilla beans (cut, blended or ground), combined with 5 ml of the chosen solvent, as this was a similar ratio to that used in industry for flavour extractions (Cameron, 2011). These were left in 100 ml glass Schott bottles, with plastic lids, also sealed with Parafilm for 72 hours unagitated at 20±2 ^{o}C. At the end of

the 72 hours, the samples were filtered using nylon syringe filters (13mm diameter, 0.54 µm pore, GRACE, New Zealand) into 1.5ml capacity glass vials for analysis on GCMS. The methods used for chromatography were the same as those outlined in *3.2.1* and all samples were analysed in triplicate. For some of the samples (pentane, water)?, the solvent was rapidly soaked up into the beans. These had to be repeated with larger volumes of solvent to ensure that there was sufficient liquid extract available for testing. This was due to the dehydrated nature of the vanilla beans, as they are dried during the curing process to around 20% moisture content (Cameron, 2011). Details of the volumes of solvent required are in Table 4.2.

Table 4.2: Volumes of each solvent used for extractions of 5 g of vanilla beans.

	Volume of Solvent (mL)		
Solvent	**Cut**	**Blended**	**Ground**
Acetonitrile	5	5	5
100% Ethanol	5	5	5
75% Ethanol	5	5	5
50% Ethanol	5	5	10
25% Ethanol	5	10	10
n-Hexane	5	5	5
Methanol	5	5	5
n-Pentane	10	5	5
Water	15	15	25

4.3 Results and Discussion

The first aspect investigated was the effect of the ethanol concentration on the volatiles extracted, with a concentration around 40% ethanol of particular interest, as this is the concentration of ethanol in most single fold vanilla extracts. The second aspect investigated was the potential use of different solvents to extract vanilla flavour, although none of the solvents were food grade, this would allow for insight into the nature of the volatiles in the vanilla beans in regard to polarity and solubility. The final aspect investigated was the difference between cut, blended and ground vanilla beans.

4.3.1 Effect of Ethanol Concentration on Volatile Content of Vanilla Extract

The various concentrations of ethanol and water were compared using the 10 most concentrated compounds extracted by each ethanol concentration for cut beans only, to limit the number of factors under investigation at one time and clarify the patterns

observed. The tentative identification (based on NIST 2008 MS library), retention time, peak area and percent of total area for each are in Tables 4.3 to 4.7.

A chromatogram for each ethanol concentration is shown in Figures 4.1 to 4.5. Based on 100% ethanol, the compounds identified were ordered by retention time and numbered alphabetically. To compare to other ethanol concentrations, the same alphabetical numbering was used and continued for any new compounds eluted in other ethanol concentrations (Tables 4.3 to 4.7).

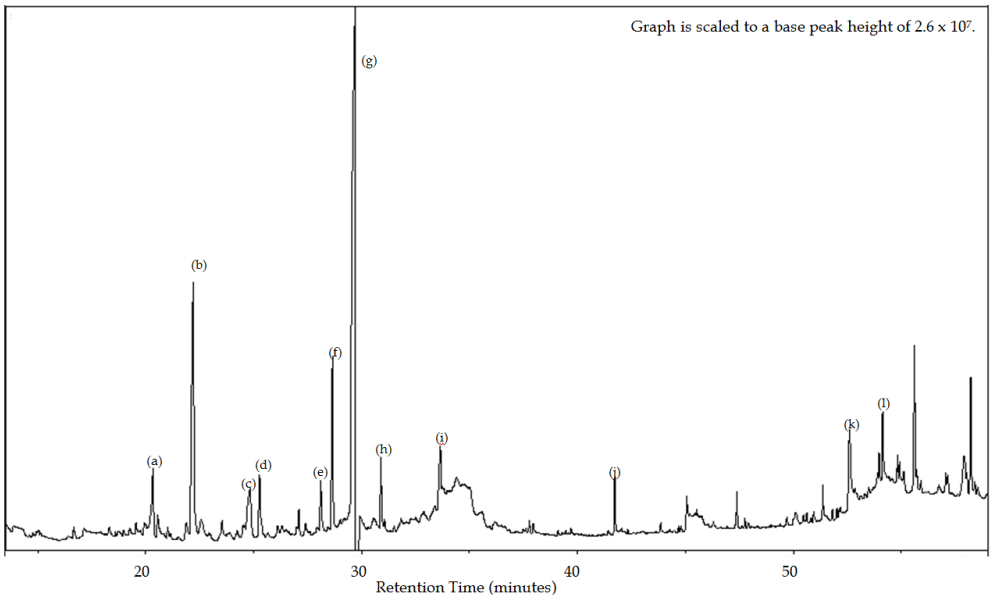


Figure 4.1: Chromatogram from GCMS of 100% ethanol vanilla extract. Labels on peaks refer to Table 4.3.

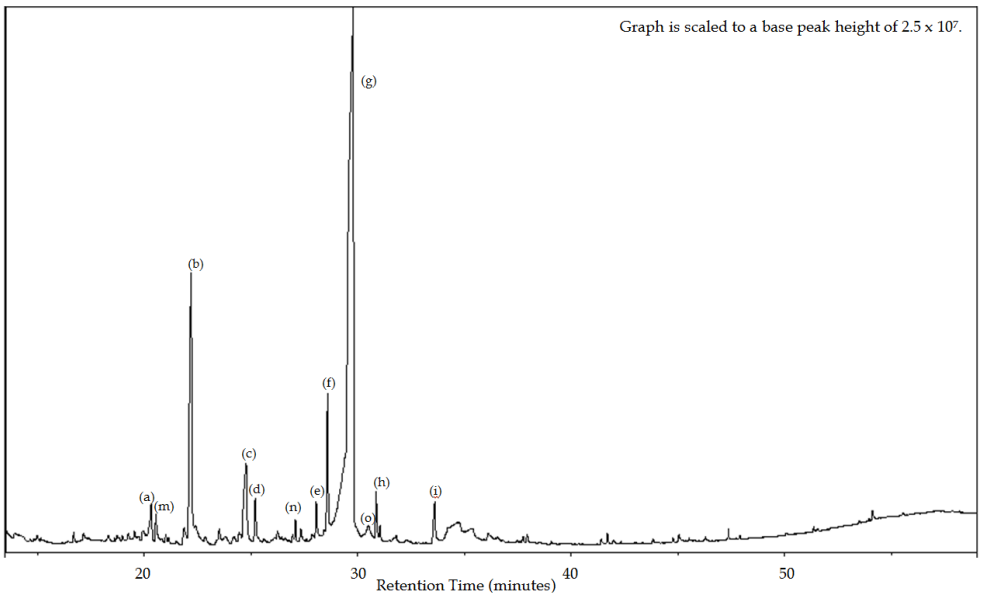


Figure 4.2: Chromatogram from GCMS of 75% ethanol vanilla extract. Labels on peaks refer to Tables 4.4.

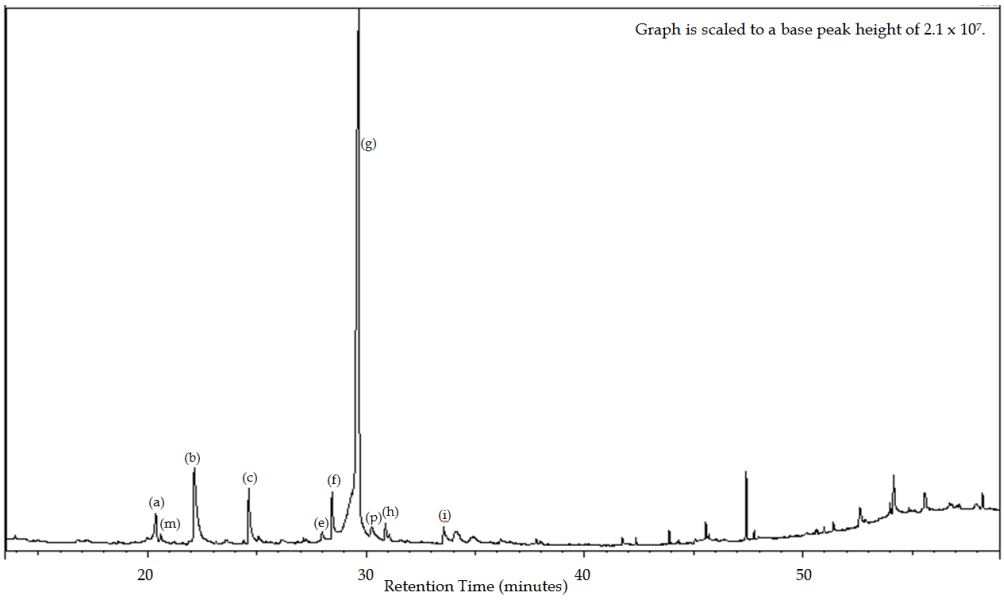


Figure 4.3: Chromatogram from GCMS of 50% ethanol vanilla extract. Labels on peaks refer to Tables 4.5.

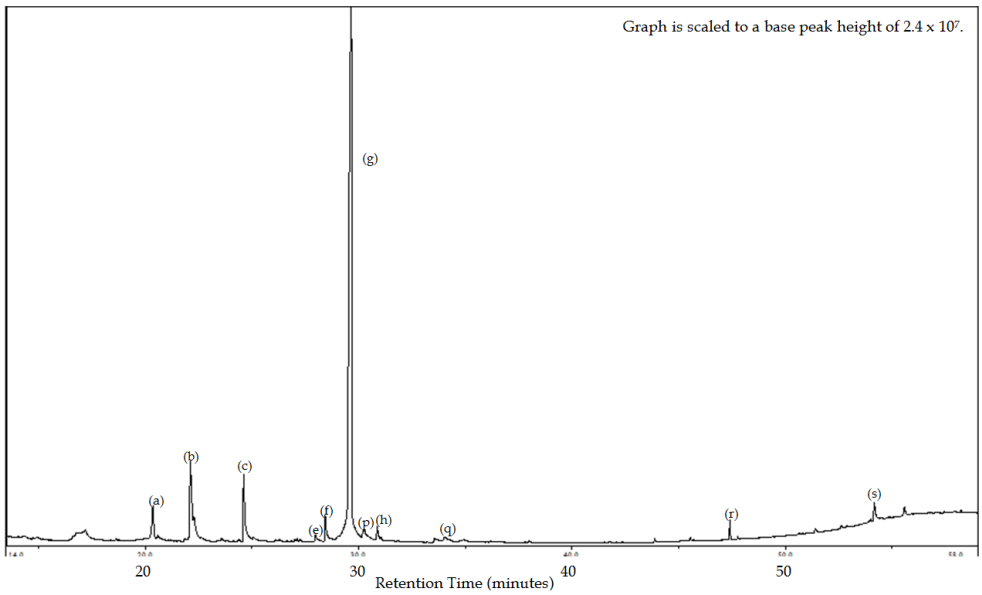


Figure 4.4: Chromatogram from GCMS of 25% ethanol vanilla extract. Labels on peaks refer to Tables 4.6.

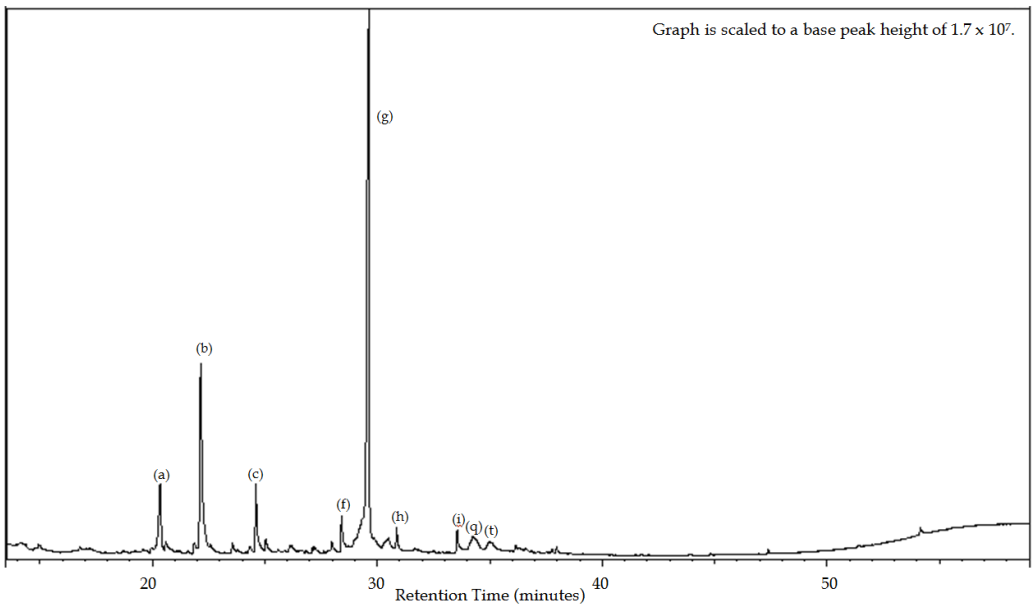


Figure 4.5: Chromatogram from GCMS of water-based vanilla extract. Labels on peaks refer to Tables 4.7.

Table 4.3: Details of the most concentrated volatile compounds extracted from vanilla beans by 100% ethanol. Compound names are identifications based on MS library (NIST 2008). Values are means ± standard error, where n=3.

	Compound Name	Retention Time (mins)	Peak Area	% Total Area
(a)	2-methoxy phenol	20.35	$24.9 \pm 0.5 \times 10^6$	2.3 ± 0.2
(b)	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	22.20	$85.1 \pm 04.7 \times 10^6$	7.7 ± 0.7
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.80	$31.0 \pm 1.8 \times 10^6$	2.8 ± 0.3
(d)	1,2,3-propanetriol monoacetate	25.25	$15.7 \pm 0.1 \times 10^6$	1.4 ± 0.1
(e)	3-hydroxy benzenemethanol	28.10	$11.3 \pm 1.7 \times 10^6$	1.0 ± 0.6
(f)	4-hydroxy benzaldehyde	28.65	$44.1 \pm 4.3 \times 10^6$	4.1 ± 0.6
(g)	Vanillin	29.70	$271.3 \pm 19.7 \times 10^6$	24.8 ± 3.1
(h)	4-hydroxy-3-methoxy benzyl alcohol	30.90	$11.6 \pm 1.2 \times 10^6$	1.05 ± 0.14
(i)	3-hydroxy-4-methoxy benzoic acid	33.65	$16.0 \pm 1.2 \times 10^6$	1.5 ± 0.2
(j)	n-hexadecanoic acid	37.95	$1.52 \pm 0.13 \times 10^6$	0.2 ± 0.0
(k)	Dihydro-5-tetradecyl-2(3H)-furanone	52.75	$60.4 \pm 24.6 \times 10^6$	4.9 ± 1.6
(l)	2,3-dihydroxypropyl-9,12-octadecadienoic acid (Z,Z) ester	54.15	$33.9 \pm 4.9 \times 10^6$	3.0 ± 0.2

Table 4.4: Details of the most concentrated volatile compounds extracted from vanilla beans by 75% ethanol. Compound names are identifications based on MS library (NIST 2008). Values are means ± standard error, where n=3.

	Compound Name	Retention Time (mins)	Peak Area	% Total Area
(a)	2-methoxy phenol	20.35	$13.6 \pm 0.9 \times 10^6$	1.6 ± 0.1
(m)	Cyclopropyl carbinol	20.55	$5.49 \pm 0.95 \times 10^6$	0.6 ± 0.1
(b)	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	22.20	$100.2 \pm 9 \times 10^6$	11.8 ± 0.8
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.75	$44.9 \pm 3.1 \times 10^6$	5.3 ± 0.4
(d)	1,2,3-propanetriol monoacetate	25.20	$11.1 \pm 1.4 \times 10^6$	1.3 ± 0.1
(n)	2-methoxy-4-vinylphenol	27.10	$2.98 \pm 0.56 \times 10^6$	0.4 ± 0.1
(e)	3-hydroxy-benzenemethanol	28.05	$9.65 \pm 1.44 \times 10^6$	1.1 ± 0.1
(f)	4-hydroxy benzaldehyde	28.60	$27.2 \pm 3.8 \times 10^6$	3.2 ± 0.3
(g)	Vanillin	29.80	$380.2 \pm 48.4 \times 10^6$	44.3 ± 4.0
(o)	Sucrose	30.50	$15.0 \pm 1.3 \times 10^6$	1.8 ± 0.3
(h)	3-hydroxy-4-methoxy benzyl alcohol	30.85	$10.6 \pm 0.4 \times 10^6$	1.2 ± 0.1
(i)	3-hydroxy-4-methoxy benzoic acid	33.60	$11.0 \pm 0.8 \times 10^6$	1.3 ± 0.0

Table 4.5: Details of the most concentrated volatile compounds extracted from vanilla beans by 50% ethanol. Compound names are identifications based on MS library (NIST 2008). Values are means ± standard error, where n=3.

	Compound Name	Retention Time (mins)	Peak Area	% Total Area
(a)	2-methoxy phenol	20.40	$16.1 \pm 0.3 \times 10^6$	2.6 ± 0.2
(m)	Cyclopropyl carbinol	20.60	$6.49 \pm 0.64 \times 10^6$	1.1 ± 0.2
(b)	2,3-dihydroxy-6-methyl-4H-pyran-4-one	22.20	$38.6 \pm 2.3 \times 10^6$	6.2 ± 0.6
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.70	$15.7 \pm 1.1 \times 10^6$	2.5 ± 0.2
(e)	3-hydroxy benzenemethanol	28.00	$4.58 \pm 0.28 \times 10^6$	0.7 ± 0.0
(f)	4-hydroxy benzaldehyde	28.50	$14.7 \pm 0.8 \times 10^6$	2.4 ± 0.2
(g)	Vanillin	29.70	$246.8 \pm 11.3 \times 10^6$	39.7 ± 2.1
(p)	2-(hydroxymethyl)-2-nitro-1,3-propanediol	30.40	$18.4 \pm 3.0 \times 10^6$	2.9 ± 0.4
(h)	4-hydroxy-3-methoxybenzyl alcohol	30.90	$8.81 \pm 0.44 \times 10^6$	1.4 ± 0.1
(i)	3-hydroxy-4-methoxybenzoic acid	33.60	$9.40 \pm 0.76 \times 10^6$	1.5 ± 0.2

Table 4.6: Details of the most concentrated volatile compounds extracted from vanilla beans by 25% ethanol. Compound names are identifications based on MS library (NIST 2008). Values are means ± standard error, where n=3.

	Compound Name	Retention Time (mins)	Peak Area	% Total Area
(a)	2-methoxy phenol	20.35	$6.66 \pm 1.28 \times 10^6$	2.0 ± 0.8
(b)	2,3-dihydroxy-6-methyl-4H-pyran-4-one	22.15	$26.8 \pm 1.6 \times 10^6$	7.8 ± 0.8
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.65	$28.0 \pm 4.8 \times 10^6$	7.9 ± 0.9
(e)	3-hydroxybenzenemethanol	28.00	$1.82 \pm 0.20 \times 10^6$	0.5 ± 0.1
(f)	4-hydroxybenzaldehyde	28.45	$6.83 \pm 1.02 \times 10^6$	1.9 ± 0.1
(g)	Vanillin	29.65	$200.1 \pm 12.3 \times 10^6$	57.4 ± 3.1
(p)	2-(hydroxymethyl)-2-nitro-1,3-propanediol	30.30	$10.7 \pm 2.1 \times 10^6$	3.0 ± 0.4
(h)	3-hydroxy-4-methoxybenzyl alcohol	30.85	$2.63 \pm 0.44 \times 10^6$	0.7 ± 0.1
(q)	3-deoxy-d-mannoic lactone	34.10	$3.16 \pm 0.69 \times 10^6$	0.9 ± 0.2
(r)	(Z)-9-tricosene	47.40	$7.54 \pm 0.15 \times 10^6$	2.1 ± 0.3
(s)	9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	54.15	$3.86 \pm 1.19 \times 10^6$	1.1 ± 0.2

Table 4.7: Details of the most concentrated volatile compounds extracted from vanilla beans by water. Compound names are identifications based on MS library (NIST 2008). Values are means ± standard error, where n=3.

	Compound Name	Retention Time (mins)	Peak Area	% Total Area
(a)	2-methoxy phenol	20.35	$15.6 \pm 1.3 \times 10^6$	6.0 ± 0.6
(b)	2,3-dihydro-3,5-dihydroxy-6-methyl-4	22.15	$27.9 \pm 7.8 \times 10^6$	9.9 ± 1.5
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.60	$6.21 \pm 2.53 \times 10^6$	2.1 ± 0.6
(f)	4-hydroxybenzaldehyde	28.40	$5.14 \pm 1.27 \times 10^6$	1.8 ± 0.3
(g)	Vanillin	29.65	$151.2 \pm 8.1 \times 10^6$	57.2 ± 3.3
(h)	3-hydroxy-4-methoxybenzyl alcohol	30.90	$2.71 \pm 0.24 \times 10^6$	1.0 ± 0.0
(i)	4-hydroxy-3-methoxybenzoic acid	33.55	$2.72 \pm 1.00 \times 10^6$	0.9 ± 0.2
(q)	3-deoxy-d-mannoic lactone	34.25	$9.01 \pm 1.61 \times 10^6$	3.3 ± 0.2
(t)	3-deoxy-d-mannonic acid	35.00	$5.59 \pm 1.24 \times 10^6$	2.0 ± 0.2

4.3.1.1 GCMS Output of Different Ethanol Concentrations of Vanilla Extracts

Of the compounds identified in the ethanol vanilla extract (Table 4.8), 11 of 20 had been identified in vanilla or vanilla extracts before (Toth *et al.*, 2010).

Table 4.8: List of compounds identified in vanilla extracts using ethanol and water extractions.

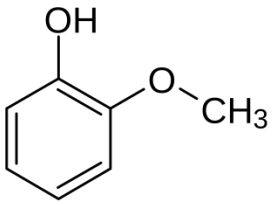
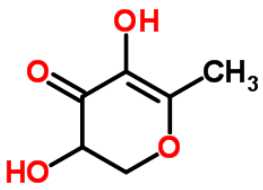
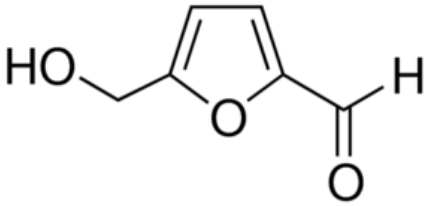
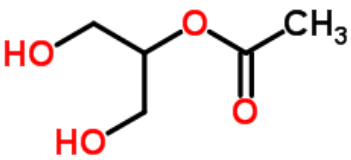
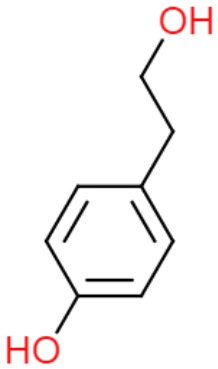
	Compound Name	Retention Time	Previous Identification
(a)	2-methoxy phenol	20.35	Yes
(b)	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	22.20	Tentative
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	24.80	Yes
(d)	1,2,3-propanetriol monoacetate	25.25	Yes
(e)	3-hydroxy benzenemethanol	28.10	Tentative
(f)	4-hydroxy benzaldehyde	28.65	Yes
(g)	Vanillin	29.70	Yes
(h)	4-hydroxy-3-methoxy benzyl alcohol	30.90	Yes
(i)	3-hydroxy-4-methoxy benzoic acid	33.65	Yes
(j)	n-hexadecanoic acid	37.95	Yes
(k)	Dihydro-5-tetradecyl-2(3H)-furanone	52.75	Tentative
(l)	2,3-dihydroxypropyl-9,12-octadecadienoic acid (Z,Z) ester	54.15	Tentative
(m)	Cyclopropyl carbinol	20.60	Tentative
(n)	2-methoxy-4-vinylphenol	27.10	Yes
(o)	Sucrose	30.50	Tentative
(p)	2-(hydroxymethyl)-2-nitro-1,3-propanediol	30.40	Tentative
(q)	3-deoxy-d-mannoic lactone	34.25	Tentative
(r)	(Z)-9-tricosene	47.40	Yes
(s)	9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	54.15	Yes
(t)	3-deoxy-d-mannonic acid	35.00	Tentative

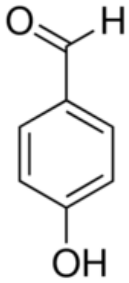
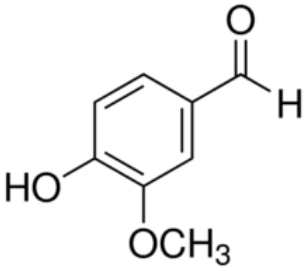
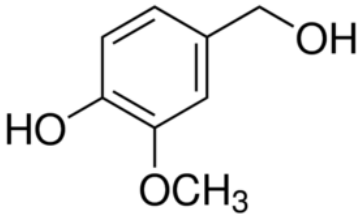
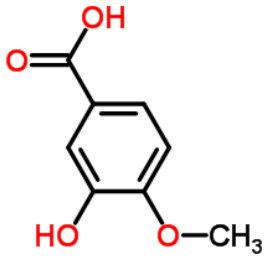
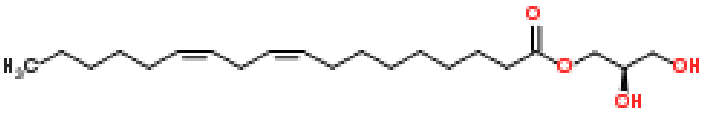
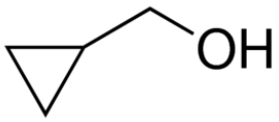
Compounds (b), (e), (k), (l) and (q) all had similar structures to compounds identified in vanilla extracts previously (Toth *et al.*, 2010), and therefore were likely to be in the vanilla extract although they had not been confirmed before. Confirmation of these compounds was not possible without the use of a reference standard, as identification of these compounds was by mass spectra alone and would need confirmation. However, it did give a strong indication of the type of compounds in the extracts.

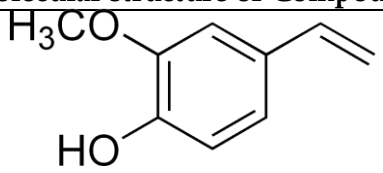
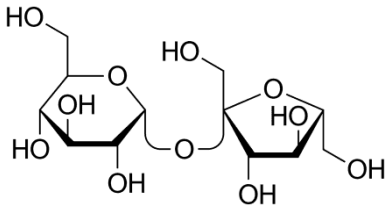
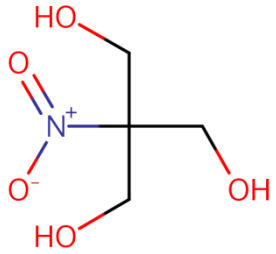
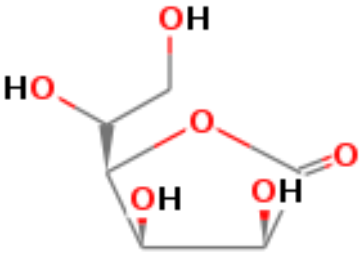
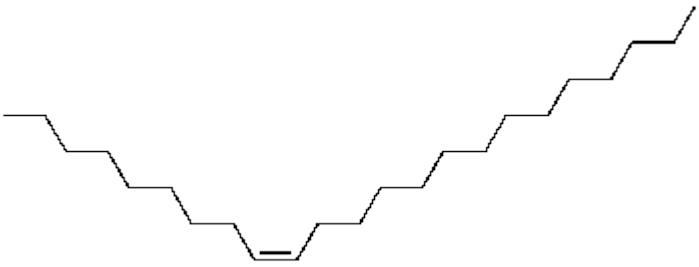
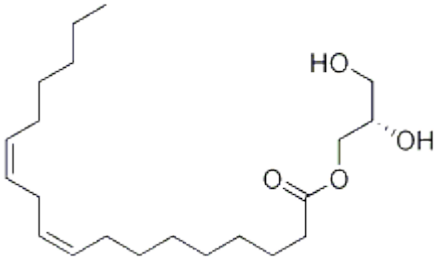
Compounds (m), (o), (p) and (t) have not been identified in vanilla extracts previously and did not have similar structures to other compounds confirmed in

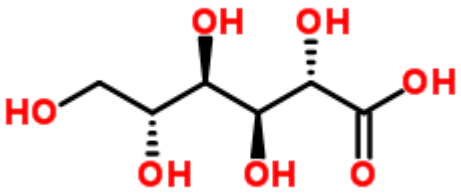
vanilla extracts (Table 4.9). Without the use of reference standards, limited by availability, the exact identity of these compounds could not be confirmed. An indication of the structure from the mass spectra could be gained but confirmation was not possible.

Table 4.9: Molecular structures of compounds identified in the vanilla extracts produced with different solvents.

	Compound Name	Molecular structure of Compound
(a)	2-methoxy phenol	
(b)	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	
(c)	5-(hydroxymethyl)-2-furancarboxaldehyde	
(d)	1,2,3-propanetriol monoacetate	
(e)	3-hydroxy benzenemethanol	

	Compound Name	Molecular structure of Compound
(f)	4-hydroxy benzaldehyde	
(g)	Vanillin	
(h)	4-hydroxy-3-methoxy benzyl alcohol	
(i)	3-hydroxy-4-methoxy benzoic acid	
(j)	n-hexadecanoic acid	
(k)	Dihydro-5-tetradecyl-2(3H)-furanone	
(l)	2,3-dihydroxypropyl-9,12-octadecadienoic acid (Z,Z) ester	
(m)	Cyclopropyl carbinol	

	Compound Name	Molecular structure of Compound
(n)	2-methoxy-4-vinylphenol	
(o)	Sucrose	
(p)	2-(hydroxymethyl)-2-nitro-1,3-propanediol	
(q)	3-deoxy-d-mannoic lactone	
(r)	(Z)-9-tricosene	
(s)	9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	

	Compound Name	Molecular structure of Compound
(t)	3-deoxy-d-mannonic acid	

The first feature to note comparing the different concentrations of ethanol was the concentration of vanillin, the highest concentration compound in all the extracts. The most vanillin was extracted by the higher concentrations of ethanol, with water extracting the least (Figure 4.6). Ethanol at 75% was found to extract significantly ($p<0.05$) more vanillin than 100% ethanol, although vanillin is more soluble in ethanol than water. Vanillin is soluble to 10 g/l in water (at 25°C) and 50 g/l in ethanol (Sigma-Aldrich, 2017). The concentrations of water and ethanol in the 75% ethanol extract must have allowed for a larger quantity of vanillin to be extracted, although the exact reasons for this are unclear. Note that the 25% ethanol and the water extraction were made using a greater volume of solvent than the other samples due to absorption of the water by the beans during the extraction (Table 4.2). This could have affected the potential final concentrations of vanillin, but did not appear to, based on the trend for the three higher concentrations of ethanol.

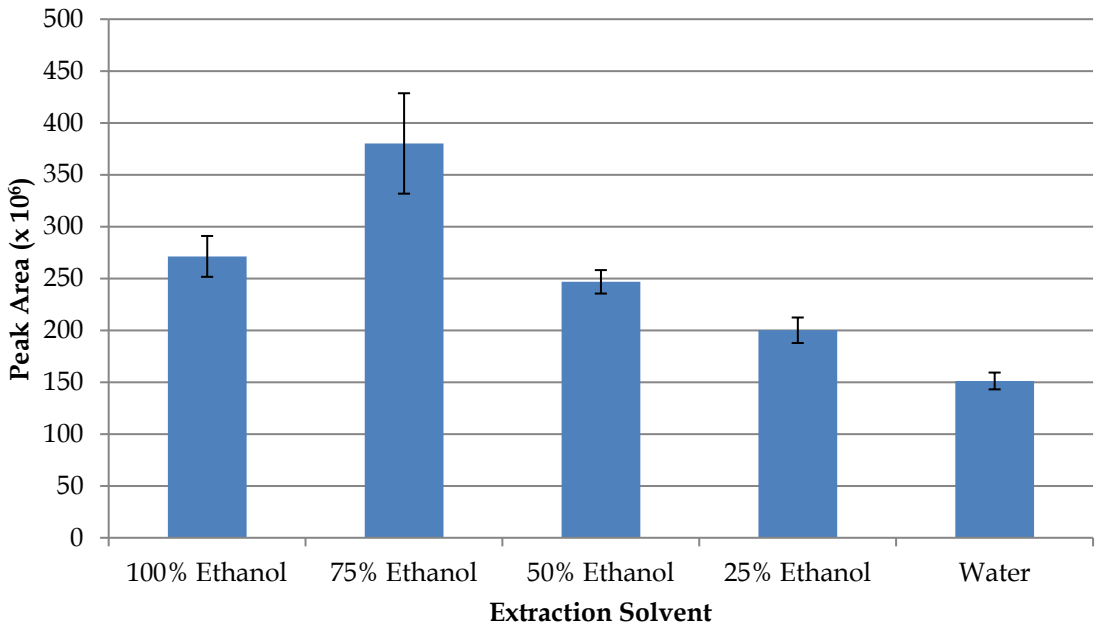


Figure 4.6: Comparison of vanillin peak area for five extractions of cut vanilla beans with different concentrations of ethanol and water.

As the concentration of ethanol decreased, so too did the peak area of all volatile compounds extracted, with water extracting the least (Figure 4.7). This can also be seen in the chromatograms, where the peak height and number of peaks decreased as the ethanol concentration decreased (Figures 4.1 to 4.5). This indicated that the volatile compounds within vanilla were more ethanol soluble than water soluble. All concentrations of water and ethanol also tended to extract the same compounds from the vanilla beans. This was likely due to both solvents being polar, and the differences noted being caused by the differences in the polarities of water and ethanol. The compounds were better extracted by the moderate polarity of the ethanol than the high polarity of the water (Table 4.10).

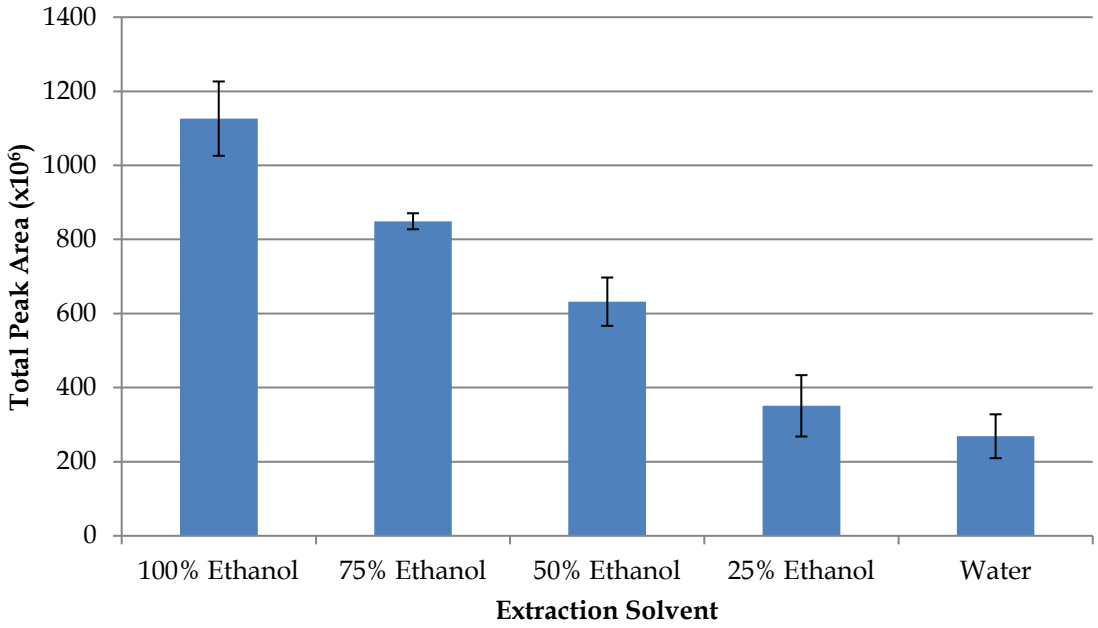


Figure 4.7: Comparison of total peak area for the vanilla volatile extractions using five different ethanol and water concentrations on cut vanilla beans.

4.3.2 Alternative Extraction Solvents

To fully investigate the volatiles within vanilla beans, a range of solvents were used for extractions, including hexane, pentane, methanol, ethanol, water and acetonitrile.

Initial analysis of the results compared the compounds extracted by each solvent. These were compared both in the patterns seen in the chromatograms produced, as well as the peak areas of the main compounds identified in each extract.

The first aspect to compare between the chromatograms of the different solvents was the base peak, the highest peak in each chromatogram, which determines the scale for the y-axis (Figures 4.8 to 4.13). For all of the solvents, vanillin was the base peak

with a retention time of 29.6 minutes. This vanillin peak height varied between the solvents. The detector was saturated for ethanol and pentane, shutting off during the run, so the total concentration of vanillin could not be determined but must have been higher than the other recorded values, which is the cause of the detector overload. For most solvents, the base peak height on the chromatogram was within the range of 24.1 x 10^6 to 27.2 x 10^6, with water being the only solvent outside this range, with a base peak height of 17.2 x 10^6. As solvents became more non-polar, more vanillin was extracted with pentane extracting the most and water extracting the least. Water was the most polar of all the solvents trialled as shown in Table 4.10. As vanillin was more soluble in non-polar solvents (Burdock, 2009d), being comprised primarily of an aldehyde and a phenol group, it was best extracted by a non-polar solvent.

Table 4.10: Physical properties of solvents used for vanilla volatile extractions.

Name	Polarity Index (P')	Boiling Point (^oC)
Pentane	0.0	36
Hexane	0.1	69
Ethanol	4.3	78
Methanol	5.1	65
Acetonitrile	5.8	82
Water	10.2	100

The more non-polar solvents – hexane and pentane – were able to extract more compounds with a longer retention time, seen as the peaks after 45 minutes. It was likely that these compounds had higher boiling points, requiring higher temperatures near the end of the temperature program in the GC oven before they eluted. This indicated they had longer carbon chains, or stronger intermolecular forces.

Acetonitrile, methanol and water had relatively few peaks seen in their chromatograms compared with the other solvents. These were the three most polar compounds of the solvents used, suggesting that the majority of the compounds in vanilla beans were non-polar.

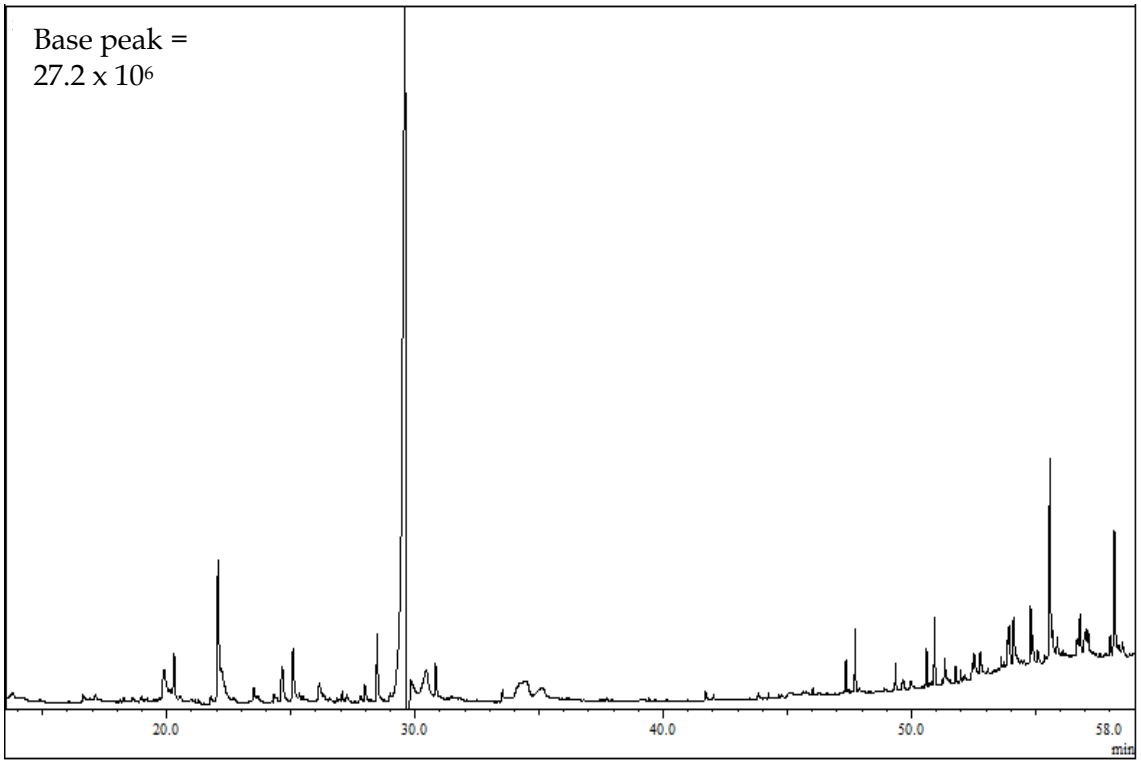


Figure 4.8: Chromatogram from GCMS of cut beans, extracted with pentane. The base peak height is labelled in the upper left corner.

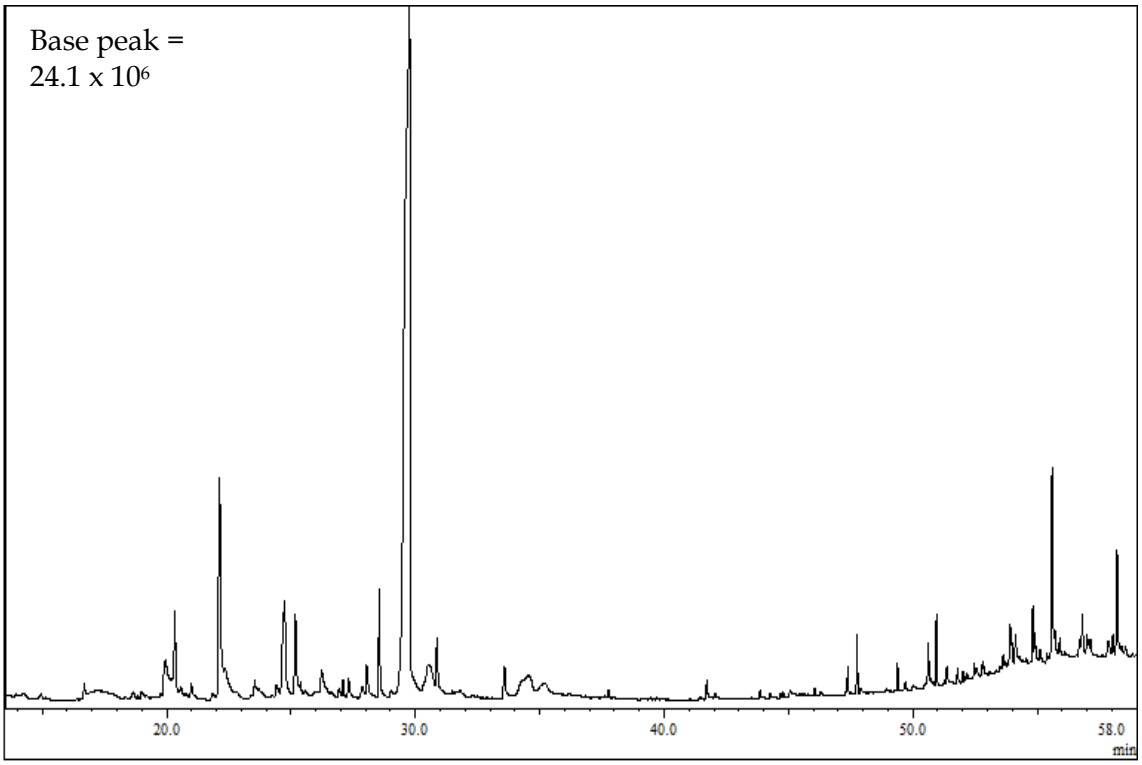


Figure 4.9: Chromatogram from GCMS of cut beans, extracted with hexane. The base peak height is labelled in the upper left corner.

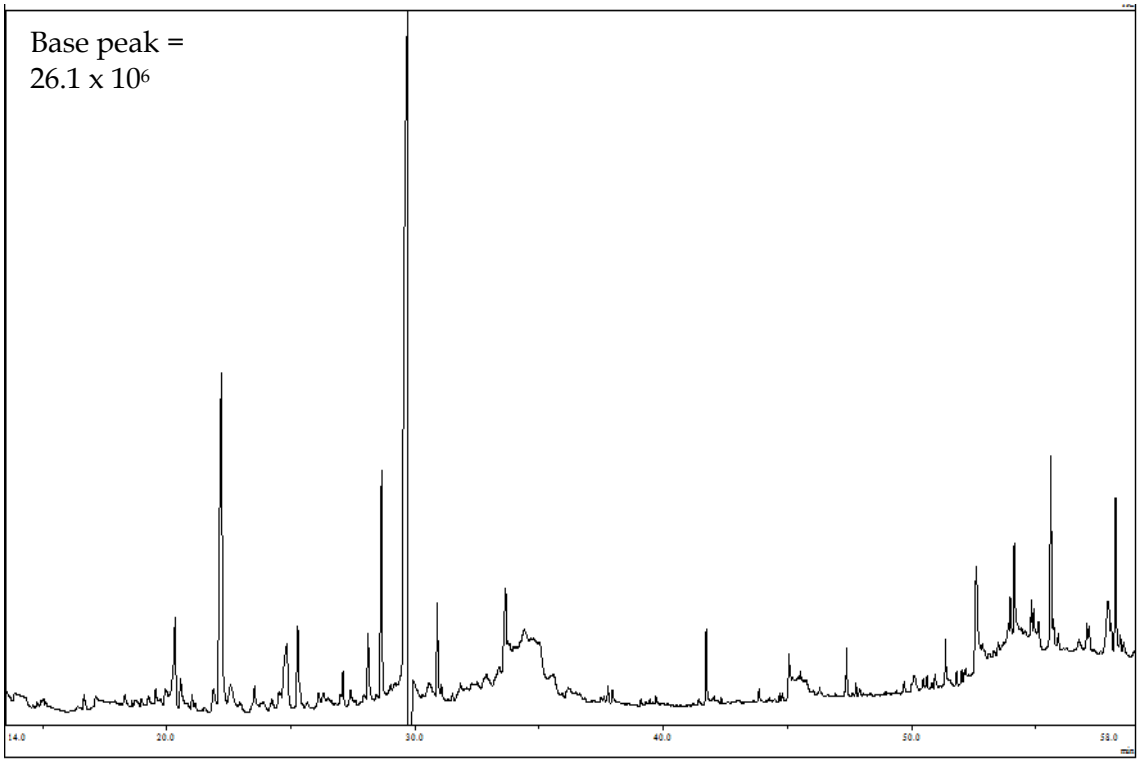


Figure 4.10: Chromatogram from GCMS of cut beans, extracted with ethanol. The base peak height is labelled in the upper left corner.

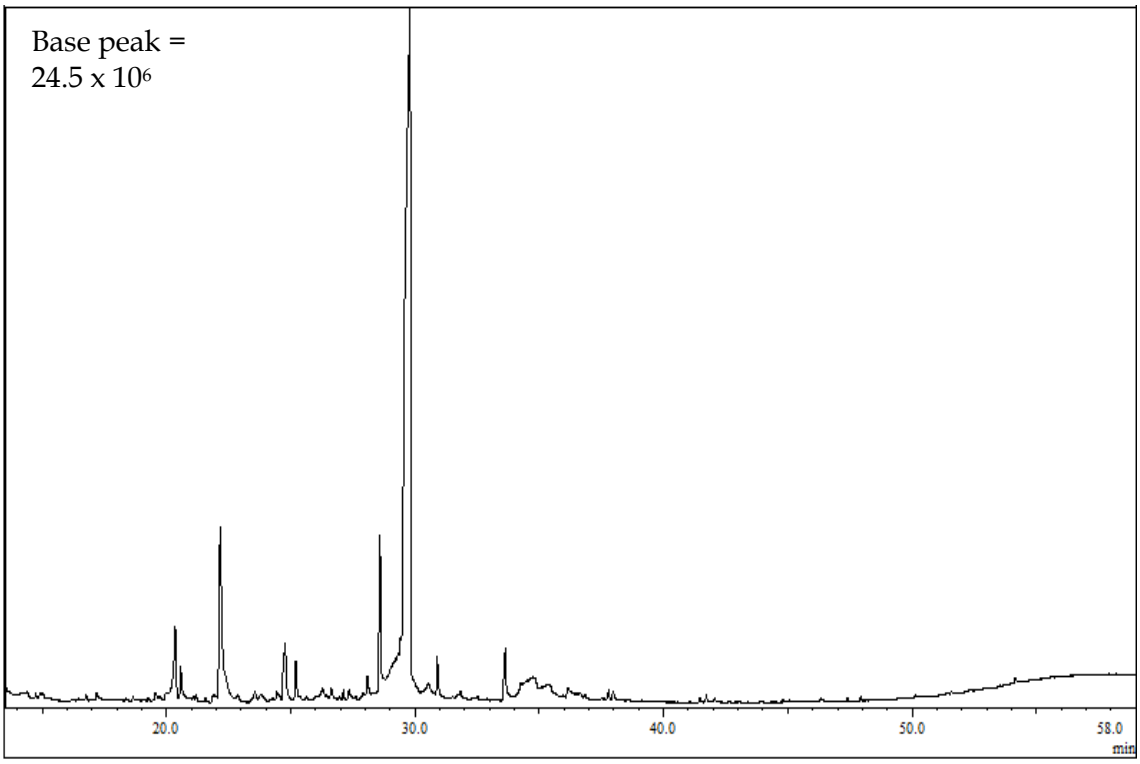


Figure 4.11: Chromatogram from GCMS of cut beans, extracted with methanol. The base peak height is labelled in the upper left corner.

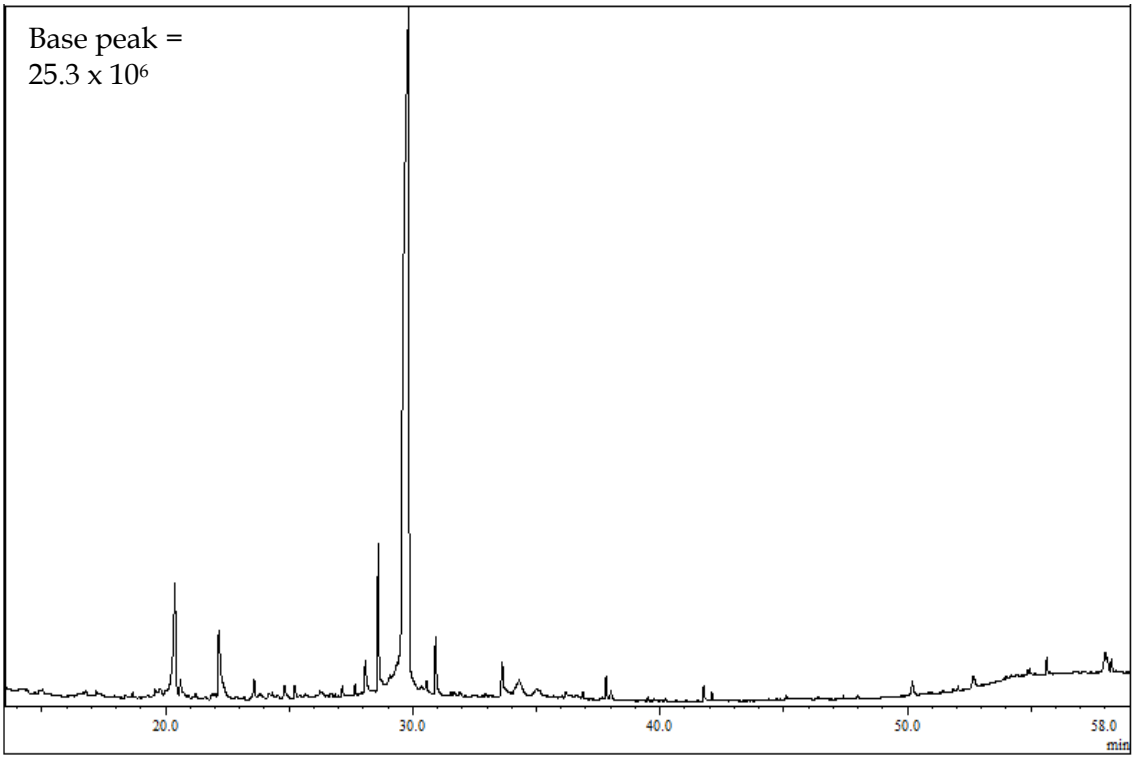


Figure 4.12: Chromatogram from GCMS of cut beans, extracted with acetonitrile. The base peak height is labelled in the upper left corner.

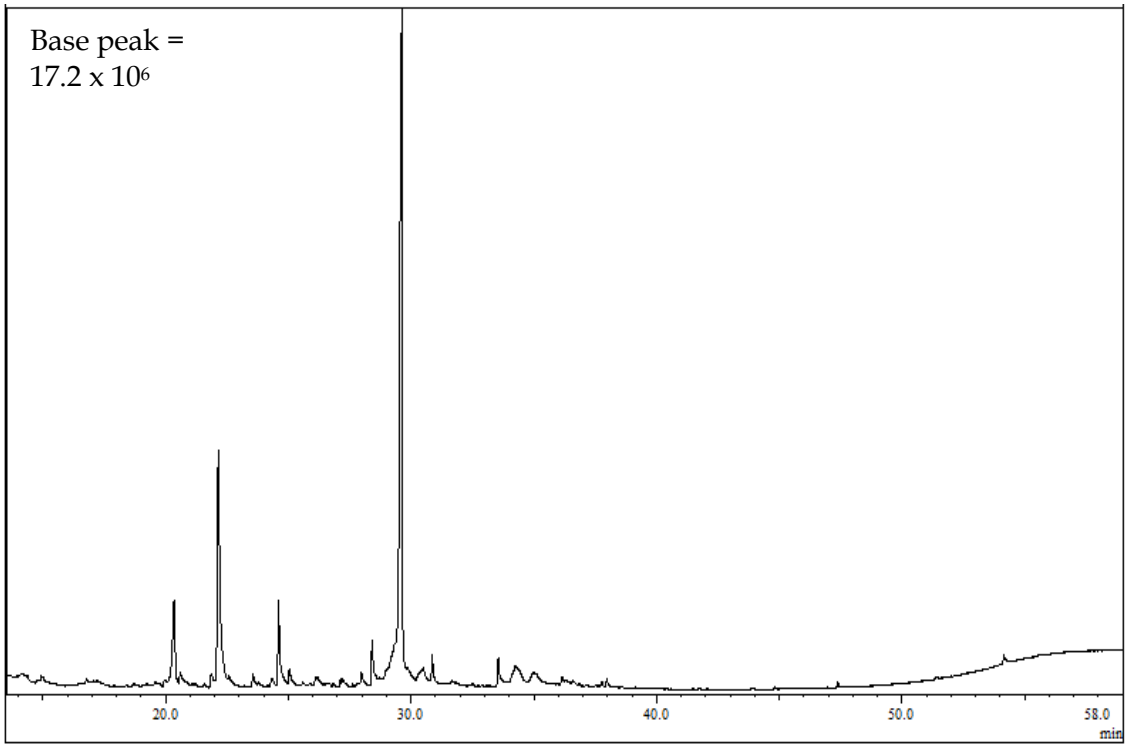


Figure 4.13: Chromatogram from GCMS of cut beans, extracted with water. The base peak height is labelled in the upper left corner.

From each of the solvents, the 10 largest peak area compounds were identified using the NIST 2008 MS library, giving 29 compounds for the six solvents. The concentration of these compounds was compared for the different solvents (Table 4.12).

Hexane was the most effective at extracting the compounds, finding 26 of the 29 compounds. In contrast, pentane was the least effective at extracting the compounds with only 14 of the common compounds detected. As hexane and pentane have very similar polarities, at 0 and 0.1 respectively (Table 4.10), they would be expected to dissolve similar compounds. The difference between these two solvents is with the boiling point, with hexane boiling at 69^{o}C compared to 36^{o}C for pentane (Yaws, 1977). This difference in the boiling points resulted in requiring a larger volume of solvent to extract the vanilla beans for the pentane compared to the hexane. The pentane disappeared from the extraction vessel, either due to being absorbed by the vanilla beans or evaporation through the sealed cap of the Schott bottle, which could have led to a lower concentration of compounds in the extract. Ethanol was found to extract a large number of compounds, similar to hexane, supporting its suitability to extract vanilla flavour for use in the food industry; ethanol is the most commonly used solvent in commercial vanilla flavour extraction (Cameron, 2011).

Pérez-Silva *et al.* (2006) also conducted a similar study, producing a vanilla extract from ground vanilla beans using three different solvents. The solvents used in the study were diethyl ether, a 1:1 (v/v) mixture of pentane and diethyl ether and a 2:1 (v/v) mixture of pentane and dichloromethane. They identified a total of 65 volatile compounds in the vanilla extracts using GCMS, which were presented in Chapter 2 (Section *2.8.2*). The compounds extracted included 25 acids, 15 phenolic compounds, 10 alcohols, four aldehydes, four heterocyclic compounds, four esters, two hydrocarbons and one ketone. Similar to the research presented here, it was found that the different solvents were able to extract different numbers of aroma compounds, with the pentane:diethyl ether mixture extracting the most (65 compounds), the diethyl ether extracting 54 compounds and the pentane:dichloromethane mixture the least (41 compounds). The proposed reason for the differences in the extracted compounds was differences in the polarity of the solvents, and their relative ability to dissolve the compounds in the vanilla extracts.

Table 4.11: Polarity indices for solvents used in the study by Perez-Silva *et al.* (2006).

Compound Name	Polarity Index (P′)
Pentane	0.0
Diethyl Ether	2.8
Dichloromethane	3.1

As can be seen in Table 4.11, the pentane:diethyl ether mix had the lowest polarity index of the mixes tested by Pérez-Silva *et al.* (2006), with the effectiveness of each solvent mix being relative to the polarity, with the most non-polar (pentane) able to extract the most compounds. This is in accordance with the findings of the current research, with pentane and hexane, as the most non-polar solvents, able to extract the most volatile compounds from the vanilla beans.

Table 4.12: Compounds identified in each solvent extract. Values are average peak areas in millions (n = 4), and Rt is retention time in minutes.

Rt	Compound Name	Pentane	Hexane	Ethanol	Methanol	Acetonitrile	Water
20.40	2-methoxy phenol	15.3	12.4	24.9	16.3	15.6	15.6
20.60	Cyclopropyl carbinol	-	3.7	4.4	8.3	4.8	3.1
22.15	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.5	4.1	85.1	47.4	10.9	27.9
24.65	5-(hydroxymethyl)-2-furancarboxaldehyde	-	2.1	31.0	21.6	4.0	6.2
25.20	1,2,3-propanetriol monoacetate	-	1.7	15.7	5.8	1.6	1.9
27.15	2-methoxy-4-vinylphenol or 4-hydroxy-2-methylacetophenone	-	0.5	4.0	1.8	4.4	0.5
28.10	3-hydroxy-benzenemethanol	-	1.0	11.3	7.1	17.0	1.9
28.50	4-hydroxybenzaldehyde	3.2	9.8	44.1	28.5	51.1	5.1
29.10	3-phenyl-2-propenoic acid, methyl ester	2.7	4.0	44.1	8.0	4.3	-
29.70	Vanillin	303.9	206.4	271.5	323.7	559.3	151.4
29.95	3-hydroxy-4-methoxy-benzaldehyde	-	9.8	271.5	-	-	-
30.35	2-(hydroxymethyl)-2-nitro-1,3-propanediol	-	-	-	-	2.0	1.5
30.50	Sucrose	-	-	4.0	18.8	-	-
30.90	3-hydroxy-4-methoxy benzyl alcohol	-	3.1	11.6	11.3	23.8	2.7
33.60	3-methoxy-4-methoxy benzoic acid	-	1.2	16.0	18.4	11.7	2.7
34.20	3-deoxy-manoic lactone	-	4.1	-	-	1.9	9.0
34.90	3-deoxy-mannonic lactone	-	4.0	-	-	-	5.6
38.10	Ethyl homovanillate	-	-	1.5	4.8	4.0	0.6
45.20	(Z,Z)-9,12-octadecadienoic acid	-	6.4	6.0	1.5	1.5	-
47.40	(Z)-9-tricosene	10.2	3.7	4.9	-	1.6	-
48.55	Hexatriacontane	13.4	9.2	-	-	-	-
50.65	1-heptacosanol	8.2	8.6	1.5	-	0.4	-
51.00	Hexatriacontane	15.8	10.0	-	-	-	-
52.75	Dihydro-5-tetradecyl-2(3H)-furanone	-	94.9	60.4	-	-	-
54.15	2,3-dihydroxypropyl-9,12-octadecadienoic acid (Z,Z) ester	15.9	12.7	33.9	-	4.0	-
55.75	Nonadecane-2,4-dione	8.6	6.7	-	-	-	-
55.90	Tricosane-2,4-dione	5.3	5.1	1.9	-	-	-
57.20	Hexadecyl-oxirane	9.6	4.6	2.0	-	0.3	-
58.20	E,E,Z-1,3,12-nonadecatriene-5,12-diol	46.8	29.9	-	-	3.0	-

Water and methanol extracted compounds with shorter retention times, before 38 minutes, and pentane, ethanol and hexane extracted the compounds with longer retention times, mostly after 45 minutes. This indicated the retention time of the compounds was dependent on the polarity of the compound, with the more polar compounds eluting sooner, in the water extract, and the more non-polar compounds eluting later, in the pentane extract. Longer chain compounds tend to have higher boiling points, and are more non-polar (Blackman *et al.*, 2016) which was also seen in the compound identifications in Table 4.11.

The compounds extracted by all solvents were 2-methoxy phenol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 4-hydroxybenzaldehyde and vanillin. The structures of these are shown in Figure 14. Three of these compounds have a phenol group as their base structure, with the other compound (2), containing a pyran group instead. The functional groups attached to the compounds are hydroxy and methoxy groups. The combination of these structures would result in the compounds having both a polar nature and a non-polar nature, thus being able to be extracted by all the solvents trialled.

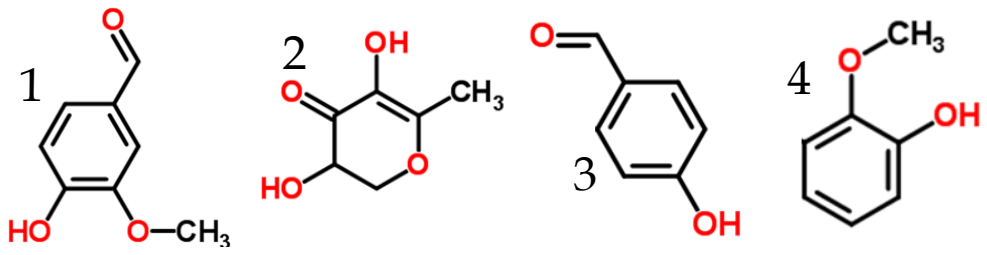


Figure 4.14: Molecular structures of four compounds found in all solvent extracts. The numbers are as follows: 1. vanillin; 2. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 3. 4-hydroxybenzaldehyde; 4. 2-methoxyphenol.

Vanillin (1) is the main flavour compound in vanilla, and is used as an artificial vanilla flavouring, described as being characteristic, creamy, vanilla-like odour with a very sweet taste (Burdock, 2009d). It is slightly soluble in water, soluble in organic solvents and oils and freely soluble in ethanol. It has a boiling point of 285°C.

2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2) has been found in vanilla before (Toth *et al.*, 2010). The aroma of this compound has been described as caramel-like (Preininger *et al.*, 2009) and it has a boiling point of 281°C.

4-hydroxy benzaldehyde (3) has also been identified in vanilla previously and is considered one of the main flavour compounds in vanilla (Toth *et al.*, 2010). It has a faint, sweet-woody-balsamic odour, occasionally considered vanillic, and a sweet taste with little other flavour (Burdock, 2009c). It is slightly soluble in water, soluble in organic solvents and freely soluble in alcohol. Its boiling point is 310^{o}C.

2-methoxyphenol (4) has been identified in vanilla previously (Toth *et al.*, 2010), and has an aroma described as phenolic, smoky, spicy, medicinal, vanilla, savoury, meaty, woody with bourbon whiskey cask nuance and a flavour described as woody, phenolic, bacon, savoury, smoky and medicinal (Burdock, 2009b). It is slightly soluble in water and slightly soluble in ethanol, with a boiling point of 203^{o}C.

These compounds did not elute solely based on boiling point, therefore the retention time of the compounds was determined by both the boiling point and the polarity.

In comparing the chromatograms with the compounds identified in Table 4.10, it can be seen that 100% ethanol, hexane and pentane were most able to extract the longer chain, non-polar compounds, seen as the greater number of peaks visible in the later retention times. The earlier eluting compounds, those with shorter carbon chains, lower boiling points and more polar natures, tended to be extracted by all the solvents, seen by the larger number of peaks in all chromatograms, as well as the arrangement of compounds in Table 4.10.

4.3.3 Effect of Vanilla Bean Size Reduction on Volatile Extraction

The 16 compounds present at the highest concentrations, identified in the previous sections were selected and compared in the ethanol, water and diluted ethanol extracts to determine the effect of vanilla bean size reduction on the extraction efficiency. The average peak areas for each compound, extraction solvent and size reduction are in Table 4.13.

For 100% ethanol and 25% ethanol, the total area under the curve was significantly different ($p<0.05$) for the different size reductions. For 75% ethanol, 50% ethanol and water there was no difference between the different preparations. The total area under the curve gave an indication of the overall number of volatile compounds extracted by each solvent and bean preparation method. As there was no repeated pattern in the results, in terms of higher extraction efficiencies with each bean preparation type, it

could be concluded that the bean preparation did not affect the total number of volatiles extracted.

Some compounds, such as 4-hydroxybenzaldehyde were most concentrated in the ground vanilla beans for water and 25% ethanol, but it was most concentrated in the blended beans for 75% ethanol and most concentrated for the cut beans for 100% ethanol. Vanillin also did not seem to follow a pattern, being most concentrated in blended and ground vanilla beans for 100% ethanol and 25% ethanol, but having no significant difference in the 75% ethanol, 50% ethanol and water. There were also no apparent trends based on retention time, with the same trends seen in the more polar compounds as well as the non-polar compounds, with longer retention times. Therefore, it was concluded that for a one-week extraction, the size of the vanilla bean pieces did not affect the concentration of the compounds extracted.

Table 4.13: Comparison of mean values for ethanol and water extracts of vanilla beans, comparing bean size reduction. C represents cut vanilla beans, B, represents blended vanilla beans and G represents freeze dried and finely ground vanilla beans. Extract types with asterisk required greater volumes of solvent for the extraction due to absorption of the water into the dried vanilla beans. The letter Y indicates that the concentrations were significantly different ($p<0.05$). The superscript letters denote samples that were significantly different, with "a" the highest.

Rt	Compound Name	100% Ethanol				75% ethanol				50% ethanol				25% ethanol				Water			
		Sig.	C	B	G	Sig.	C	B	G	Sig.	C	B	G*	Sig.	C	B*	G*	Sig.	C*	B*	G*
20.4	2-methoxy phenol	Y	24.9^{a}	30.9^{a}	12^{b}		13.6	14.2	19.2		16.1	13.4	21.7	Y	6.7^{c}	18.7^{b}	33.3^{a}		15.6	16.6	18.2
20.6	Cyclopropyl carbinol	Y	4.4^{a}	6.2^{a}	0^{b}		5.5	4.8	3.8		6.5	4.3	3.2	Y	1.2^{c}	4.8^{a}	3.7^{b}		3.1	3.3	4.6
22.2	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Y	85.1^{a}	54.1^{b}	0^{c}	Y	100.2^{a}	54.2^{b}	78.2^{ab}		38.6	48.3	47.1	Y	26.8^{b}	40^{a}	43.7^{a}		27.9	35.6	25
24.7	5-(hydroxymethyl)-2-furancarboxaldehyde	Y	31^{a}	22.4^{a}	0^{b}		44.9	51.2	34.1		15.7	18.9	13	Y	28^{a}	8.9^{c}	13.6^{b}		6.2	9.9	3.8
25.2	1,2,3-propanetriol monoacetate	Y	15.7^{a}	11.5^{a}	1.9^{b}	Y	11.1^{a}	3.7^{b}	9.5^{a}	Y	3.1^{b}	5.4^{a}	4.5^{ab}	Y	0.6^{b}	7.9^{a}	8.6^{a}		1.9	5.6	3.5
27.2	2-methoxy-4-vinylphenol	Y	4^{a}	3.4^{a}	0^{b}		2.9	3.5	1.8		1.1	1.3	1.8		0.5	1.6	2.5	Y	0.5^{b}	1^{a}	0.5^{b}
28.1	3-hydroxy-benzenemethanol		11.3	14.6	9.6	Y	9.7^{b}	14^{a}	6.3^{b}		4.6	5.5	6.6	Y	1.8^{c}	8.2^{b}	11.9^{a}		1.9	3.8	2.6
28.5	4-hydroxybenzaldehyde	Y	44.1^{a}	37.4^{ab}	24^{b}	Y	27.2^{b}	48.2^{a}	20.7^{b}		14.7	18.7	17.2	Y	6.8^{c}	23.8^{b}	35.4^{a}	Y	5.1^{b}	10.3^{a}	5.7^{b}
29.1	3-phenyl-2-propenoic acid, methyl ester		44.1	6.4	0		4.2	11.7	0		0	0	0		0	0	4.2	Y	0^{b}	0.9^{a}	0^{b}
29.7	Vanillin	Y	271.5^{b}	423.2^{a}	328.1^{a}		380.2	528.3	303.2		247.3	234.8	291.7	Y	200.3^{b}	365.4^{a}	372.2^{a}		151.4	158.1	173
30.9	3-hydroxy-4-methoxy benzyl alcohol	Y	11.6^{b}	18.9^{a}	9.8^{b}	Y	10.6^{b}	26.5^{a}	10^{b}		8.8	6.9	7.7	Y	2.6^{b}	11^{a}	13.6^{a}	Y	2.7^{c}	5.6^{a}	4.4^{b}
33.6	3-hydroxy-4-methoxy benzoic acid	Y	16^{a}	19.1^{a}	5.2^{b}		11	12.2	9.8	Y	9.4^{ab}	12.7^{a}	5.7^{b}	Y	1.5^{c}	9.2^{b}	17.8^{a}	Y	2.7^{b}	7.2^{a}	3^{b}
34.2	3-deoxy-mannoic lactone	Y	0^{b}	34.5^{a}	0^{b}		9.2	29.8	6.4	Y	7.9^{a}	0^{b}	7.1^{a}		3.2	11.8	0	Y	9^{b}	11.9^{a}	10.6^{ab}
38.1	Ethyl homovanillate	Y	1.5^{a}	0^{b}	0^{b}		1.5	2.4	1.4		1.7	1.8	1.3	Y	0.5^{b}	1.5^{a}	1.8^{a}	Y	0.6^{a}	0^{b}	0.7^{a}
47.4	(Z)-9-tricosene	Y	4.9^{b}	0^{b}	25.7^{a}		1.5	0	3	Y	0^{b}	0^{b}	2^{a}	Y	7.5^{a}	1.1^{c}	4.3^{b}	Y	0^{b}	0^{b}	0.6^{a}
54.2	2,3-dihydroxypropyl-9,12-octadecadienoic acid (Z,Z) ester	Y	33.9^{a}	0^{b}	0^{b}	Y	4.5^{b}	0^{b}	34.1^{a}	Y	0^{b}	0^{b}	22.4^{a}		3.9	5.6	15.3	Y	0^{b}	0^{b}	2.3^{a}
Total Area under chromatogram		Y	1126.2^{b}	1956.5^{ab}	3720.3^{a}		849.1	944.7	882.2		632	634.2	676.2	Y	350.9^{b}	696.3^{a}	770.2^{a}	Y	268.8	983.2	312.6

4.4 Conclusion

It was found that the most vanillin was extracted using 75% ethanol compared to 100% ethanol, 50% ethanol, 25% ethanol and water. The same pattern was seen in the overall concentration of volatiles extracted, with 75% ethanol extracting the greatest concentration of total compounds.

Different compounds were extracted by six different solvents, with hexane and pentane extracting the most compounds, and water extracting the least. Hexane and pentane extracted more non-polar compounds with longer retention times in the GC and ethanol was found to extract similar compounds to hexane, making it the most suited for flavour extraction in the food industry.

Comparing hand cut, blended and ground freeze-dried vanilla beans, it was found that there was no pattern in the concentrations of volatile compounds extracted from each, therefore for a one-week extraction the size of the vanilla bean pieces does not affect the extraction efficiency.

5. Sensory Analysis of Natural Vanilla Extracts

5.1 Introduction

As a natural food flavouring, there are a number of different factors that could affect the final flavour of vanilla extracts, including growing region, curing process and the flavour extraction process. The flavour extraction process used by different companies may vary, and information about this is sparse as many companies are unwilling to share the specifics of the methods used to create their product. At best, the final ethanol content, vanillin concentration and the extraction solvent can be used to estimate the processing method used.

To determine the effect that different factors have on the flavour of vanilla extracts, extracts can be analysed by gas chromatography, sensory analysis or a combination of these methods, such as with gas chromatography-olfactory (GC-O).

Extensive studies have been conducted in the past looking at the volatiles present in vanilla extracts, and how they are affected by the growing region, the curing process and the flavour extraction process. Gas chromatography (Pardio *et al.*, 2009), gas chromatography-mass spectrometry (Lhugenot *et al.*, 1971; Adedeji *et al.*, 1993; Sostaric *et al.*, 2000; Takahashi *et al.*, 2013a; Li *et al.*, 2014) or high-pressure liquid chromatography (Archer, 1989; Lamprecht *et al.*, 1994; Pyell *et al.*, 2002; Lavine *et al.*, 2012) have been used. Some studies have looked at the specific aroma of the volatiles found in the vanilla extracts using GC-O (Pérez-Silva *et al.*, 2006; Brunschwig *et al.*, 2012; Zhang and Mueller, 2012), HPLC-MS combined with aroma analysis by participants (Schwarz and Hofmann, 2009) and electronic nose analysis (Hariom *et al.*, 2006; Naidu *et al.*, 2012).

There have been fewer studies looking at the sensory perception of vanilla extracts. A range of methods have been used, including descriptive analysis to investigate bean quality and species (Takahashi *et al.*, 2013a; Takahashi *et al.*, 2013b), quantitative descriptive analysis (QDA) to investigate enzyme assisted curing (Naidu *et al.*, 2012) and to compare the sensory profile to volatile compounds in vanilla extracts (Bruschwig *et al.* 2015), descriptive sensory analysis to investigate an improved extraction process (Hariom *et al.*, 2006) and untrained participants to investigate a range of factors within the curing process (Van Dyk *et al.*, 2010).

No studies have been published that investigated multiple factors simultaneously or how these factors affect the sensory profile of the vanilla extract, in order to determine which factor has the most influence on the final sensory profile of the vanilla extract. The aims of this research were:

- To determine the effect of the vanilla bean growing region on the sensory profile of vanilla extracts
- To determine the effect of the flavour extraction process on the sensory profile of vanilla extracts
- To compare the sensory profile of commercial vanilla extracts to determine the main factor(s) causing differences in the aroma and flavour

5.2 Materials and Methods

5.2.1 Materials

In 2015 eleven different natural vanilla extracts were purchased from supermarkets in New Zealand and Singapore to get a representative sample of commercially available extracts. The extracts were manufactured in Australia, New Zealand and the US, the details of which can be seen in Table 5.1. The extracts were ethanol based, except for one which was a glycerol-based extract.

To control for the effect of flavour extraction, ethanol extracts were also made from vanilla beans sourced from five regions around the world (India, Madagascar, Papua New Guinea, Tonga and Uganda). The extraction process is described in 5.2.2 and the vanilla beans' details are in Table 5.1.

5.2.2 Ethanol Extractions of Vanilla Beans

Vanilla beans from five different regions were cut up by hand with a knife into pieces 3-5mm in length. 200 ± 2 g of each type of cut vanilla bean was put into a one litre capacity Schott bottle. The vanilla bean types were from India, Madagascar, Papua New Guinea, Tonga and Uganda (Table 5.1).

A 55% w/w ethanol solution was prepared with 96% ethanol (Anchor Ethanol Limited, New Zealand) and reverse osmosis (RO) water. As a check of the ethanol concentration, a sample of the 55% ethanol solution was diluted 1:10 with RO water and frozen at -20 ± 2^{o}C for analysis by GC (Section 3.2.3).

Table 5.1: Details of vanilla extracts and vanilla beans used in sensory analysis. The abbreviations will be used throughout the chapter to denote the various vanilla extracts.

Sample Name	Abbreviation	Country of Bean Origin	Country of Extract Manufacture
A. Commercial Vanilla Extracts			
Heilala 5-Fold Extract	H5	Tonga	New Zealand
Heilala Glycerol Extract	HG	Tonga	New Zealand
Heilala Infusion 2	HI2	Tonga	New Zealand
Heilala Infusion 3	HI3	Tonga	New Zealand
Heilala Single Fold Extract	H1	Tonga	New Zealand
Queen Finest Vanilla Extract with Seeds - Vava'u	QT	Tonga	Australia
LorAnn Gourmet Pure Madagascar Bourbon Vanilla Extract	L	Madagascar	USA
Nielson Massey Madagascar Bourbon Vanilla Extract	NM	Madagascar	USA
Queen Natural Organic Vanilla Essence-Extract	QO	Madagascar	Australia
Virginia Dare Pure Vanilla Extract	VD	Madagascar	USA
Whittington's Natural Vanilla Extract – Double Strength	W	Madagascar and Papua New Guinea	Australia
B. Laboratory Vanilla Extracts			**Vanilla Bean Supplier**
India	I	India	Beanilla.com
Madagascar	M	Madagascar	Heilala Vanilla Ltd.
Papua New Guinea	PNG	Papua New Guinea	VanillaproductsUSA
Tonga	T	Tonga	Heilala Vanilla Ltd.
Uganda	U	Uganda	Beanilla.com

After the addition of 500ml of the ethanol to the 200 g of vanilla beans the glass bottles were inverted twice to mix the ethanol and the beans. The extracts were left in a 20 ± 2 ^{o}C temperature-controlled room for seven days (168 hours), without any stirring or agitation. It was determined by a previous research project (Swan, 2008) that the concentration of volatiles in vanilla extracts reaches maximum after 48 hours, so seven days was chosen to imitate the processing conditions used by Heilala Vanilla Ltd., based on this information.

The spent vanilla beans were separated from the extract using a stainless-steel sieve. All the extract was recovered from the beans. The weight of the empty bottles, the extract and the spent beans were recorded.

The vanilla extract samples were stored at 4 ± 2°C for approximately one month until they were to be analysed for sensory profiles. A sample of the final extract was diluted 1:10 with RO water for analysis of ethanol concentration using GC and an undiluted sample was analysed for vanillin concentration using HPLC (Section *3.2.2*). These samples were frozen at -20 ± 2°C until they could be analysed.

5.2.3 Analysis of vanilla extracts for ethanol, vanillin and volatile composition

The concentration of ethanol in the extracts was measured using the method on the GC described in Section *3.2.3.* The concentration of vanillin and other key phenolics was determined using HPLC, with the method described in Section *3.2.2.* The overall volatile content was analysed with GCMS, described in Section *3.2.1.*

5.2.4 Sensory Analysis

The sensory analysis for all the samples presented in this chapter (Table 5.1) was carried out in two randomly selected blocks, with half the samples in each block, over a period of three weeks. The methods used for the sensory analysis, as well as the data analysis of the results are described in Section *3.1*.

The panel performance was determined using a range of measures including t-tests to compare in-session results with a duplicate at the end of each session and ANOVA to determine significant factors. All analyses were carried out using SPSS (Version 21, IBM, USA). Further analysis of patterns within the samples was carried out with PCA and Hierarchical Cluster Analysis using XLStat (Version 2015.4.01.20270, Microsoft, USA).

5.3 Results and Discussion

To determine the effect of each factor on the aroma and flavour of natural vanilla extracts, the samples were grouped based on growing region (Sample I, M, PNG, T and U), extraction process (Sample H1, H5, HI2, HI3 and HG) and the commercial samples (Sample H1, L, NM, QO, QT, VD and W). The samples were then analysed as one group to determine the factor of greatest influence. These groups were analysed first with ANOVA, to determine significant effects, then Tukey's HSD to determine significant differences between products and finally with principal component analysis

(PCA) to investigate how the products were being separated within each grouping. All data related to the PCA is presented in Appendix 4.

The extracts produced from beans from different growing regions were first analysed for ethanol and vanillin concentration to check the extraction performance and compare the produced extracts with the purchased vanilla extracts.

5.3.1 Vanilla Extracts Produced from different growing regions

The initial moisture content of the vanilla beans varied from 14.42 to 46.30% (w/w), with PNG beans the driest and Tonga beans the wettest (Appendix 3). A similar mass of extract was obtained from each of the extractions, with the least 327.73 g for the Madagascar beans and the most 362.20 g for the Uganda beans. The percent yield (mass of extract against mass of solvent) of extract from each type of bean was as follows: 56.15% for I, 53.22% for M, 54.08% for PNG, 55.74% for T and 58.69% for U. The small range in the results indicated that the moisture content of the initial vanilla beans did not affect the amount of extract produced. A full mass balance can be found in Appendix 3.

5.3.2 Vanillin Concentration using HPLC

All samples were tested for vanillin concentration using HPLC and standardised to the same vanillin concentration for presentation to the sensory panel as described in Section *3.1.3.1.* As vanillin is the highest concentration compound within vanilla extracts, it was chosen for the basis of the standardisation, as it gave an indication of the relative strengths of each vanilla extract.

5.3.2.1 Commercial Extracts

The commercial vanilla extracts were tested for vanillin concentration using HPLC, with the results presented in Table 5.2. Most of the extracts were single-fold extracts, with the only exceptions being HG, H5 and W. A single-fold vanilla extract is defined as "the extractive matter of 13.35 ounces of vanilla beans to a gallon of liquid" (FDA, 1993), which equates to 1.5 mg/ml of vanillin. There is no regulation of vanillin concentration in New Zealand, but the majority of vanilla extracts worldwide follow this recommendation for a single-fold extract.

From the commercial single fold extracts, only H1 was found to meet this single-fold concentration at 1.83 mg/ml vanillin (Table 5.2). The lowest vanillin concentration

was QO at 0.96 mg/ml vanillin. W was a double-fold extract, and contained 3.31 mg/ml of vanillin, higher than the 3 mg/ml standard. The highest concentration sample was H5 at 10.40 mg/ml with HG similar at 8.68 mg/ml of vanillin. Both these extracts are sold as 5-fold extracts and should contain a minimum of 7.5 mg/ml vanillin as per the FDA standard.

Table 5.2: Vanillin concentration of the commercial vanilla extracts used for sensory testing. Data are means ± standard error where samples were analysed in triplicate.

Sample Name	Vanillin concentration (mg/ml)
HG	8.68 ± 0.14
H1	1.83 ± 0.00
H5	10.40 ± 0.04
HI2	3.67 ± 0.03
HI3	1.69 ± 0.00
L	1.39 ± 0.01
NM	1.15 ± 0.01
QO	0.96 ± 0.01
QT	1.20 ± 0.09
VD	1.35 ± 0.02
W	3.31 ± 0.02

5.3.2.2 Laboratory Vanilla Extracts

The vanillin concentration of the extracts differed in the laboratory made extracts (Table 5.3), with M and T having the highest vanillin concentration at 4.60 mg/ml and 4.34 mg/ml respectively. In comparison, the PNG extract only contained 1.23 mg/ml of vanillin, with the I and U extracts containing intermediate concentrations at 2.40 mg/ml and 3.38 mg/ml. Based on the concentration of the final extract, the approximate concentration of vanillin in the original vanilla beans, per dry weight, was calculated (Table 5.3). The PNG vanilla beans contained the least vanillin per dry weight, with approximately 2.4 mg vanillin/ g bean and the Tonga vanilla beans contained the most with approximately 13.9 mg vanillin/g bean.

Toth *et al.* (2010) reported the concentration of vanillin in vanilla beans to be higher for most growing regions than was found in this study (Table 5.4). As the nature of the vanilla beans extracted by Toth *et al.* (2010) is unknown (moisture content, extraction solvent, curing method) it is hard to draw comparisons, but no one growing region differed greatly from the others in this past study. The present research found that Tonga had more vanillin than the other growing regions, and Papua New Guinea the least vanillin.

Table 5.3: Concentration of vanillin in extracts from different growing regions and approximate vanillin concentrations in the vanilla beans from different growing regions, as determined from the extraction yields. Reported vanillin concentration is from Toth *et al.* (2010). The nature of the vanilla products in this review are unknown.

Origin of Bean	Vanillin in Extract (mg/ml)	Vanillin in beans (mg vanillin/g dry weight)	Reported vanillin in beans (mg/g) (Toth *et al.* 2010).
India	2.40 ± 0.01	5.1	12.2-28.6
Madagascar	4.60 ± 0.07	9.3	15.9-22.4
Papua New Guinea	1.23 ± 0.04	2.4	9.0-16.6
Tonga	4.34 ± 0.07	13.9	10.4-12.2
Uganda	3.38 ± 0.13	8.5	11.9

The PNG vanilla may have contained less vanillin (2.4 mg/g) as it was a different species than the other four vanilla beans. The PNG vanilla was *Vanilla tahitensis*, a less common species that developed around Tahiti (Cameron, 2011), rather than *Vanilla planifolia* as with the other beans. This different species has been found to contain lower concentrations of vanillin in the past (Toth *et al.*, 2010).

A number of factors can also lead to a lower concentration of vanillin in the vanilla beans, including lower quality vanilla beans (Ramachandra Rao and Ravishankar, 2000), vanilla beans that have been left on the vine too long (Cameron, 2011) or poor curing of the vanilla beans after harvest (Pérez-Silva *et al.*, 2011).

5.3.3 Ethanol Concentration Using GC

All samples were tested for ethanol concentration as an indicator of the extraction process used to produce the extracts.

Of the commercial vanilla extracts, W and H5 had the highest ethanol concentration, at 49.49 and 49.46% v/v respectively. As both of these extracts were greater than single fold extracts, the higher percentage of ethanol would have been required to either extract more flavour, or the single fold extracts are simply just dilutions of this more concentrated extract. QT had the lowest concentration of ethanol, at 12.38 ± 0.34 % v/v, with sugar listed as one of the main ingredients, which would have helped stabilise the product against spoilage instead of the ethanol in the other products.

Table 5.4: Ethanol concentration of all vanilla extracts used for sensory testing. Data are means ± standard error where samples were analysed in triplicate.

Sample Name	Ethanol concentration (% v/v)
HG	N/A
H1	29.21 ± 1.67
H5	49.46 ± 0.31
HI2	26.19 ± 0.46
HI3	29.42 ± 1.21
L	24.99 ± 0.76
NM	31.96 ± 0.13
QO	33.83 ± 1.49
QT	12.38 ± 0.34
VD	29.32 ± 0.13
W	49.49 ± 1.49
I	61.86 ± 0.57
M	55.92 ± 0.32
PNG	57.01 ± 0.47
T	56.99 ± 0.61
U	54.99 ± 0.79

For the extracts produced from different growing regions, the ethanol concentration was higher than for the commercial vanilla extracts, ranging from 54.99 to 61.86 % v/v. This indicated that the process used to produce these extracts used a higher initial concentration of ethanol than the commercial vanilla extracts.

5.3.4 Panel Performance

Prior to analysis of the sensory data for patterns and relationships in the samples, ANOVA was used to check the panel performance by looking at the significance of the main effects and the interaction between these effects, namely the panellists, products and sessions.

The main effect of product was significant for all attributes other than vanilla aroma and vanilla flavour (Table 5.5). Vanillin was used as the reference material for both vanilla aroma and flavour and all samples were standardised based on vanillin concentration. As the samples were rated similarly for vanilla aroma and flavour, this confirmed that the participants were producing reliable results.

Table 5.5: Results from a general linear model on data collected for the aroma profile of the vanilla extracts. Values are p-values, with significant values in italics (p<0.05).

	Product	Panellist	Session	Product: Panellist	Product: Session
Overall Aroma	*0.000*	*0.000*	0.937	0.820	0.746
Artificial Fruity Aroma	*0.000*	*0.000*	0.960	0.490	0.569
Bourbon Aroma	*0.000*	*0.000*	0.741	0.224	0.875
Caramel Aroma	*0.006*	*0.000*	0.285	0.349	0.847
Raisin Aroma	*0.000*	*0.000*	0.136	0.985	0.964
Spicy Aroma	*0.000*	*0.000*	0.073	0.014	0.757
Vanilla Aroma	0.288	*0.000*	0.522	0.619	0.746
Overall Flavour	*0.000*	*0.000*	*0.034*	0.848	0.867
Sweet Flavour	*0.031*	*0.000*	0.201	0.661	0.680
Vanilla Flavour	0.725	*0.000*	0.397	0.105	0.814
Butterscotch Flavour	*0.014*	*0.000*	*0.032*	*0.013*	0.890
Raisin Flavour	*0.000*	*0.000*	*0.001*	0.922	0.341
Bitter Flavour	*0.000*	*0.000*	0.333	0.896	0.487
Straw Flavour	*0.001*	*0.000*	0.091	*0.002*	0.644
Woody Flavour	*0.000*	*0.000*	*0.000*	0.149	0.742
Bourbon Flavour	*0.000*	*0.000*	*0.002*	0.467	0.856

The main effect, panellist, was significant ($p<0.05$) for all attributes (Table 5.5). This indicated that the panellists were providing a different response to each other for all the attributes. However, the panellists were rating the samples consistently between each session, as this was only significant for overall flavour ($p=0.03$), butterscotch flavour ($p=0.03$), raisin flavour ($p=0.00$), woody flavour ($p=0.00$) and bourbon flavour ($p=0.00$). A non-significant value for session indicated that the panel was able to produce similar results across the sessions, so were producing consistent responses. Using a generic descriptive analysis technique for the panel training, these values indicated that the panel was producing reliable results, consistent with their own scores (Kemp *et al.*, 2009).

A further measure of the panel performance was to check the consistency of score rating across the session with a duplicate sample presented at the end of each session. The mean scores were compared with the identical samples presented in the same set. The Students's t-test was carried out to determine the differences (Table 5.6) and of the 48 session-attribute combinations, only one was found to differ – straw flavour for

session 3. This indicated that the panel was providing consistent responses, and the intensity scores could be interpreted and compared reliably.

Table 5.6: Student's t-test comparing the in-session responses with those of the repeat sample at the end of each session, for all sessions and all attributes during the sensory analysis of various commercial vanilla extracts as well as vanilla extracts created from vanilla beans from different growing regions. Values shown are p-values, with significant values in italics ($p<0.05$).

Attribute	Student's t-test scores		
	Session 1	Session 2	Session 3
Overall Aroma	0.336	0.216	0.709
Artificial Fruity Aroma	0.929	0.608	0.654
Bourbon Aroma	0.645	0.376	0.227
Caramel Aroma	0.737	0.697	0.105
Raisin Aroma	0.627	0.474	0.132
Spicy Aroma	0.898	0.825	0.189
Vanilla Aroma	0.906	0.387	0.467
Overall Flavour	0.157	0.596	0.947
Sweet Flavour	0.311	0.102	0.784
Vanilla Flavour	0.307	0.328	0.739
Butterscotch Flavour	0.937	0.392	0.247
Raisin Flavour	0.179	0.657	0.541
Bitter Flavour	0.632	0.564	0.835
Straw Flavour	0.547	0.668	*0.040*
Woody Flavour	0.726	0.610	0.930
Bourbon Flavour	0.465	0.224	0.941

From the results of the ANOVA and the Student's t-test, it was concluded that the panellists were providing reliable results and were able to differentiate between the products for most attributes.

5.3.5 Sensory analysis of Samples from Different Growing Regions

The laboratory made vanilla extract samples from five different growing regions were compared to isolate the effect of growing region. After using ANOVA to determine significant differences, the mean intensity scores were compared for patterns and trends. To further investigate the patterns, principal component analysis (PCA) was used to map out the samples.

5.3.5.1 Mean Sensory Scores of samples from Different Growing Regions

After ANOVA to determine significance, a multiple paired comparison was performed using Tukey's HSD. The attributes caramel aroma, vanilla aroma and straw

flavour were not able to differentiate between the vanilla extracts from different growing regions, but the 13 other attributes differed significantly (Table 5.7 and Table 5.8).

PNG had a higher overall aroma rating than the other extracts and U had a lower overall aroma rating (Table 5.7). These two extracts tended to be highest and lowest for all other attributes, respectively, although PNG rated lowest for raisin aroma at 2.4.

For flavour, there tended to be two groups in the ratings (Table 5.8). I and PNG were higher in overall flavour (5.6 and 5.9) as well as most other flavour attributes, other than sweet flavour (2.7 and 3.2). In comparison M, T and U rated low for most attributes, and high in sweet flavour.

Table 5.7: Multiple paired comparison results comparing vanilla extracts from different growing regions on aroma attributes. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test, with samples tested in triplicate.

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
I	4.8^{ab}	2.0^{b}	2.7^{ab}	2.7^{a}	3.0^{bc}	2.9^{b}	2.8^{a}
M	4.0^{c}	2.3^{ab}	3.1^{a}	2.9^{a}	3.6^{ab}	2.4^{bc}	2.9^{a}
PNG	5.5^{a}	2.2^{b}	3.1^{a}	2.4^{a}	2.4^{c}	3.5^{a}	2.8^{a}
T	4.7^{bc}	2.8^{a}	2.2^{b}	2.9^{a}	4.1^{a}	2.3^{bc}	3.3^{a}
U	3.9^{c}	2.1^{b}	2.3^{b}	2.4^{a}	3.3^{b}	2.1^{c}	2.8^{a}

Table 5.8: Multiple paired comparison results comparing vanilla extracts from different growing regions on flavour attributes. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test, with samples tested in triplicate.

	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
I	5.6^{a}	3.2^{bc}	2.7^{b}	1.9^{b}	3.8^{a}	2.8^{a}	3.0^{a}	3.4^{a}	2.3^{ab}
M	4.4^{b}	4.1^{a}	3.3^{a}	2.8^{a}	3.2^{ab}	2.4^{b}	2.7^{a}	2.5^{b}	2.5^{ab}
PNG	5.9^{a}	3.0^{c}	3.2^{ab}	1.7^{b}	3.0^{b}	2.9^{a}	2.8^{a}	3.3^{a}	2.7^{a}
T	4.3^{b}	3.7^{ab}	3.4^{a}	2.9^{a}	2.8^{b}	1.8^{c}	2.5^{a}	2.0^{b}	2.0^{b}
U	4.1^{b}	3.3^{bc}	2.9^{ab}	2.1^{b}	2.9^{b}	1.9^{c}	3.1^{a}	2.1^{b}	2.2^{ab}

5.3.5.2 Principal Component Analysis of Vanilla extracts from Different Growing Regions

Principal component analysis (PCA) was used to visualise the separation between the vanilla extracts based on the sensory characteristics. It provided a plot to allow for visualisation of the relative arrangement of the extracts to each other, based on a reduced number of components which were driving the variation between the samples.

The aroma attributes and flavour attributes were analysed separately, to produce clearer PCA bi-plots.

a) Aroma PCA

For the aroma attributes, the first two components were found to have eigenvalues greater than one (Appendix 4). Component 1 explained 58.8% of the variation in the data and component 2 explained 24.7% of the variation, therefore 83.5% of the variation in the data was explained by the first two components. Figure 5.1 shows the bi-plot of these two components.

Table 5.9: Factor loadings for aroma attributes in the PCA of the vanilla extracts for different growing regions. Values in bold are the highest factor loading for each attribute.

	PC1	PC2
Overall Aroma	-0.552	**0.772**
Artificial Fruity Aroma	**0.718**	0.631
Bourbon Aroma	**-0.638**	0.234
Caramel Aroma	**0.715**	0.322
Raisin Aroma	**0.994**	-0.064
Spicy Aroma	**-0.819**	0.570
Vanilla Aroma	**0.848**	0.498

PC1 was positively correlated with artificial fruity aroma, caramel aroma, raisin aroma and vanilla aroma, and negatively correlated with bourbon aroma and spicy aroma (Table 5.9). PC2 was positively correlated with overall aroma. As PC2 was only correlated with one attribute – overall aroma – this led to the one-sided arrangement in Figure 5.1.

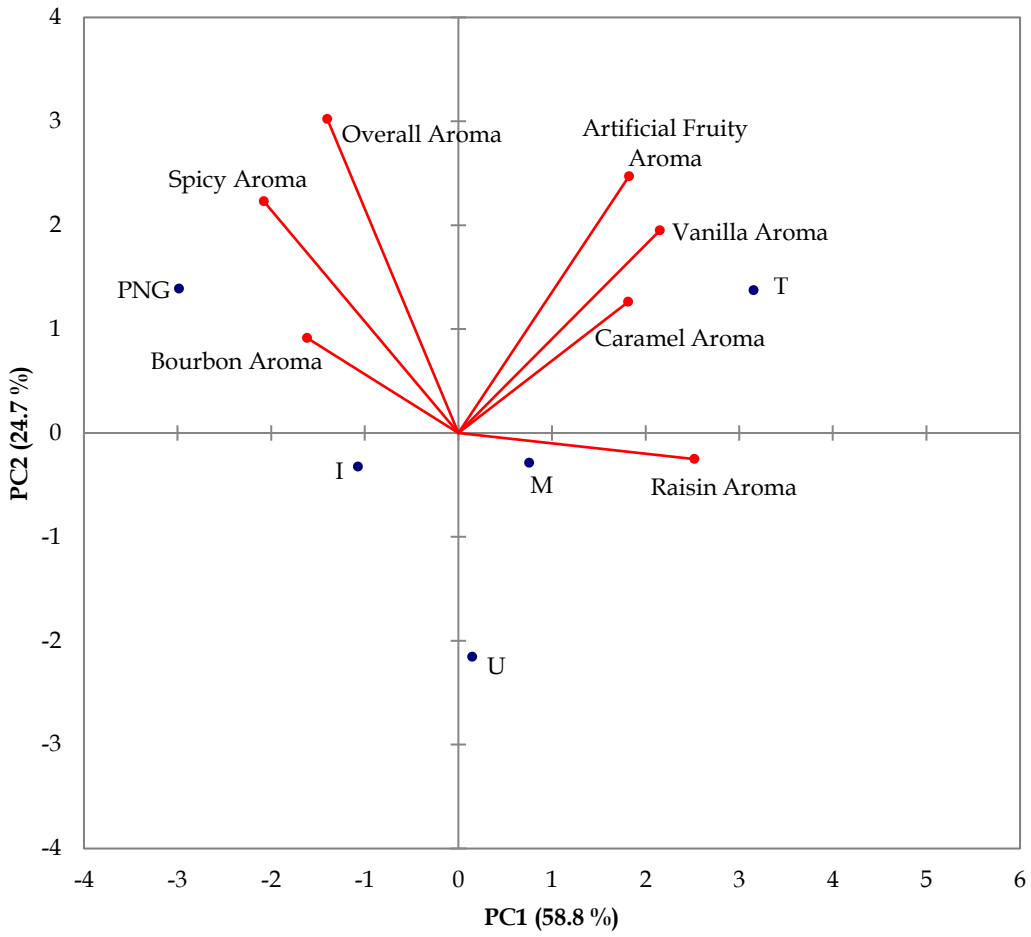


Figure 5.1: Bi-plot of the first two principal components identified through PCA of all aroma attributes for vanilla extracts from different growing regions. The first component had an eigenvalue of 4.12 and the second component had an eigenvalue of 1.73. The cumulative proportion of variation explained by the components was 83.5%. The vanilla extracts are marked by the blue dots.

The correlations matrix can be seen in Table 5.10. Spicy aroma was positively correlated with overall aroma and vanilla aroma was positively correlated with artificial fruity aroma.

The products were well separated by the principal components, as can be seen in Figure 5.1. This indicated that the aroma profile of each extract was unique. However, the difference was not significant for caramel aroma and vanilla aroma based on the ANOVA (Table 5.7).

Table 5.10: Correlation matrix for aroma attributes in the PCA of the vanilla extracts from different growing regions. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	0.065	0.304	-0.266	-0.624	**0.899**	-0.032
Artificial Fruity Aroma	0.065	**1**	-0.283	0.610	0.670	-0.248	**0.931**
Bourbon Aroma	0.304	-0.283	**1**	-0.050	-0.586	0.646	-0.553
Caramel Aroma	-0.266	0.610	-0.050	**1**	0.739	-0.382	0.666
Raisin Aroma	-0.624	0.670	-0.586	0.739	**1**	-0.851	0.795
Spicy Aroma	**0.899**	-0.248	0.646	-0.382	-0.851	**1**	-0.412
Vanilla Aroma	-0.032	**0.931**	-0.553	0.666	0.795	-0.412	**1**

T was characterised as being relatively high in artificial fruity aroma, vanilla aroma, caramel aroma and raisin aroma, and relatively low in overall aroma, bourbon aroma and spicy aroma. The PNG extract in contrast was relatively high in overall aroma, bourbon aroma and spicy aroma and relatively low in artificial fruity aroma, vanilla aroma, caramel aroma and raisin aroma. The U extract was relatively low in all attributes, as it was positioned on the lower half of the bi-plot. As the processing conditions were controlled, these differences most likely were caused by either the growing region, the bean quality or the curing method (Cameron, 2011). The PNG extract was also made from a different variety of vanilla beans – *Vanilla planifolia tahitensis* rather than *Vanilla planifolia* like the other extracts, which also could have led to differences between the extracts.

b) Flavour PCA

For the flavour attributes, the first three components were found to have eigenvalues greater than one at 5.25, 2.15 and 1.03. PC1 explained 58.4% of the variation, PC2 explained 23.9% of the variation and PC3 explained 11.5% of the variation in the data (Appendix 4). The total variation explained by the first two components was 82.3%, so to keep the interpretation simple only the first two components were focussed on.

PC1 was positively correlated with overall flavour, raisin flavour, bitter flavour and woody flavour and negatively correlated with sweet flavour and butterscotch flavour (Table 5.11). PC2 was positively correlated with straw flavour and negatively correlated with vanilla flavour and bourbon flavour.

The factor loadings led to a one-sided PCA plot, with all of the factors other than straw flavour tending to be positioned on the lower half of the bi-plot (Figure 5.3). It should be noted that straw flavour was not able to significantly differentiate the products in the ANOVA (Table 5.8).

Table 5.11: Factor loadings for flavour attributes in the PCA of the vanilla extracts for different growing regions. Values in bold are the highest factor loading for each attribute.

	PC1	PC2	PC3
Overall Flavour	**0.889**	-0.344	-0.149
Sweet Flavour	**-0.728**	-0.411	0.504
Vanilla Flavour	-0.639	**-0.722**	-0.265
Butterscotch Flavour	**-0.890**	-0.266	0.369
Raisin Flavour	0.690	0.012	**0.708**
Bitter Flavour	**0.879**	-0.472	0.067
Straw Flavour	0.540	**0.726**	0.080
Woody Flavour	**0.929**	-0.337	0.150
Bourbon Flavour	0.572	**-0.636**	-0.124

Bitter flavour and woody flavour were highly positively correlated. Sweet flavour was positively correlated with butterscotch flavour and vanilla flavour was negatively correlated with straw flavour (Table 5.12).

Table 5.12: Correlation matrix for flavour attributes in the PCA of the different growing regions vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	-0.638	-0.282	-0.750	0.543	**0.937**	0.109	**0.927**	0.614
Sweet Flavour	-0.638	**1**	0.630	**0.940**	-0.182	-0.416	-0.562	-0.468	-0.110
Vanilla Flavour	-0.282	0.630	**1**	0.663	-0.638	-0.239	**-0.888**	-0.391	0.129
Butterscotch Flavour	-0.750	**0.940**	0.663	**1**	-0.353	-0.633	-0.653	-0.682	-0.397
Raisin Flavour	0.543	-0.182	-0.638	-0.353	**1**	0.650	0.376	0.747	0.226
Bitter Flavour	**0.937**	-0.416	-0.239	-0.633	0.650	**1**	0.133	**0.986**	0.789
Straw Flavour	0.109	-0.562	**-0.888**	-0.653	0.376	0.133	**1**	0.258	0.048
Woody Flavour	**0.927**	-0.468	-0.391	-0.682	0.747	**0.986**	0.258	**1**	0.714
Bourbon Flavour	0.614	-0.110	0.129	-0.397	0.226	0.789	0.048	0.714	**1**

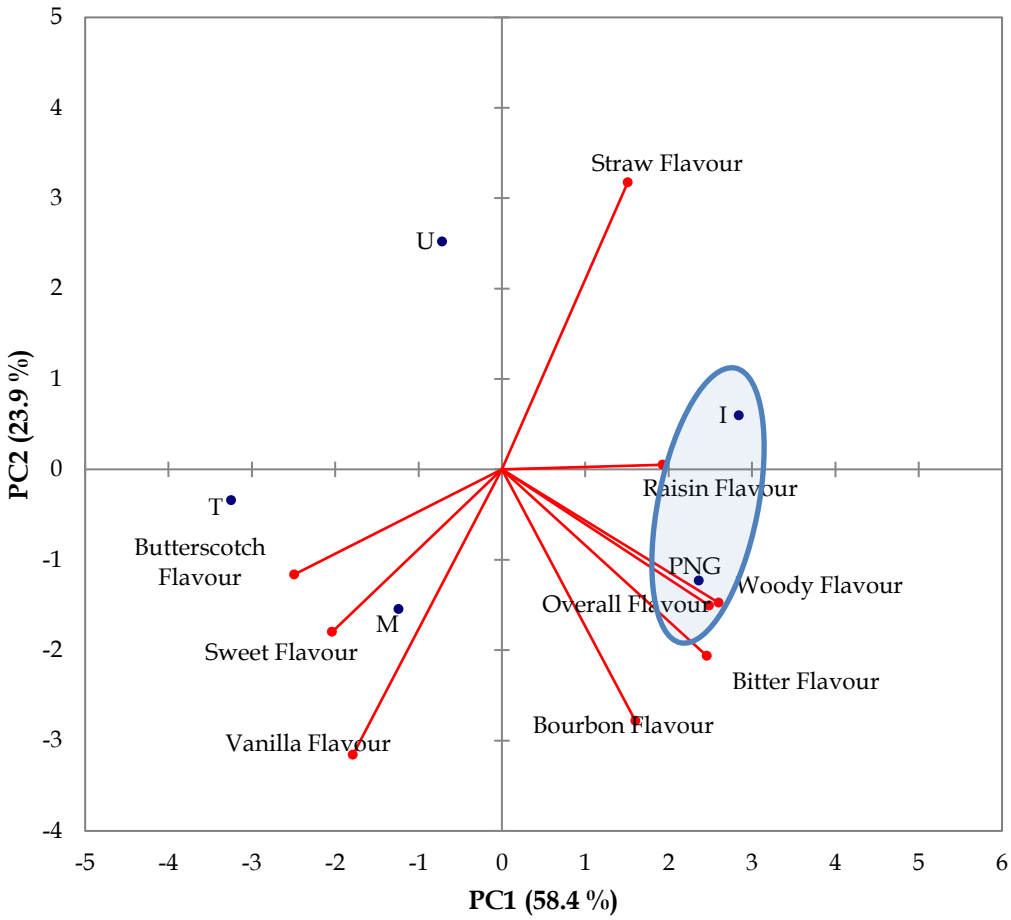


Figure 5.3: Bi-plot of the first two principal components identified through PCA of all flavour attributes of vanilla extracts from different growing regions. The first component had an eigenvalue of 5.25 and the second component had an eigenvalue of 2.15. The cumulative variation explained by the components was 82.2%. The blue circle surrounds the I and PNG extracts. The vanilla extracts are marked by the blue dots.

Of the five different vanilla extracts, there were two groupings based on the sensory profiles (Figure 5.3). The first group, circled in blue, contained I and PNG and the second group, less closely associated, contained M, T and U. This is slightly different to the aroma profile, which found that the products were well separated.

In particular, the I and PNG extracts had very similar flavour profiles, being characterised as relatively

high in raisin flavour, bitter flavour, woody flavour, bourbon flavour and overall flavour. They were also relatively low in butterscotch flavour, sweet flavour and vanilla flavour.

The M, T and U extracts were less similar but all three tended to be relatively low in overall flavour, raisin flavour, bitter flavour and woody flavour and relatively high in

sweet flavour and butterscotch flavour. They were separated based on PC2 having a range of ratings for vanilla flavour, straw flavour and bourbon flavour.

5.3.5.3 Conclusions on sensory profiles of vanilla extracts from different growing regions

Vanilla extracts from vanilla beans grown in different regions had different sensory characteristics, as shown in PCA plots. Tonga and Madagascar had similar aroma and flavour profiles, being high in vanilla aroma, caramel aroma and raisin aroma, as well as sweet flavour, butterscotch flavour and vanilla flavour. India and Papua New Guinea were also similar to each other, being high in bourbon aroma and spicy aroma as well as raisin flavour, bitter flavour and woody flavour. Uganda was more similar to the Tonga and Madagascar group for both aroma and flavour.

5.3.6 Sensory Analysis of Heilala Vanilla Extracts to Compare Extraction Conditions

To compare the vanilla extracts produced with different extraction processes and solvents, a range of samples from Heilala Vanilla Ltd. were supplied. Five different samples were chosen, extracted with different concentrations of ethanol, or extracted with glycerol.

H5 is a five-fold extract and is produced from the first ethanol extraction of the vanilla beans. HI2 and H13 are produced from subsequent extractions of the vanilla beans resulting in different concentration of vanillin and ethanol. H1 is the single fold extract produced from a blend of H5, HI2 and HI3. HG is an extract produced with glycerol as the solvent instead of ethanol to produce a halal vanilla extract. Due to confidentiality, the exact concentration of ethanol used for each Heilala extract cannot be presented, but information is presented in Table 5.3 for the final vanillin concentration and Table 5.4 for the final ethanol concentration.

5.3.6.1 Mean Sensory Scores of Heilala Vanilla Extracts

The mean values for the sensory scores of the five Heilala samples can be seen in Table 5.13 for aroma and Table 5.14 for flavour, along with the results of the multiple paired comparison, which were produced after determining significance of differences using ANOVA.

For overall aroma, HG and HI3 rated the lowest of the five samples. HG was extracted with glycerol rather than ethanol as the other Heilala extracts were. HI3 was extracted with the lowest concentration of ethanol of the four ethanol extracts, with a final ethanol concentration of 29.42 ± 1.21 % v/v. This difference in the extraction solution may have caused the overall aroma profile of these two extracts to reduce, which affected the rating for all the attributes, even with the samples standardised to the same vanillin concentration.

Of the five samples, H5 rated the highest for most aroma attributes. This extract was the 5-fold extract, the highest vanillin concentration of all the samples (10.40 ± 0.04 mg/ml) and the highest ethanol concentration (49.46 ± 0.31 % v/v). This higher concentration of ethanol may have extracted more volatiles from the vanilla beans, resulting in higher ratings for the attributes.

This difference observed between H5, and HG and HI3 indicated that the ethanol concentration of the extraction solution had a significant effect on the aroma profile of the extract produced. H5 rated highest for all of the aroma attributes other than artificial fruity aroma and HG rated lowest for all of the aroma attributes other than artificial fruity aroma (Table 5.13). HI3 rated lowest of the ethanol extracts for the aroma attributes. This indicated that the higher concentrations of ethanol, as in H5, were better able to extract the aroma compounds than the lower concentrations of ethanol, as in HI3, or glycerol, as in HG.

For the flavour attributes, the multiple paired comparison and the mean values can be found in Table 5.14. HG and HI3 tended to rate the lowest for all attributes and H5 tended to rate the highest, similar to the aroma attributes. This would be due to the same effects as for the aroma – the H5 with the high ethanol concentration was best at extracting flavour, and the HI3 and HG with lower ethanol content and glycerol extraction, were not as good at extracting the flavour compounds.

Table 5.137: Multiple paired comparison results comparing vanilla extracts using different extraction solvents on aroma attributes. Means within the same column with different letters are significantly different (p<0.05) as determined by Tukey's HSD test.

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
H1	4.5^{bc}	2.9^{abc}	2.8^{abc}	2.8^{abc}	3.4^{bc}	2.4^{bc}	3.2^{ab}
HG	3.5^{d}	3.3^{ab}	1.6^{e}	2.4^{c}	2.2^{ef}	1.6^{d}	3.2^{ab}
H5	4.9^{ab}	2.4^{c}	2.9^{abc}	3.1^{ab}	4.5^{a}	2.8^{ab}	3.0^{b}
HI2	3.8^{cd}	3.1^{abc}	2.2^{cde}	3.2^{a}	3.6^{b}	2.4^{bc}	3.4^{ab}
HI3	3.5^{d}	2.5^{bc}	2.0^{de}	2.8^{abc}	2.5^{def}	2.0^{cd}	3.2^{ab}

Table 5.14: Multiple paired comparison results comparing vanilla extracts using different extraction methods on flavour attributes. Means within the same column with different letters are significantly different (p<0.05) as determined by Tukey's HSD test.

	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
H1	3.9^{c}	3.8^{b}	3.3^{ab}	2.7^{bc}	2.2^{de}	1.8^{c}	2.4^{ab}	1.9^{bcd}	1.9^{bc}
HG	3.7^{c}	3.8^{b}	3.1^{b}	2.5^{bc}	1.6^{e}	1.8^{c}	2.1^{b}	1.8^{cd}	1.5^{c}
H5	4.3^{bc}	4.0^{ab}	3.5^{ab}	2.9^{ab}	2.9^{abc}	2.3^{abc}	2.6^{ab}	2.3^{abc}	2.3^{ab}
HI2	4.4^{abc}	4.2^{ab}	3.7^{ab}	3.4^{a}	3.0^{a}	2.1^{bc}	2.6^{ab}	2.2^{abcd}	2.1^{bc}
HI3	3.8^{c}	3.8^{b}	3.2^{ab}	2.9^{ab}	1.7^{de}	1.8^{c}	2.1^{b}	1.7^{d}	1.6^{c}

5.3.6.2 Principal Component Analysis of Heilala Vanilla Extracts

a) Aroma PCA

For the aroma attributes, the principal component analysis (PCA) found two principal components, the first with an eigenvalue of 4.92 and the second with an eigenvalue of 1.22. The first component explained 70.2% of the variation in the data, and PC2 explained 17.4% of the variation, together explaining 87.6% of the variation.

PC1 was positively correlated with overall aroma, bourbon aroma, caramel aroma raisin aroma and spicy aroma (Table 5.15). PC2 was positively correlated with vanilla aroma, with a factor loading of 0.780. The attribute artificial fruity aroma was not correlated with either of these two principal components.

Table 5.15: Factor loadings for aroma attributes in PCA of vanilla extracts from Heilala. Values in bold are the highest factor loading for each attribute.

	PC1	PC2
Overall Aroma	**0.928**	-0.184
Artificial Fruity Aroma	**-0.656**	0.299
Bourbon Aroma	**0.938**	-0.027
Caramel Aroma	**0.730**	0.631
Raisin Aroma	**0.955**	0.169
Spicy Aroma	**0.970**	0.241
Vanilla Aroma	-0.596	**0.780**

Bourbon aroma and raisin aroma were positively correlated with each other, and both were positively correlated with overall aroma. Bourbon aroma and spicy aroma were positively correlated, as were raisin aroma and spicy aroma. Therefore, overall aroma – the overall intensity impact of the samples – was primarily determined by the bourbon aroma, raisin aroma and spicy aroma.

The bi-plot of PC1 and PC2 can be seen in Figure 5.4. HG is in the lower left-hand quadrant indicating that it was low in overall aroma, bourbon aroma, caramel aroma, raisin aroma and spicy aroma relative to the other samples. In contrast, H5 was high in PC1, thus it was relatively high in those same attributes. This separation of the products would be from the difference in the extraction solvent used for these two samples. HG used about 70% glycerol as the extraction solvent and H5 used about 65%

ethanol as the extraction solvent. These two extraction methods will extract different combinations of compounds from the same beans, as was indicated in Chapter 4.

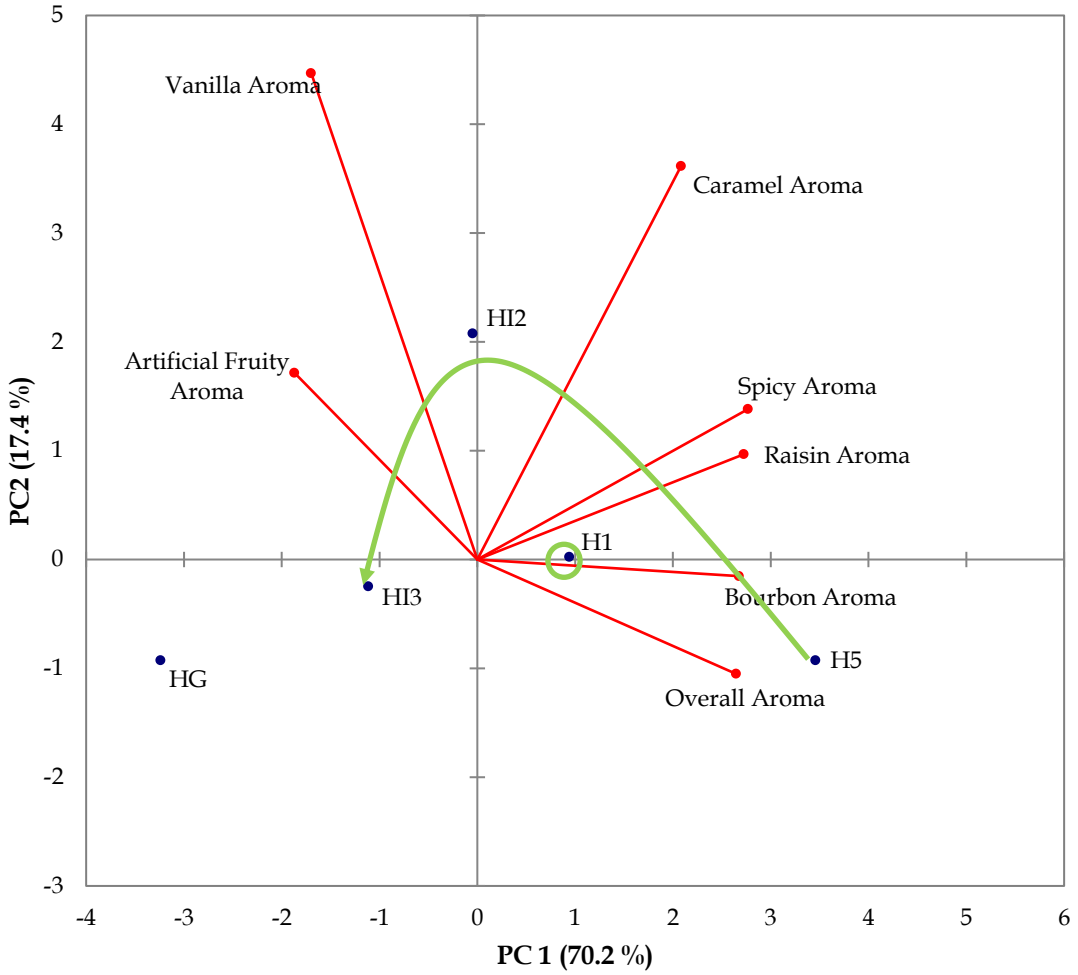


Figure 5.3: Bi-plot of the principal components identified through PCA of the aroma attributes for Heilala vanilla extract samples. The first component had an eigenvalue of 4.92 and the second component had an eigenvalue of 1.22. The cumulative variation explained by these components was 87.6%. The green arrow shows the progression from most ethanol to least ethanol used for flavour extraction. The vanilla extracts are marked by the blue dots.

The green arrow in Figure 5.3 indicates the progression from H5 to HI2 to HI3, the samples that were produced with decreasing ethanol concentration. Within the centre of these three samples was H1, as H1 was made of a blend of the other three extracts (HI2, HI3 and H5). The progression from H5 to HI3 indicated that the first extraction was able to extract more of the overall aroma, bourbon aroma, caramel aroma, raisin aroma and spicy aroma, and less of the vanilla aroma. The second extract, HI2, was more defined by vanilla aroma, being the highest in vanilla aroma of the five samples. The final extract, HI3, was relatively low in overall aroma, bourbon aroma, caramel aroma, raisin aroma and spicy aroma. It was expected that H5 would have the highest

vanilla aroma, being the highest in vanillin concentration, but with the standardisation, the other aroma attributes may have dominated more in the aroma profile of the extract, making the vanilla aroma seem weaker in comparison.

b) Flavour PCA

For the flavour attributes of the Heilala vanilla extracts, the first two components in the PCA had eigenvalues greater than one. The first component explained 83.2% of the variation and had an eigenvalue of 7.49 and the second component had an eigenvalue of 1.15 and explained 12.8% of the variation, summing to 95.95% of the variation in the data set being explained. Full details of the PCA can be found in Appendix 4.

Table 5.16: Correlation matrix for PCA of flavour attributes of Heilala vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	**0.927**	**0.948**	0.844	**0.910**	**0.899**	0.733	0.819	0.812
Sweet Flavour	**0.927**	**1**	0.797	**0.910**	0.708	0.699	0.461	0.550	0.541
Vanilla Flavour	**0.948**	0.797	**1**	0.820	**0.984**	**0.880**	**0.889**	0.878	**0.925**
Butterscotch Flavour	0.844	**0.910**	0.820	**1**	0.712	0.556	0.522	0.458	0.548
Raisin Flavour	**0.910**	0.708	**0.984**	0.712	**1**	**0.898**	**0.949**	**0.940**	**0.966**
Bitter Flavour	**0.899**	0.699	**0.880**	0.556	**0.898**	**1**	0.784	**0.947**	**0.909**
Straw Flavour	0.733	0.461	**0.889**	0.522	**0.949**	0.784	**1**	**0.920**	**0.961**
Woody Flavour	0.819	0.550	0.878	0.458	**0.940**	**0.947**	**0.920**	**1**	**0.970**
Bourbon Flavour	0.812	0.541	**0.925**	0.548	**0.966**	**0.909**	**0.961**	**0.970**	**1**

PC1 was positively correlated with all the attributes, and PC2 was not correlated with any of the attributes, as they were primarily determined by PC1 (Table 5.17).

Table 5.17: Factor loadings for flavour attributes in PCA of vanilla extracts from Heilala. Values in bold are the highest factor loading for each attribute.

	PC1	PC2
Overall Flavour	**0.960**	0.243
Sweet Flavour	**0.794**	0.582
Vanilla Flavour	**0.991**	0.052
Butterscotch Flavour	**0.766**	0.591
Raisin Flavour	**0.988**	-0.111
Bitter Flavour	**0.929**	-0.139
Straw Flavour	**0.889**	-0.366
Woody Flavour	**0.922**	-0.357
Bourbon Flavour	**0.940**	-0.325

There were multiple correlations between the attributes (Table 5.16). In Table 5.16 the attributes are presented in the order that they are detected during tasting, with the attributes that are detected immediately listed first and the attributes that are detected after 5-8 seconds listed last. The later flavour attributes were positively correlated with each other.

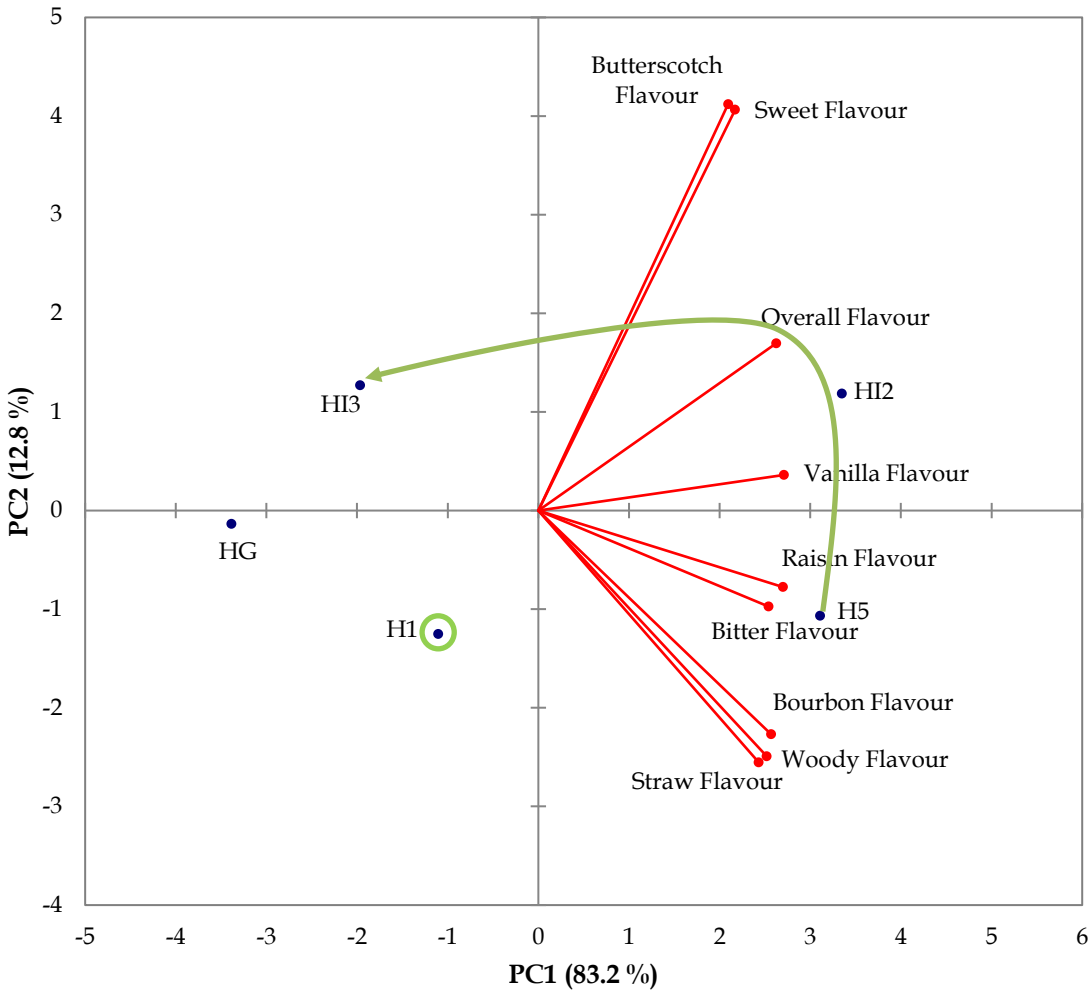


Figure 5.4: Bi-plot of the principal components identified through PCA of the flavour attributes for Heilala vanilla extract samples. The first component had an eigenvalue of 7.49 and the second component had an eigenvalue of 1.15. The cumulative variation explained by the components was 96.0%. The vanilla extracts are marked by the blue dots.

In the PCA bi-plot (Figure 5.4) all of the eigenvectors were on the right-hand side of the plot. Very similar to what was seen in the aroma attributes there was a pattern in the extracts from H5 to HI2 to HI3, shown by the green arrow on Figure 5.4. This followed the order of decreasing ethanol concentration during extraction and showed that as the ethanol decreased in the solvent, the flavour that was extracted also decreased, with HG showing less of each flavour attribute than the other extracts. H1 was once again positioned between all the other extracts, being created as a blend of the other extracts.

5.3.6.3 Conclusions on the sensory profile of Heilala Vanilla Extracts

The sample that rated highest for most attributes was H5, the extract extracted with the highest ethanol concentration. There was then a progression from H5 to HI2 to HI3 to HG, which reflected the ethanol concentration used to extract the aroma and flavour. HG had the lowest rating for the aroma and flavour attributes, indicating that glycerol was not as suited to extract vanilla aroma and flavour as ethanol.

5.3.7 Sensory Analysis of Commercial Vanilla Extracts

Growing region and extraction solvent had a significant effect on the aroma and flavour profile of natural vanilla extracts, as reported in the previous sections. An investigation into the differences in commercially available vanilla extracts was also carried out. The aims of this section were to determine if the trained panel was able to differentiate between commercial vanilla extracts, and to identify what factors have an impact on any differences observed. The commercial samples were all ethanol based, with all but one of the seven extracts at single fold concentration; W was a double-fold extract, containing 3.31 ± 0.02 mg/ml of vanillin (Table 5.2).

5.3.7.1 Mean Sensory Scores of Commercial Vanilla Extracts

From the ANOVA, it was found that 13 of the 16 aroma and flavour attributes were able to differentiate the seven commercial vanilla extracts. The attributes that did not show differences in the commercial vanilla extracts were vanilla flavour, butterscotch flavour and straw flavour. The results of the multiple paired comparisons for these samples and attributes are in Table 5.18 (aroma) and Table 5.19 (flavour).

For overall aroma H1 and W were found to be the lowest, rated as 4.5 and 4.6 respectively. All other samples were rated similarly, with values ranging from 4.9 for QT to 5.6 for QO. For artificial fruity aroma W was rated the highest (3.7) with all other samples rating very similarly to each other. Bourbon aroma was also able to differentiate the products, with QO (3.8) as the highest and H1 (2.8) and QT the lowest (2.5). NM rated the highest for caramel aroma (3.1) and QT the lowest (2.4). For raisin aroma, H1 rated the highest (3.4), but there were no significant differences, other than with QO and W (2.6 and 1.9). NM rated the highest for spicy aroma (3.3), with little variation in the other samples for this attribute (between 2.4 and 2.9). All samples were rated similarly for vanilla aroma between 2.9 and 3.8, with only two different groupings. The samples had been standardised based on vanillin concentration, so it

Table 5.18: Multiple paired comparison results comparing commercial vanilla extracts on aroma attributes. Means within the same column with different letters are significantly different (p<0.05) as determined by Tukey's HSD test.

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
H1	4.5^{bc}	2.9^{bc}	2.8^{bcd}	2.8^{abc}	3.4^{ab}	2.4^{bc}	3.2^{ab}
L	5.2^{ab}	2.8^{bc}	3.1^{abc}	2.8^{abc}	2.9^{abcd}	2.6^{bc}	3.5^{ab}
NM	5.5^{a}	2.7^{bc}	3.7^{ab}	3.1^{ab}	3.2^{abc}	3.3^{a}	3.7^{a}
QO	5.6^{a}	2.6^{bc}	3.8^{a}	2.7^{abc}	2.6^{cde}	2.9^{ab}	3.8^{a}
QT	4.9^{ab}	2.7^{bc}	2.5^{cde}	2.4^{c}	2.8^{bcd}	2.8^{ab}	2.9^{b}
VD	5.2^{ab}	2.8^{bc}	3.7^{ab}	2.5^{bc}	2.7^{bcd}	2.5^{bc}	3.5^{ab}
W	4.6^{b}	3.7^{a}	3.3^{abc}	2.5^{bc}	1.9^{e}	2.7^{abc}	3.0^{b}

Table 5.198: Multiple paired comparison results comparing commercial vanilla extracts on flavour attributes. Means within the same column with different letters are significantly different (p<0.05) as determined by Tukey's HSD test.

	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
H1	3.9^{d}	3.8^{b}	3.3^{ab}	2.7^{ab}	2.2^{cde}	1.8^{c}	2.4^{ab}	1.9^{bcd}	1.9^{cd}
L	5.0^{abc}	3.9^{b}	3.5^{ab}	2.6^{ab}	2.2^{bcde}	2.3^{abc}	2.4^{ab}	2.2^{abcd}	2.8^{ab}
NM	5.2^{a}	4.3^{ab}	3.9^{a}	2.4^{ab}	2.9^{ab}	2.5^{ab}	2.8^{a}	2.5^{ab}	3.3^{a}
QO	5.2^{a}	4.0^{ab}	3.6^{ab}	2.1^{b}	2.4^{abcd}	2.2^{abc}	2.6^{ab}	2.4^{abc}	3.1^{a}
QT	5.2^{a}	4.8^{a}	3.2^{ab}	2.4^{ab}	1.9^{de}	2.1^{bc}	2.4^{ab}	2.4^{b}	2.4^{bc}
VD	5.2^{ab}	4.3^{ab}	3.5^{ab}	2.3^{ab}	2.1^{de}	2.3^{abc}	2.6^{ab}	2.4^{bac}	3.1^{a}
W	4.8^{abc}	4.2^{ab}	3.6^{ab}	2.6^{ab}	1.8^{de}	2.7^{a}	2.5^{ab}	2.7^{a}	3.4^{a}

was expected that all the samples would rate the same for vanilla aroma, however the differences indicate that there are other chemical compounds other than vanillin that are also affecting the rating for vanilla aroma. Overall, NM and QO had similar sensory profiles, although NM was higher in raisin aroma. H1 and W were also very similar except W was significantly lower in raisin aroma.

A smaller range of values was recorded for flavour than aroma, however there were still significant differences between the products (Table 5.19). As there were more distinct groups for flavour than aroma, this indicated that the panellists were more consistent in their ratings for flavour than aroma, with a lower standard deviation, allowing for better differentiation between the products even with smaller differences between them.

For overall flavour NM, QO and QT were rated the highest at 5.2 and H1 the lowest at 3.9. QT was also rated highest for sweet flavour (4.8) and H1 and L the lowest (3.8 and 3.9). NM was slightly higher than the other samples for vanilla flavour, but they were all rated in the range from 3.2-3.9, due to the standardisation of the samples on vanillin, which was the reference material for this attribute. QO was lower than the other samples for butterscotch flavour (2.1) but the difference was not significant. NM was highest for raisin flavour (2.9). W was the highest in bitter flavour (2.7) and H1 the lowest (1.8), but there was little difference between most of the samples. Straw flavour was not able to differentiate between the samples. W was highest for woody flavour (2.7) and NM, QO, VD and W were highest for bourbon flavour with H1 the lowest. Overall H1 tended to be lowest for most flavour attributes and QO and NM the highest, although QO was lowest for butterscotch flavour. Further data analysis was next completed to better visualise the patterns occurring in the data using PCA.

5.3.7.2 Principal Component Analysis of Commercial Vanilla Extracts

a) Aroma PCA

For aroma, the first two principal components had eigenvalues greater than one – PC1 was 3.74 and PC2 was 1.68. PC1 was found to explain 53.5% of the total variation and PC2 24.0% of the variation, which accounted for 77.5% of the total variation in this dataset.

Of the attributes, principal component one was positively correlated with overall aroma (0.901), bourbon aroma (0.713), caramel aroma (0.711), spicy aroma (0.711) and

vanilla aroma (0.936) and negatively correlated with artificial fruity aroma (-0.642). PC2 was positively correlated with raisin aroma (0.906) and negatively correlated with artificial fruity aroma (-0.503) and bourbon aroma (-0.607), although it could be concluded that artificial fruity aroma was not correlated with either of the principal components as the value was too low (Table 5.20). Full details of the correlations and factor loadings are in Appendix 4.

Table 5.20: Factor loadings for aroma attributes in PCA of commercial vanilla extracts. Values in bold are the highest factor loading for each attribute.

	PC1	PC2
Overall Aroma	**0.901**	-0.267
Artificial Fruity Aroma	**-0.642**	-0.503
Bourbon Aroma	**0.713**	-0.607
Caramel Aroma	**0.711**	0.349
Raisin Aroma	0.376	**0.906**
Spicy Aroma	**0.697**	-0.179
Vanilla Aroma	**0.936**	-0.119

Table 5.21: Correlation matrix for PCA of aroma attributes of commercial vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	-0.616	0.738	0.370	0.055	0.668	**0.856**
Artificial Fruity Aroma	-0.616	**1**	-0.092	-0.288	-0.637	-0.283	-0.532
Bourbon Aroma	0.738	-0.092	**1**	0.339	-0.225	0.447	**0.816**
Caramel Aroma	0.370	-0.288	0.339	**1**	0.624	0.503	0.645
Raisin Aroma	0.055	-0.637	-0.225	0.624	**1**	0.070	0.256
Spicy Aroma	0.668	-0.283	0.447	0.503	0.070	**1**	0.455
Vanilla Aroma	**0.856**	-0.532	**0.816**	0.645	0.256	0.455	**1**

Vanilla aroma was positively correlated with overall aroma and bourbon aroma (Table 5.21). Overall aroma and bourbon aroma were not correlated with each other. This means that the overall aroma impact of the samples was largely determined by the vanilla aroma, with some influence on the rating of vanilla aroma coming from the bourbon aroma.

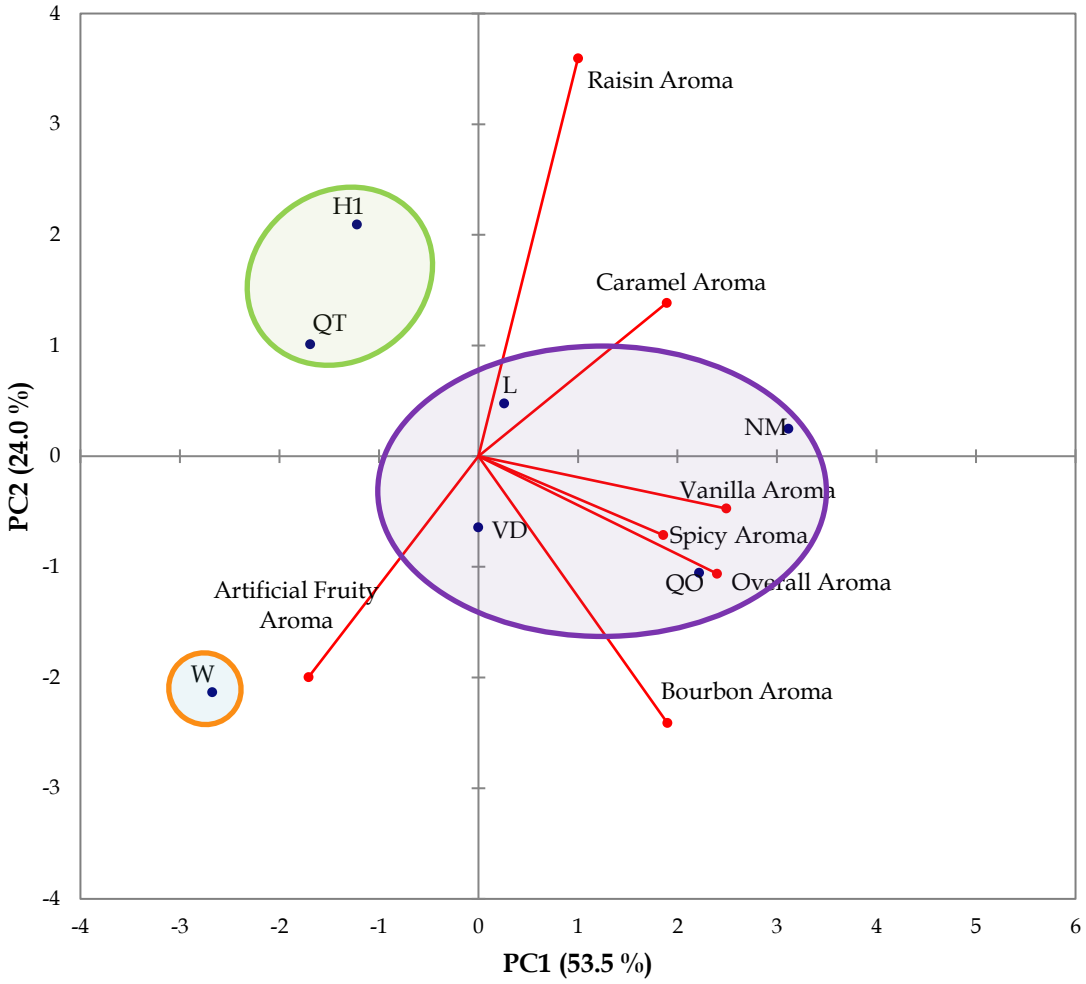


Figure 5.5: Bi-plot of the principal components identified through PCA of the aroma attributes for commercial vanilla extract samples. The first component had an eigenvalue of 7.49 and the second component had an eigenvalue of 1.15. The cumulative variation explained by the components was 77.5%. The Tongan vanilla extracts are circled in green, the Madagascar extracts are circled in purple and the Papua New Guinea-Madagascar blend is circled in orange. The vanilla extracts are marked by the blue dots.

Heirarchical cluster analysis revealed three main groupings, which were circled in Figure 5.5. The group circled in green contained the extracts H1 and QT, both of which are made from Tongan vanilla beans. The second group (purple) contained L, NM, QO and VD, all of which are made from Madagascan vanilla beans. The final group, in orange, contained W, which was the only extract made from both Madagascan vanilla

beans and Papua New Guinean vanilla beans (*Tahitensis* variety). It was clear that vanilla extracts made from vanilla beans of different growing regions produced different sensory profiles.

The group of extracts in green was relatively low in overall aroma, bourbon aroma, caramel aroma and vanilla aroma, and relatively high in raisin aroma. The group in orange was relatively low in all the attributes, other than artificial fruity aroma, which defined sample W. The group in purple was split into two parts –VD and L, which were relatively moderate for all attributes and QO and NM, which were relatively high in overall aroma, bourbon aroma, caramel aroma, spicy aroma and vanilla aroma, located in the far right-hand side of PC1 (Figure 5.5). This confirmed the findings in the effect of growing region (Section *5.3.4*) that vanilla extracts from different growing regions produce different sensory profiles. Although the exact extraction conditions for the commercial samples used in this experiment are unknown, the effect of growing region was strong enough to overcome any differences and be the main driving factor in differentiating between the sensory profiles of the samples.

b) Flavour PCA

Principal component analysis of the commercial extracts in terms of flavour found three components with eigenvalues greater than one. As the third component was not correlated with any of the attributes, only the first two were considered for presentation in this thesis. PC1 had an eigenvalue of 4.87 and explained 54.1% of the variation and PC2 had an eigenvalue of 2.01 and explained 22.4% of the variation, explaining a total of 76.5% of the variation.

Table 5.22: Factor loadings for flavour attributes in the PCA of the commercial vanilla extracts. Values in bold are the highest factor loading for each attribute.

	PC1	PC2	PC3
Overall Flavour	**0.799**	-0.350	0.311
Sweet Flavour	0.363	**-0.653**	0.590
Vanilla Flavour	**0.868**	0.357	-0.321
Butterscotch Flavour	**-0.687**	-0.207	-0.447
Raisin Flavour	0.426	**0.827**	0.219
Bitter Flavour	**0.806**	-0.273	-0.506
Straw Flavour	**0.730**	0.561	0.266
Woody Flavour	**0.853**	-0.456	-0.016
Bourbon Flavour	**0.886**	-0.100	-0.403

PC1 was positively correlated with overall flavour, vanilla flavour, butterscotch flavour, bitter flavour, straw flavour, woody flavour and bourbon flavour and PC2 was positively correlated with sweet flavour and raisin flavour (Table 5.22).

The correlations between the attributes are shown in Table 5.23. Overall flavour was positively correlated with woody flavour. Woody flavour was positively correlated with bitter flavour and bourbon flavour. Bitter flavour was positively correlated with vanilla flavour, woody flavour and bourbon flavour. Vanilla flavour was positively correlated with bitter flavour and straw flavour. Straw flavour was positively correlated with vanilla flavour and raisin flavour. The complex correlations within the attributes represent the correlations between the principal components and the attributes, and were seen more clearly in Figure 5.7, the PCA bi-plot.

Table 5.23: Correlation matrix for flavour attributes in the PCA of the commercial vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	0.640	0.442	-0.618	0.166	0.573	0.388	**0.780**	0.645
Sweet Flavour	0.640	**1**	-0.091	-0.243	-0.214	0.210	0.136	0.628	0.104
Vanilla Flavour	0.442	-0.091	**1**	-0.503	0.602	**0.764**	**0.761**	0.614	**0.841**
Butterscotch Flavour	-0.618	-0.243	-0.503	**1**	-0.422	-0.211	-0.651	-0.446	-0.493
Raisin Flavour	0.166	-0.214	0.602	-0.422	**1**	0.030	**0.844**	-0.008	0.169
Bitter Flavour	0.573	0.210	**0.764**	-0.211	0.030	**1**	0.331	**0.816**	**0.935**
Straw Flavour	0.388	0.136	**0.761**	-0.651	**0.844**	0.331	**1**	0.386	0.453
Woody Flavour	**0.780**	0.628	0.614	-0.446	-0.008	**0.816**	0.386	**1**	**0.764**
Bourbon Flavour	0.645	0.104	**0.841**	-0.493	0.169	**0.935**	0.453	**0.764**	**1**

In the cluster analysis, the samples were again grouped by growing region of the vanilla beans, shown by the circles in Figure 5.6, similar to the results for aroma. The Tonga extracts, H1 and QT, were grouped to the left in the green circle. The Madagascar extracts, NM, QO, VD and L, were grouped to the right in the purple circle. W, the blended Papua New Guinea and Madagascar extract was in the lower right-hand side, nearer to the Madagascar grouping.

H1 was low in most flavour attributes, but higher in butterscotch flavour, similar to QT, although there was some separation between these two samples on the PCA bi-plot (Figure 5.6) as QT was higher in sweet flavour and lower in raisin flavour and straw flavour relative to H1, separating the samples by PC2. The Madagascar extracts in contrast were relatively high in all flavour attributes, except for butterscotch flavour. The exception to this was L, which was positioned more to the left of the plot, indicating that it was lower in most attributes compared to the other Madagascar extracts. W, the blend extract, was more similar to the Madagascar extracts, but was more dominated by sweet flavour, woody flavour and overall flavour than the Madagascar extracts, which were more characterised by raisin flavour, straw flavour and vanilla flavour.

As it was not known what processing method was used for the extracts, the effect that this could have on the extracts cannot be determined. However, there is a clear effect being introduced by the growing region of the vanilla beans used to create each vanilla extract, as was seen in section *5.3.5*.

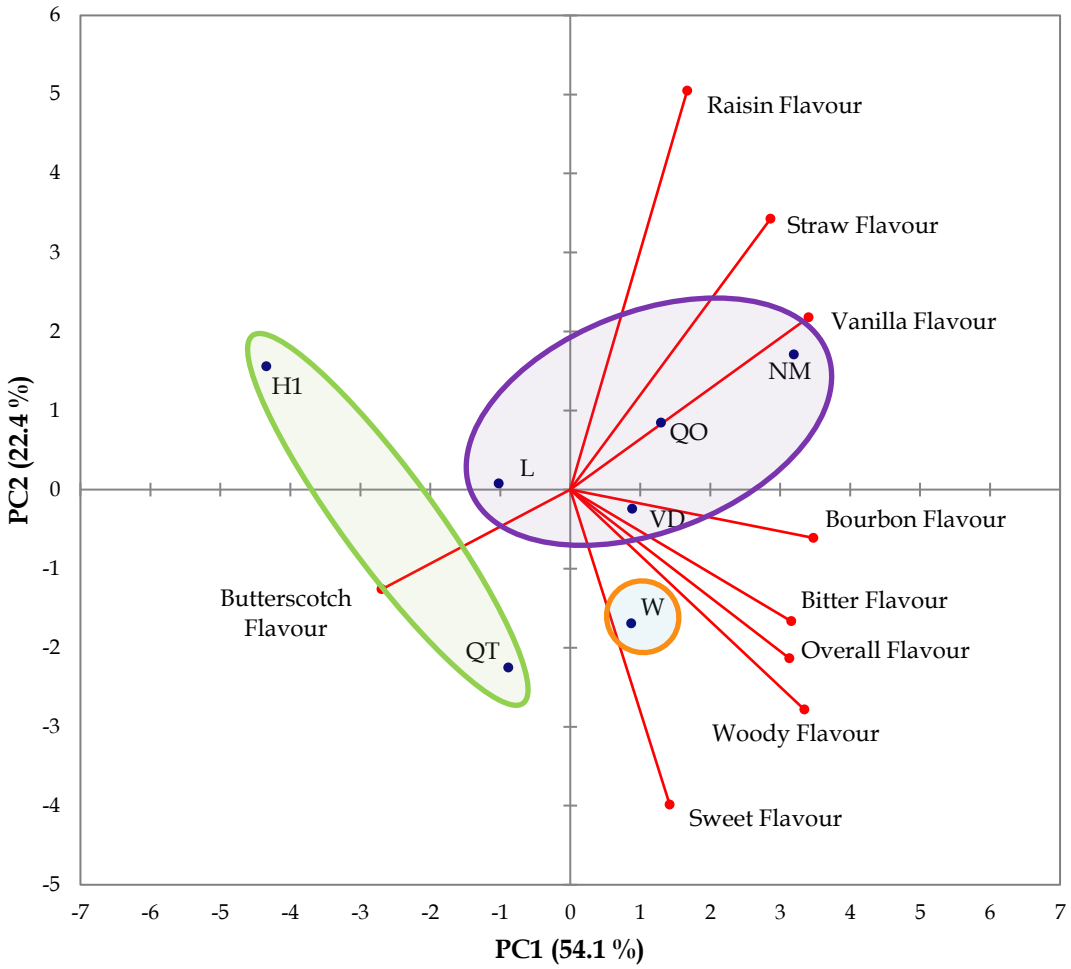


Figure 5.6: Bi-plot of the principal components identified through PCA of the flavour attributes for commercial vanilla extract samples. The first component had an eigenvalue of 4.87 and the second component had an eigenvalue of 2.01. The cumulative proportion of variation explained by the components was 76.5%. Tongan vanilla extracts are circled in green, Madagascan extracts are circled in purple and Papua New Guinea-Madagascar blend extracts are circled in orange. The vanilla extracts are marked by the blue dots.

5.3.7.3 Conclusions on sensory profile of Commercial Vanilla Extracts

The commercial vanilla extracts were able to be differentiated by the trained panel based on the 16 aroma and flavour attributes used. The variation in the intensity scores for the attributes was lower for flavour than aroma; the panellists were able to differentiate between the samples better for aroma than flavour.

The samples tended to group based on growing region of the vanilla beans used to manufacture each extract, with clear groups for Tonga, Madagascar and the Papua New Guinea-Madagascar blend. This was seen in both aroma and flavour. As the processing methods for each extract was not known, any effects that this had could not be identified.

5.3.8 Sensory Analysis of All the Natural Vanilla Extracts

Having separated the vanilla extracts out into groups to investigate the effects of origin and extraction solvent as well as looking at a comparison of commercially available single fold ethanol vanilla extracts, these factors needed to be combined for an overall analysis. This would allow for a side by side comparison of the relative effect of each of these factors, so that the main factors that affect the aroma and flavour profile of a vanilla extract could be determined.

5.3.8.1 Principal Component Analysis of All the Natural Vanilla Extracts

a) Aroma PCA

For aroma, the first three components were found to have eigenvalues greater than one, with values of 2.74, 1.75 and 1.54 respectively. The proportion of the variation explained by each was 39.1% for PC1, 25.0% for PC2 and 22.0% for PC3. The resulting bi-plots can be seen in Figure 5.7, Figure 5.8 and Figure 5.9.

The correlations between variables and PCs can be seen in Table 5.24. PC1 was positively correlated with overall aroma, bourbon aroma and spicy aroma. PC2 was positively correlated with caramel aroma and raisin aroma and PC3 was positively correlated with artificial fruity aroma and vanilla aroma.

Table 5.24: Factor loadings for aroma attributes in the PCA of the natural vanilla extracts. Values in bold are the highest factor loading for each attribute.

	PC1	PC2	PC3
Overall Aroma	**0.948**	-0.137	-0.065
Artificial Fruity Aroma	-0.170	-0.246	**0.823**
Bourbon Aroma	**0.911**	-0.123	0.099
Caramel Aroma	0.171	**0.889**	0.300
Raisin Aroma	0.017	**0.911**	-0.231
Spicy Aroma	**0.883**	-0.044	-0.292
Vanilla Aroma	0.409	0.175	**0.785**

Between the attributes, there were a range of correlations, as per Table 5.25. There was a positive correlation between overall aroma with bourbon aroma and spicy aroma, a positive correlation between bourbon aroma and spicy aroma, and a positive correlation between caramel aroma and raisin aroma. These three sets of correlations largely represent the three principal components identified in the analysis, as can be seen comparing Table 5.24 and Table 5.25.

Table 5.25: Correlation matrix for PCA of aroma attributes of all vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	-0.144	**0.821**	-0.028	-0.033	**0.845**	0.319
Artificial Fruity Aroma	-0.144	**1**	-0.044	0.023	-0.332	-0.289	0.342
Bourbon Aroma	**0.821**	-0.044	**1**	0.067	-0.095	**0.707**	0.391
Caramel Aroma	-0.028	0.023	0.067	**1**	**0.667**	0.101	0.405
Raisin Aroma	-0.033	-0.332	-0.095	**0.667**	**1**	0.000	-0.056
Spicy Aroma	**0.845**	-0.289	**0.707**	0.101	0.000	**1**	0.045
Vanilla Aroma	0.319	0.342	0.391	0.405	-0.056	0.045	**1**

i) PC1 and PC2

There was a separation of the vanilla extracts with the aroma attributes based on the region that the vanilla beans were grown (Figure 5.7), although this was not as apparent in the cluster analysis. The circle on the left (green) surrounds all of the Tongan originated vanilla extracts and the circle on the right (blue) surrounds most of the Madagascar originated extracts.

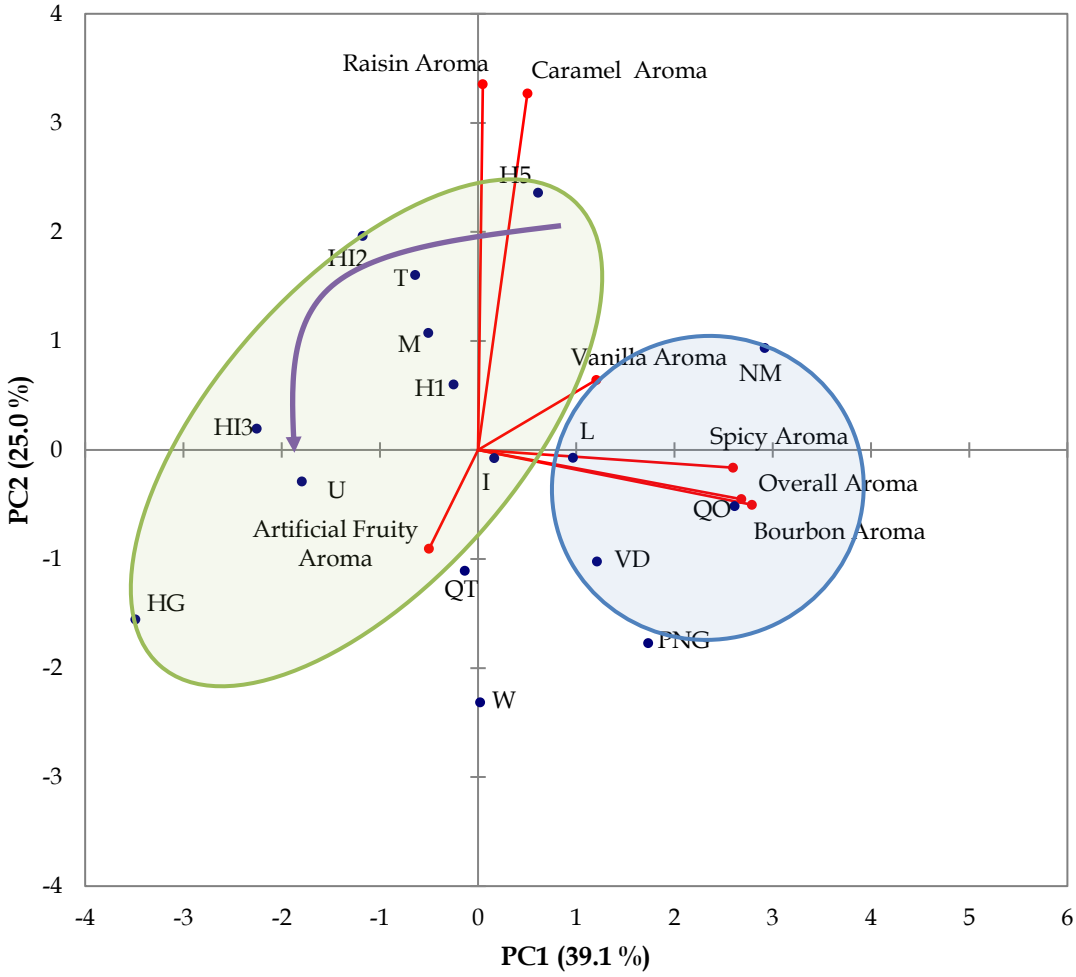


Figure 5.7: Bi-plot of the first two principal components identified through PCA of all aroma attributes looking at all vanilla extract samples presented for sensory analysis. The first component had an eigenvalue of 2.73 and the second component had an eigenvalue of 1.75. The cumulative variation explained by the components was 64.0%. The blue circle surrounds the Madagascar originated extracts, the green circle surrounds the Tonga originated extracts and the purple arrow shows the trend in the Tongan extracts. The vanilla extracts are marked by the blue dots.

The Tongan extracts tended to be lower in bourbon aroma, spicy aroma and overall aroma, and higher in raisin aroma and caramel aroma. The HG extract was towards the periphery of this group, as it was made with glycerol as the extraction solvent, rather than ethanol as all the other extracts were. The different polarity of the solvents would

have caused different aroma and flavour compounds to have been extracted. The HG sample was characterised as relatively high in artificial fruity aroma. It was also relatively low in caramel aroma and raisin aroma when compared to the other extracts from the same region and compared with the other, ethanol-based Heilala extracts.

In the Tongan group a trend within the Heilala extracts was observed – from H5 at the top, as the first extract created from the vanilla beans, with the highest ethanol concentration, to HI3 at the left, as the last extract created in the process, with the lowest ethanol concentration. This is shown by the arrow on Figure 5.7. H1, the single fold commercial extract, was positioned in the middle of the other extracts, as it was created as a blend of the other extracts. This was noted in Section *5.3.5.2,* the section looking only at these Heilala (H) samples. The T extract, made especially for this study, also had a similar sensory profile to the Heilala samples and was located in the centre of the H extracts and the Tonga group in green.

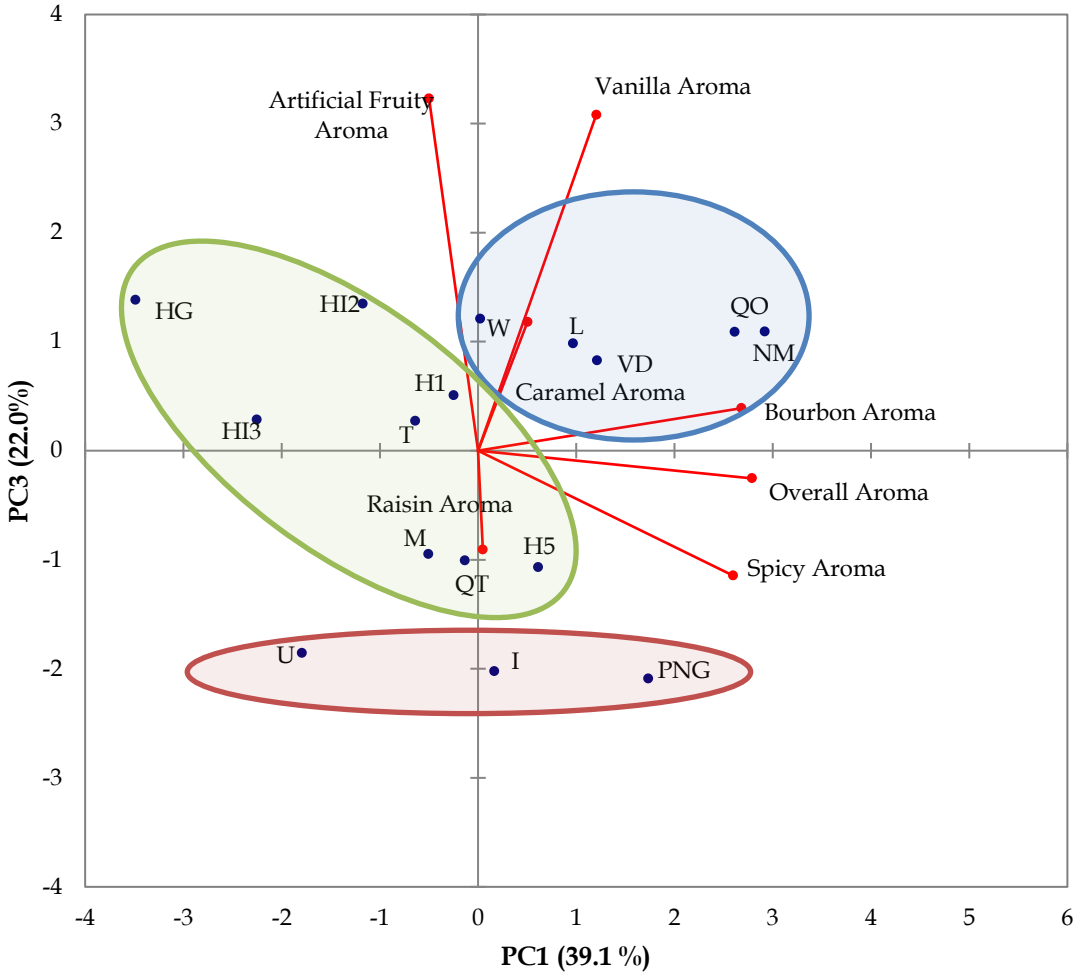
The Madagascar extracts tended to be positioned on the right-hand side of Figure 5.7, with a relatively high bourbon aroma, spicy aroma and overall aroma, and relatively low artificial fruity aroma. The Madagascar extract (M) was grouped with the Tongan extracts, in the green circle, rather than with the other Madagascan extracts, as was expected. The difference in the Madagascar extracts could be due to the different processing or extraction methods. .

ii) PC1 and PC3

Similar patterns in the arrangement of the samples were observed in Figure 5.8 as for Figure 5.7, although PC1 and PC3 were now presented instead of PC1 and PC2. PC3 was positively correlated with artificial fruity aroma and vanilla aroma. The Tongan extracts were grouped on the left side of the plot, in the green circle, and the Madagascar extracts were grouped in the upper right-hand side of the plot, in the blue circle. The PNG extract, I extract and U extract were all positioned on the lower half of the bi-plot, in the red circle, separated from the Madagascar and Tonga extracts. The

PCA bi-plot indicated that the extracts were able to be differentiated by the trained panel based on the region that the vanilla beans originated from.

Figure 5.8: Bi-plot of the first principal component and the third principal component identified through PCA of all aroma attributes looking at all vanilla extract samples presented for sensory analysis. The first component had an eigenvalue of 2.73 and the third component had an eigenvalue of 1.54. The cumulative variation explained by the components was 61.0%. The blue circle surrounds the Madagascar originated extracts, the green circle surrounds the Tonga originated extracts and the red circle surrounds the remaining extracts. The vanilla extracts are marked by the blue dots.



The main separation of the extracts for PC3 was the grouping of U, I and PNG, low in PC3. This indicated that they were lower in artificial fruity aroma and vanilla aroma than the other vanilla extracts. The other extracts were not able to be separated based on PC3.

For the most part, the extracts were separated based on the region that the vanilla beans originated from, but there was also an effect based on the extraction solvent

used, leading to the conclusion that there are multiple factors that determine the final aroma profile of a vanilla extract.

b) Flavour PCA

The first two components for flavour had eigenvalues greater than one, with PC1 scoring 4.50 and PC2 scoring 2.32. PC1 explained 50.0% of the variation and PC2 explained 25.8% of the variation, leading to a combined percent of 75.8% of the variation explained through the PCA.

Overall flavour was positively correlated with bitter flavour, woody flavour and bourbon flavour, and negatively correlated with butterscotch flavour (Table 5.26). Sweet flavour was positively correlated with vanilla flavour. Butterscotch flavour was negatively correlated with overall flavour, bitter flavour, straw flavour and woody flavour.

Table 5.26: Correlation matrix for PCA of flavour attributes of all vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05

Variables	Overall flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall flavour	**1**	-0.022	0.101	**-0.643**	0.334	**0.832**	0.448	**0.836**	**0.686**
Sweet Flavour	-0.022	**1**	**0.625**	0.467	-0.420	-0.184	-0.390	-0.280	0.275
Vanilla Flavour	0.101	**0.625**	**1**	0.372	-0.210	0.072	-0.250	-0.181	**0.581**
Butterscotch Flavour	**-0.643**	0.467	0.372	**1**	-0.188	**-0.520**	**-0.545**	**-0.627**	-0.364
Raisin Flavour	0.334	-0.420	-0.210	-0.188	**1**	0.455	**0.803**	**0.604**	0.089
Bitter Flavour	**0.832**	-0.184	0.072	**-0.520**	0.455	**1**	0.480	**0.929**	**0.684**
Straw Flavour	0.448	-0.390	-0.250	**-0.545**	**0.803**	0.480	**1**	**0.635**	0.344
Woody Flavour	**0.836**	-0.280	-0.181	**-0.627**	**0.604**	**0.929**	**0.635**	**1**	**0.512**
Bourbon Flavour	**0.686**	0.275	**0.581**	-0.364	0.089	**0.684**	0.344	**0.512**	**1**

One correlation created a group of attributes; overall flavour, bitter flavour, woody flavour and bourbon flavour were all positively correlated - as one of these attributes increased, the other attributes would also increase. This indicated that overall flavour was driving the response for these attributes.

In Table 5.27 PC1 was positively correlated with overall flavour, raisin flavour, bitter flavour, straw flavour and woody flavour and negatively correlated with butterscotch flavour. PC2 was positively correlated with sweet flavour, vanilla flavour and bourbon flavour.

Table 5.27: Factor loadings for flavour attributes in the PCA of the natural vanilla extracts. Values in bold are the highest factor loading for each attribute.

	PC1	PC2
Overall flavour	**0.850**	0.345
Sweet Flavour	-0.371	**0.775**
Vanilla Flavour	-0.147	**0.882**
Butterscotch Flavour	**-0.741**	0.195
Raisin Flavour	**0.647**	-0.337
Bitter Flavour	**0.888**	0.263
Straw Flavour	**0.777**	-0.266
Woody Flavour	**0.949**	0.026
Bourbon Flavour	0.595	**0.729**

The pattern for the flavour of the vanilla extracts was most clear in Figure 5.10, compared to the other PCA plots in this section when the groupings from cluster analysis were circled. There was a clear group of Madagascar extracts in the upper right-hand quadrant of the plot (blue circle) and a group of Tonga extracts on the left side of the plot (green circle).

The Madagascar extracts (L, VD, W, M, QO and NM) were characterised as relatively high in overall flavour, bitter flavour, woody flavour, bourbon flavour, vanilla flavour and sweet flavour, and relatively low in butterscotch flavour. It should be noted that as L lies to the left of the y-axis, it was moderate for all these attributes rather than relatively high, but still more similar to the Madagascar extracts than the Tonga extracts.

The Tonga extracts (HI3, HG, H1, T, HI2, H5 and QT) were characterised as relatively high in butterscotch flavour, and relatively low in all other attributes as a whole but there was more separation between the samples within the Tonga group

than the Madagascar group. A trend in the Heilala extracts could be seen, with the H5 near the centre of the plot, leading to HI3, near the left edge of the plot, close to HG. The Heilala single fold extract was in the centre of the grouping, as expected, as it was made as a blend of the other extracts. This was noted in Section *5.3.5.2*.

This, along with the patterns seen in the previous plots (Figure 5.7, 4.8 and 4.9), showed that HG was most similar to HI3 of the ethanol extracts, which may indicate that glycerol as a solvent extracted similar compounds as the lower ethanol content used to extract HI3.

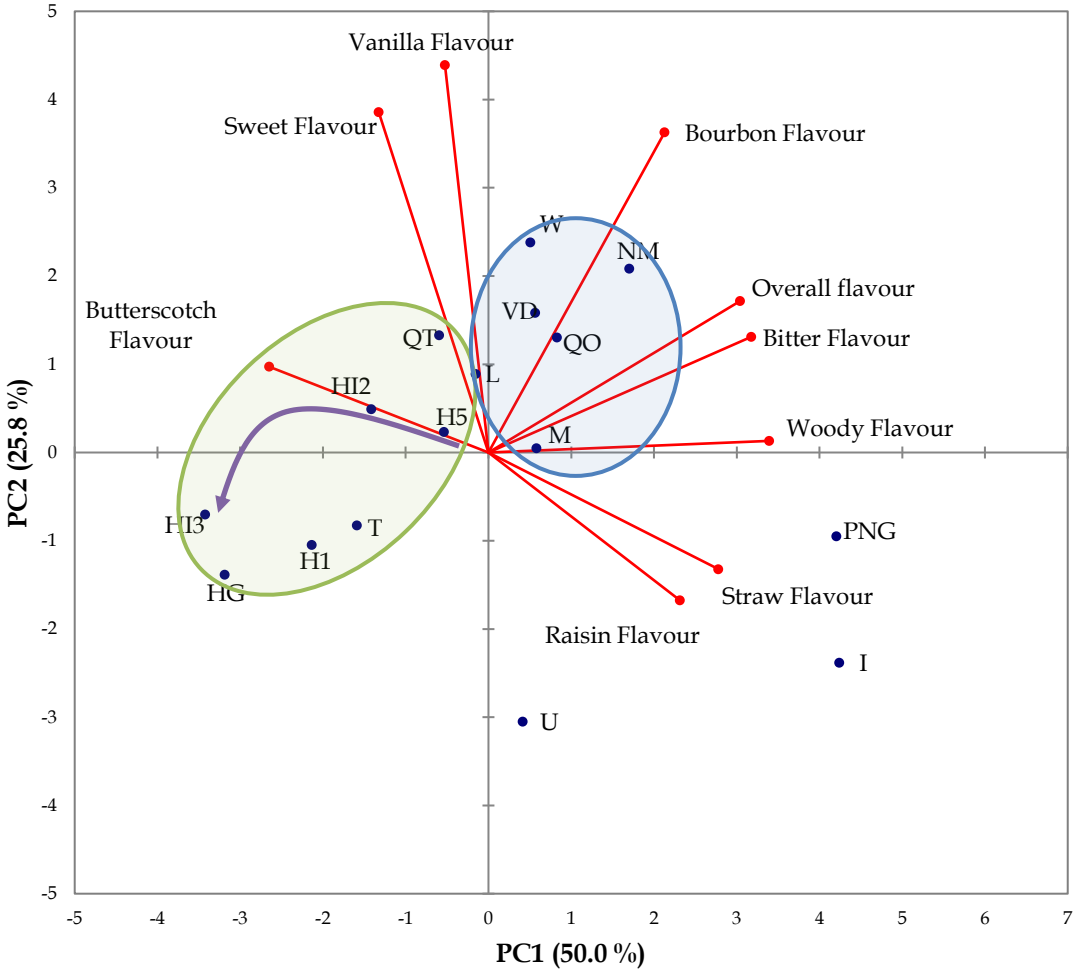


Figure 5.10: Bi-plot of the first two principal components identified through PCA of all flavour attributes for looking at all samples presented to the trained panel. The first component had an eigenvalue of 4.50 and the second component had an eigenvalue of 2.32. The cumulative variation explained by the components was 75.8%. The blue circle surrounds the Madagascar originated extracts and the green circle surrounds the Tonga originated extracts. The vanilla extracts are marked by the blue dots.

The extracts U, PNG and I were separated from the Tonga and Madagascar groups in Figure 5.10, indicating that the extracts were able to be differentiated by flavour based on region of bean growth. These samples were relatively high in straw flavour and raisin flavour, and relatively low in butterscotch flavour, sweet flavour and vanilla flavour. They were moderate in overall flavour, bourbon flavour, bitter flavour and woody flavour.

It was interesting that the PNG extract and W extract were not placed near each other in Figure 5.10 even though they both contained Papua New Guinea vanilla beans. The difference could be due to the ratio of Madagascar vanilla beans in the W extract, resulting in it having a more Madagascar-like sensory profile. There could have also been a difference in the processing conditions used, which had an influence on the flavour profile, different to the impact of the growing region or the vanilla bean species.

5.3.8.2 Conclusions on sensory profile of All the Natural Vanilla Extracts

Both the growing region and the flavour extraction solvent were found to affect the aroma and flavour profile of the vanilla extracts. For aroma, the extraction solvent seemed to have a greater influence on differentiating samples from each other. For the flavour, both the growing region and the extraction solvent resulted in clear differentiation between the samples.

5.4 Conclusions and Recommendations

Comparing vanilla extracts produced under the same conditions, but from different growing regions, the trained sensory panel was able to separate the extracts into different growing regions based on aroma and flavour profile. The regions tended to form groups, with the most similar being Tonga and Madagascar in one group and India and Papua New Guinea in the other group. The Uganda extract was more similar to the Tonga/Madagascar group.

For the effects of the extraction solvent, it was found that the higher the ethanol content used to extract the vanilla beans, the higher the intensity for the overall aroma and overall flavour. The extracts tended to be more dominated by the sweet type aromas/flavours as the ethanol was decreased, with the relative strength of the vanilla aroma, artificial fruity aroma, butterscotch flavour and sweet flavour increasing. The glycerol extracted solution had the lowest overall aroma and overall flavour of all the

extracts investigated, indicating that this solvent produced a different vanilla extract to those produced with ethanol.

The commercial samples were able to be differentiated by the trained panel for both aroma and flavour, with all the attributes other than vanilla flavour, butterscotch flavour and straw flavour able to find differences between the extracts. The commercial vanilla extracts tended to be grouped on PCA based on the region that the vanilla beans were grown, with the Tonga extracts and the Madagascar extracts creating clear groupings. W, the extract that contained both Madagascar vanilla beans and Papua New Guinea vanilla beans was closer to the Madagascar group, but positioned on the outside of this group, suggesting that the Papua New Guinea vanilla beans were causing it to have a different sensory profile. This was seen for both aroma and flavour.

When all of the extracts were compared together, it was found that they were grouped primarily based on the region that the vanilla beans were grown in, with clear groupings of Tonga extracts and Madagascar extracts. However, when comparing the effect of the growing region and the extraction solvent, the dominant factor could not be determined. Both influenced the final aroma and flavour profile of the vanilla extracts.

6. Volatile Analysis of Vanilla Extracts using GCMS and Correlations with Sensory Characteristics

6.1 Introduction

Vanilla beans must be cured for several months after harvest to develop the full flavours that define this valuable spice (Cameron, 2011). A flavour extract is typically extracted from the cured beans using ethanol (Havkin-Frenkel and Belanger, 2011). One way to monitor changes in flavour is the use of instrumentation such as gas chromatography mass spectrometry (GCMS), which allows for the identification and quantification of the compounds within vanilla extracts. High pressure liquid chromatography (HPLC) can also quantify the compounds but cannot identify them without the use of an external standard or with an attached mass spectrometer.

Sensory analysis can be used to provide a descriptive profile of a flavour extract (Chapter 5). However, it tends to be more time intensive and variable than instrumental measures, with people being used as the instruments (Kemp *et al.*, 2009). If it were known which compounds within the vanilla extract were providing the sensory characteristics, it would be possible to monitor changes during the processing of the vanilla beans to ensure that the same sensory profile was maintained for subsequent products.

Over 500 chemical compounds have been identified and quantified in vanilla extracts (Archer, 1989; Cicchetti and Chaintreau, 2009b; Toth *et al.*, 2010; Takahashi *et al.*, 2013a), and numerous studies have investigated the sensory profile of vanilla extracts (Hariom *et al.*, 2006; Van Dyk *et al.*, 2010; Naidu *et al.*, 2012; Takahashi *et al.*, 2013a; Takahashi *et al.*, 2013b) however there has only been one published study on the correlation between sensory profiles and individual compounds identified by instrumental techniques. Brunschwig *et al. (*2016) compared a range of Tahitian vanilla extracts, of the *Vanilla tahitensis* variety with Madagascan *Vanilla planifolia* vanilla extract using GCMS and QDA sensory analysis. They found that there were significant differences in the volatile content of the vanilla beans depending on where the beans were grown as well as the species and variety of the vanilla bean. Brunschwig *et al. (*2016) reported correlations between the volatile compounds and the nine aroma attributes rated by the sensory panel, but interactions between the compounds made it

hard to determine any clear relationships. Much previous research has also looked at the correlation between sensory and analytical instrumental data on other food products such as fruit smoothies (Keenan *et al.*, 2012), lager (Techakriengkrai *et al.*, 2006) and olive oil (Borras *et al.*, 2016) with a high degree of success, so an application to vanilla extract should provide useful information for future use.

The specific aims of this chapter were:

- To identify and quantify the concentration of potential aroma and flavour compounds in vanilla extracts using GCMS
- To investigate correlations between the sensory attributes described by a trained sensory panel and the types and concentrations of aroma and flavour compounds measured with GCMS or HPLC using PLS (Partial Least Squares)

6.2 Materials and Methods

6.2.1 Analysis of Vanilla Extracts by GCMS and Trained Sensory Panel

The vanilla extracts presented in Table 6.1 were analysed by GCMS using the methods in *Section 3.2.1* as well as by the trained sensory panel using the methods in *Section 3.1* (sensory results presented in Chapter 5). All samples analysed by GCMS were injected in quadruplicates and the concentrations of the compounds taken as an average of all four injections.

6.2.1.1 Standards for Quantification of Key Compounds Identified by GCMS in Vanilla Extracts

Table 6.2 provides a list of the 22 chemical standards used to quantify key compounds identified in vanilla extracts with the GCMS. These compounds were chosen as they were present in more than three of the vanilla extracts, they were reported to have an aroma in literature or they were one of the largest ten peaks in the GCMS chromatograms of the vanilla extracts.

Table 6.1: Details of 15 vanilla extracts analysed by GCMS and trained sensory panel. The abbreviations will be used throughout the chapter to denote the various vanilla extracts.

Sample Name	Abbreviation	Country of Bean Origin	Country of Manufacture
A. Commercial Vanilla Extracts			
Heilala 5-Fold Extract	H5	Tonga	New Zealand
Heilala Infusion 2	HI2	Tonga	New Zealand
Heilala Infusion 3	HI3	Tonga	New Zealand
Heilala Single Fold Extract	H1	Tonga	New Zealand
Queen Finest Vanilla Extract with Seeds - Vava'u	QT	Tonga	Australia
LorAnn Gourmet Pure Madagascar Bourbon Vanilla Extract	L	Madagascar	USA
Nielson Massey Madagascar Bourbon Vanilla Extract	NM	Madagascar	USA
Queen Natural Organic Vanilla Essence-Extract	QO	Madagascar	Australia
Virginia Dare Pure Vanilla Extract	VD	Madagascar	USA
Whittington's Natural Vanilla Extract – Double Strength	W	Madagascar and Papua New Guinea	Australia
B. Laboratory Vanilla Extracts			**Vanilla Bean Supplier**
India	I	India	Beanilla.com
Madagascar	M	Madagascar	Heilala Vanilla Ltd.
Papua New Guinea	PNG	Papua New Guinea	VanillaproductsUSA
Tonga	T	Tonga	Heilala Vanilla Ltd.
Uganda	U	Uganda	Beanilla.com

Table 6.2: List of reference standards used for GCMS and HPLC analysis of natural vanilla extracts, with supplier and purity.

Compound Number	Compound IUPAC Name	Common Name	Solubility[1,2,3]		Melting Point (°C)[1,3]	Supplier	Purity
			Water	Ethanol			
(7)	2-methoxy-4-methyl phenol	Creosol	Slight	Soluble	5.5	Sigma-Aldrich	≥98%
(4)	2-methoxyphenol	Guaiacol	15 g/L	Miscible	28	Acrōs organics	>99%
	3,4-dimethoxybenzaldehyde		Slightly soluble in hot water	Soluble	40-43	Aldrich	99%
(5)	3-hydroxy-2-methyl-4H-pyran-4-one	Maltol	Sparingly soluble	Soluble	161-162	Sigma-Aldrich	≥99%
(12)	3-hydroxy-4-methoxybenzaldehyde	Isovanillin	Slight	Soluble	113-116	Aldrich	≥95%
(6)	3-methyl-2-furoic acid		Soluble	Soluble	133-137	Aldrich	97%
(15)	4-hydroxy-3,5-dimethoxybenzaldehyde	Syringaldehyde	Insoluble	9.5 g/L	110-1113	Aldrich	98%
(9)	4-hydroxy-3-benzaldehyde	Vanillin	10 g/L	Soluble	81-83	Sigma	>97%
	4-hydroxy-3-benzyl alcohol		Hot water soluble	Soluble	110-117	Aldrich	98%
(13)	4-hydroxy-3-methoxy acetophenone	Apocynin, Acetovanillone	5 g/L	Soluble	115	Sigma-Aldrich	≥98%
(3)	4-hydroxy-3-methoxybenzoic acid	Vanillic acid	1.5 g/L	Soluble	210-213	Fluka	≥97.0%
(11)	4-hydroxy-3-methoxybenzyl alcohol	Vanillyl alcohol	Insoluble	Soluble	113	Fluka	>98%
(8)	4-hydroxybenzaldehyde		8.45 g/L	Soluble	112-116	Aldrich	>98%
(14)	4-hydroxybenzoic acid		5 g/L	Soluble	214.5	Aldrich	>99%
(10)	4-methoxybenzoic acid	p-anisic acid	530 g/L (37°C)		185	Aldrich	>99%
(2)	4-methyl phenol	p-cresol	24 g/L	Free	35.5	Sigma-aldrich	>99%
	5-(hydroxymethyl)furfural		Soluble	Soluble	30-34	Aldrich	>99%
	Benzaldehyde		Slight	Soluble	-26	Sigma-Aldrich	≥99.5%

Compound Number	Compound IUPAC Name	Common Name	Solubility[1,2,3]		Melting Point (°C)[1,3]	Supplier	Purity
			Water	Ethanol			
	Benzoic acid		2.9 g/L	Soluble	122.4	Sigma-Aldrich	≥99.5%
	Ethyl 4-hydroxy-3-methoxyphenyl acetate	Ethyl homovanillate	0.89 g/L	Soluble	314-315	Aldrich	97%
(1)	Hexanoic acid		10 ml/L	Soluble	-3.4	Aldrich	≥99.5%
	Methyl benzoate		Insoluble	Miscible	-15	Sigma-Aldrich	≥99.5%
	Pentanal	Valeraldehyde	Slight	1 L/L	-60	Sigma-Aldrich	≥97.5%

[1] Sigma-Aldrich (2017)
[2] Burdock (2009)
[3] O'Neil (2006)

The reference standards were diluted with either water or ethanol, as appropriate, to produce standard curves for each compound as described in *3.2.1*. The standard curves were used to determine the concentration of the reference compounds in the vanilla extracts as analysed by GCMS. The concentration range of the standard curves was selected based on Toth *et al.* (2010). If the compound was not identified in any of the extracts, based on the retention time of the most concentrated dilution, the full standard curve was not produced. Standard curves are presented in Figures A1 to A15 in Appendix 2.

The concentration of vanillin in the vanilla extracts exceeded the detection limits of the GCMS, and saturated the detector for eight of the fifteen extracts, so the concentration was determined by HPLC, as per *3.2.2*. Three of the vanilla extracts (QT, HI3 and VD) were compared for vanillin concentration as determined by HPLC and GCMS to check that the two methods were providing the same results.

6.2.2 Aroma testing of the reference chemicals

The 15 chemical reference standards (Table 6.2) were presented to four of the trained panellists for aroma descriptions. The standards were undiluted and were presented in 100ml brown glass jars with a plastic lid. For the liquid samples 1 ml of undiluted reference standard was placed in the bottom of the brown glass jar and for the powder sample, 1 g of solid was placed into the brown glass jar. Seven compounds of the 22 were only soluble in ethanol, which was found to mask the aroma of the compound, thus necessitating the use of undiluted samples for aroma analysis.

The panellists were presented with the reference standards in individual sensory booths with both the samples and the room temperature at 20±2^{o}C. The samples were separated into three blocks, which were presented over three sessions, with duplicates presented to each panellist. The panellists received the samples one at a time, in a randomised order within each block. A minimum of two minutes was given between samples, and the coffee powder (Chapter 5) was provided as a palate cleanser. They were asked to carefully sniff the headspace of the sample and then describe the aroma of the reference standard.

6.2.3 Sensory analysis

The sensory panel ratings of aroma and flavour from *Chapter 5 Sensory Analysis of Vanilla Extracts* was used for this section for the vanilla extract samples as per Table 6.1.

6.2.4 Correlation of Sensory to GCMS Data

Both the sensory ratings and the GCMS concentrations of compounds were analysed using XLStat (Version 2015.4.01.20270, Microsoft, USA) using a partial least squares (PLS) regression. The concentration of the reference compounds was standardised based on the vanillin concentration, adjusting the concentration of all extracts to 1.5 mg/ml of vanillin, the equivalent of a single fold vanilla extract. This matched with the sensory data, in which the samples were all diluted to the same vanillin concentration for presentation to the panellists for testing.

6.3 Results and Discussion

6.3.1 GCMS Analysis of Vanilla Extracts

6.3.1.1 Compounds in Vanilla Extracts

All 15 vanilla extracts were analysed by GCMS and the peaks identified using the NIST 2008 Library. The chromatograms for each vanilla extract are presented in Figures 6.1a, 6.1b, 6.1c and 6.2. From the identified peaks, any peaks that contributed more than 0.5% of the total peak area were considered for further investigation. The list was then further narrowed down based on comparisons of identifications of the peaks between the different vanilla extracts, searching literature for other occurrences of the compounds and checking if they were likely breakdown products from other compounds within the vanilla extracts or from the GC column degrading. From this reduced list of compounds, the 22 most concentrated were purchased and tested for concentration in the vanilla extracts. The details of the reference standards are in Table 6.2.

Each vanilla extract was found to have a different volatile profile based on the appearance of the chromatogram (Figure 6.1a - c and Figure 6.2). QT and W were dominated by the peak at 26.9 minutes, identified by the MS library as vanillin, the most concentrated compound in vanilla extracts. HI3, H1 and L had relatively few volatile peaks compared to other extracts. The volatile content would be affected by a

range of different factors, such as the growing conditions, curing methods, flavour extraction method and vanilla bean species. Without being able to control these factors, the exact cause of the variation cannot be identified.

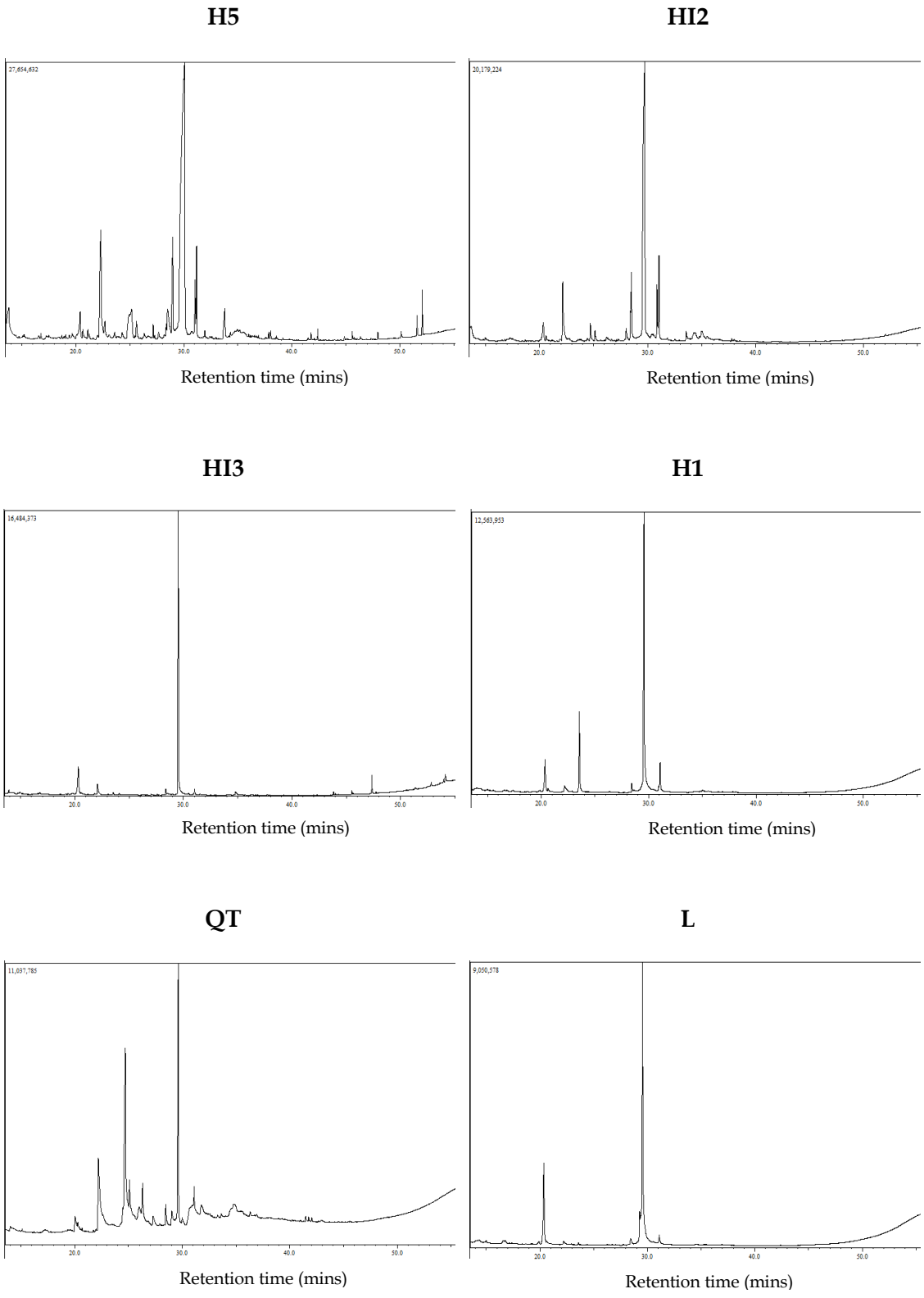


Figure 6.1a: GCMS chromatograms for each of the 15 vanilla extracts studied in this chapter.

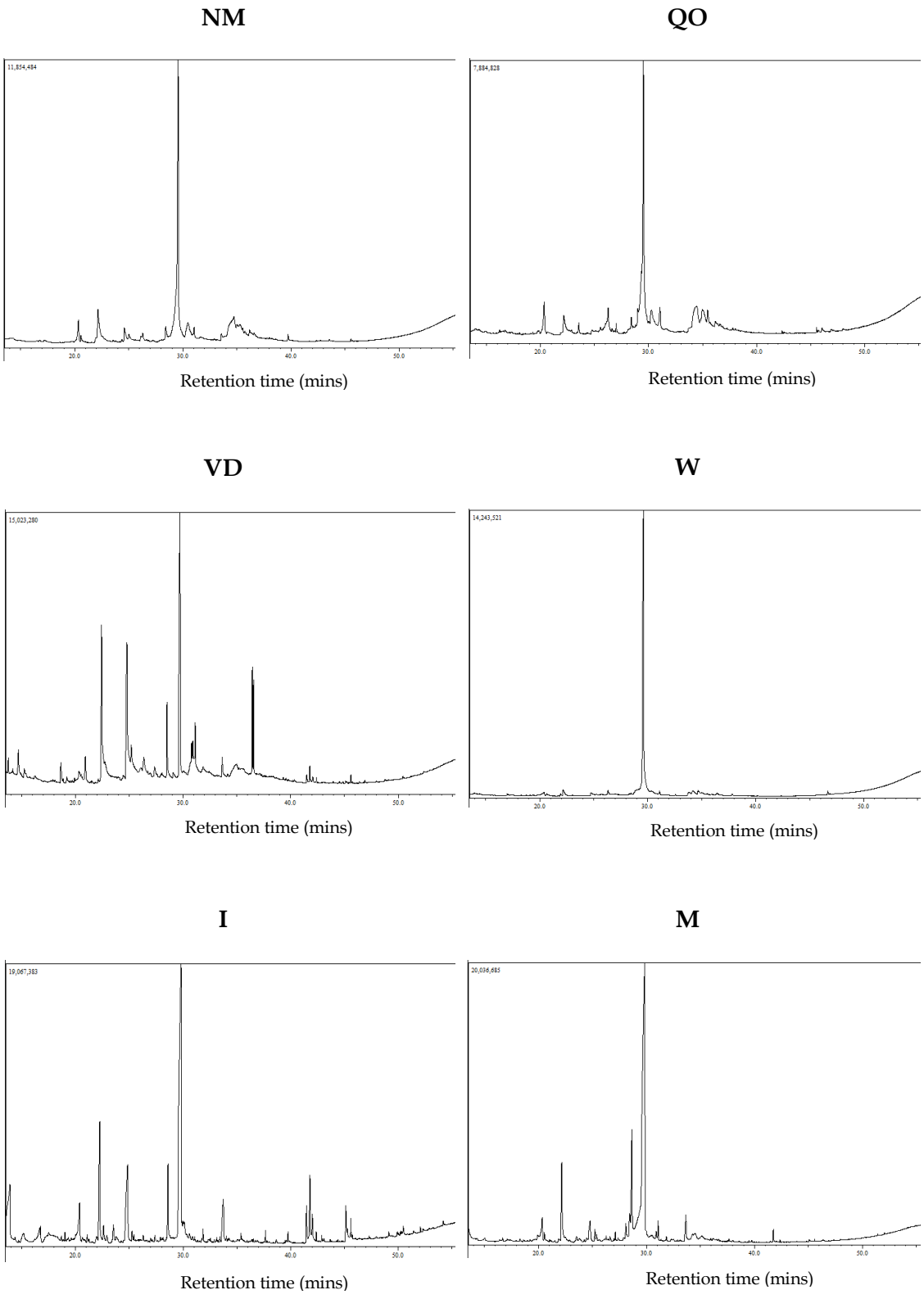


Figure 6.1b: GCMS chromatograms for each of the 15 vanilla extracts studied in this chapter.

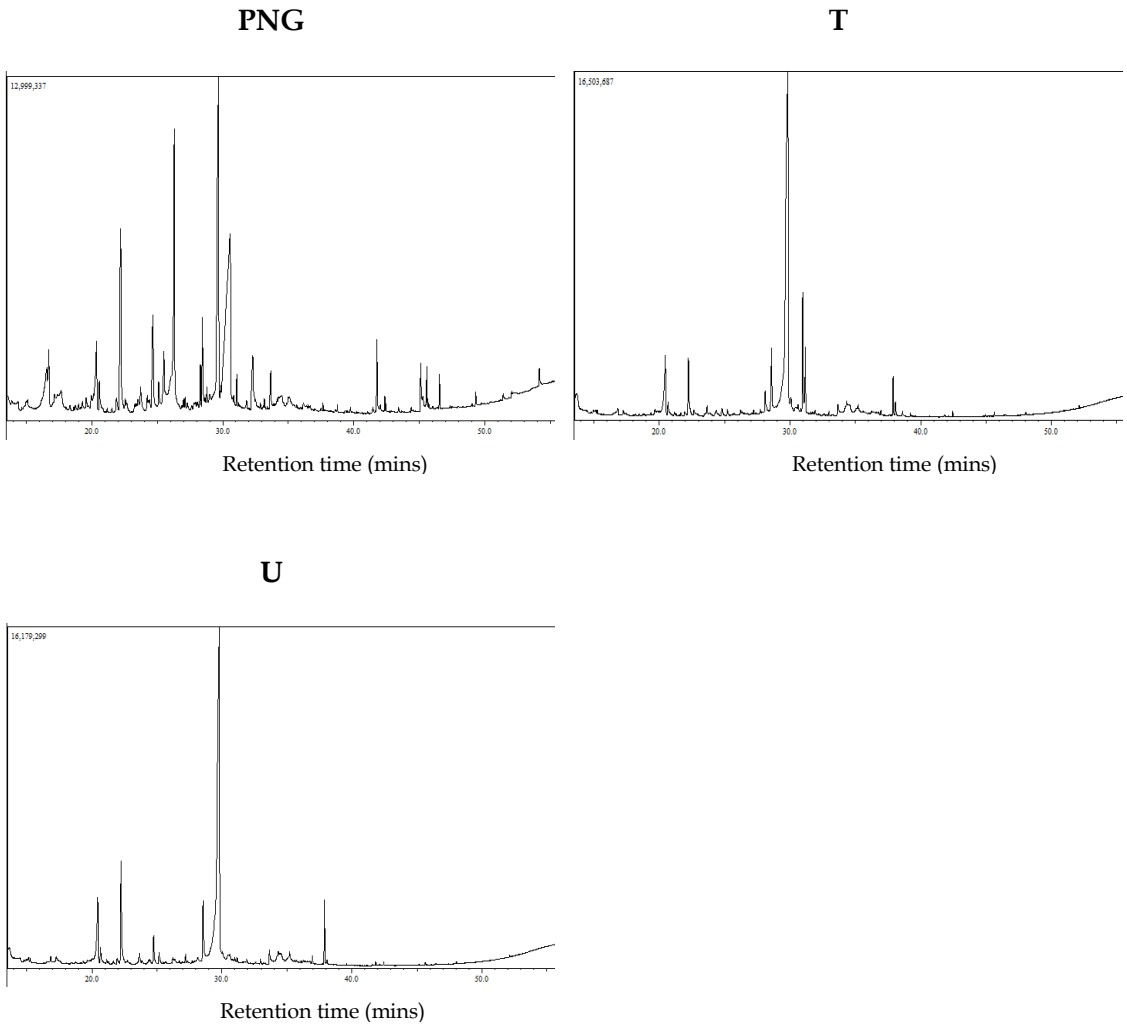


Figure 6.1c: GCMS chromatograms for each of the 15 vanilla extracts studied in this chapter.

6.3.1.2 Quantification of Compounds in Vanilla Extracts

All of the samples tested by sensory analysis, apart from HG, were analysed using GCMS and HPLC to determine the concentration of the 22 compounds in Table 6.2. The retention time of the glycerol in HG meant that the peak from this was on top of many of the peaks of interest (up to 27 min retention time) so useful information could not be collected. The concentration of each reference standard in each vanilla extract is presented in Table 6.4. The compounds 3,4-dimethoxy benzaldehyde, ethyl homovanillate, valeraldehyde, 5-hydroxymethyl furfural, benzoic acid, benzaldehyde were not found in any of the vanillas although they had been found in vanilla extracts previously (Toth *et al.*, 2010). They had been identified in the samples using mass spectra but the retention times did not match, therefore the compounds were not present. Of the compounds quantified, 3-methyl-2-furoic acid and isovanillin had not been identified in vanilla extract or products before. All others have been found in at

least one vanilla product previously (Toth *et al.*, 2010), detailed in Appendix 1, Table A1.

The compound present in all vanilla extracts at the highest concentration was vanillin, typically considered the main flavour compound in vanilla (Havkin-Frenkel and Belanger, 2011). The second highest concentration compound quantified was 3-methyl-2-furoic acid (6), with concentrations ranging from 0.017 to 1.19 mg/ml in all the vanilla extracts (Table 6.4). This compound had not been identified in vanilla extracts previously although a similar compound, 2-furoic acid was found at concentrations between 0.02 and 0.19 mg/kg in Madagascan and Ugandan vanilla bean extracts (Zhang and Mueller, 2012). Vanillic acid (3) was found in all of the samples, ranging from 0.021 mg/ml in W to 0.557 mg/ml in I (Table 6.4). As vanillin and vanillic acid are easily converted from one to the other, a high concentration of both was expected. Some compounds, such as 4-hydroxybenzoic acid (14), were identified more clearly with HPLC than GCMS (data not shown); the concentration of these compounds was too low to be detected by GCMS in some of the samples and the 4-hydroxybenzoic acid peak on the GCMS was not well separated from other compounds with similar retention times.

Table 6.3: Concentration of compounds in vanilla extracts, as determined by GCMS and HPLC. Samples were analysed in quadruplicate. Rt refers to the retention time (minutes) in the GMCS. N/D refers to compounds that were not detected.

			Concentration (mg/ml)													
No.	**Compound Name**	**Rt**	**H5**	**HI2**	**HI3**	**H1**	**QT**	**L**	**NM**	**QO**	**VD**	**W**	**I**	**M**	**PNG**	**T**	**U**
(1)	Hexanoic acid	16.95	0.017	0.005	0.003	N/D	0.001	0.003	0.002	0.003	N/D	N/D	0.108	0.014	0.111	0.008	0.014
(2)	p-cresol	20.1	0.015	N/D	0.003	N/D	N/D	N/D	0.001	N/D	N/D	N/D	N/D	0.010	0.024	N/D	N/D
(3)	Vanillic acid	20.2	0.723	0.245	0.117	0.129	0.098	0.121	0.118	0.114	0.124	0.021	0.557	0.414	0.143	0.322	0.282
(4)	Guaiacol	20.25	0.213	0.160	0.103	0.087	0.078	0.181	0.064	0.100	0.043	0.009	0.222	0.174	0.285	0.221	0.182
(5)	Maltol	21.05	0.036	0.000	N/D	N/D	0.011	0.001	0.003	N/D	0.038	N/D	0.010	0.007	0.007	0.012	0.007
(6)	3-methyl-2-furoic acid	22.15	1.193	0.100	0.063	0.045	1.297	0.017	0.125	0.090	0.581	0.038	0.785	0.630	0.746	0.280	0.702
(7)	Creosol	23.35	0.050	0.006	0.003	0.110	0.039	0.002	N/D	0.011	0.008	N/D	N/D	0.022	N/D	0.016	0.021
(8)	4-hydroxybenzaldehyde	28.4	0.511	0.043	0.008	0.014	0.127	0.014	0.033	0.039	0.138	0.004	N/D	0.163	N/D	0.112	0.196
(9)	Vanillin	29.1	10.397	3.666	1.693	1.833	1.203	1.392	1.147	0.963	1.354	3.314	2.404	4.597	1.233	4.342	3.383
(10)	p-anisic acid	29.95	0.037	0.047	N/D	0.063	0.092	N/D	N/D	0.006	0.023	0.029	N/D	N/D	N/D	0.329	0.139
(11)	Vanillyl alcohol	30.8	0.215	0.017	0.002	0.004	N/D	N/D	0.024	N/D	N/D	N/D	0.047	0.044	N/D	0.350	0.028
(12)	Isovanillin	31.62	0.299	0.013	0.005	0.025	0.254	0.008	0.011	0.017	0.051	0.003	0.013	0.030	0.041	0.081	0.013
(13)	Acetovanillone	31.75	0.137	N/D	N/D	N/D	N/D	N/D	N/D	0.001	N/D	N/D	0.050	0.016	0.042	0.007	0.007
(14)	4-hydroxybenzoic acid	32.15	0.171	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	0.073	0.037	0.396	N/D	0.014
(15)	Syringaldehyde	35.75	N/D	N/D	N/D	N/D	N/D	N/D	0.047	0.153	N/D	N/D	0.017	0.013	0.019	0.005	0.009

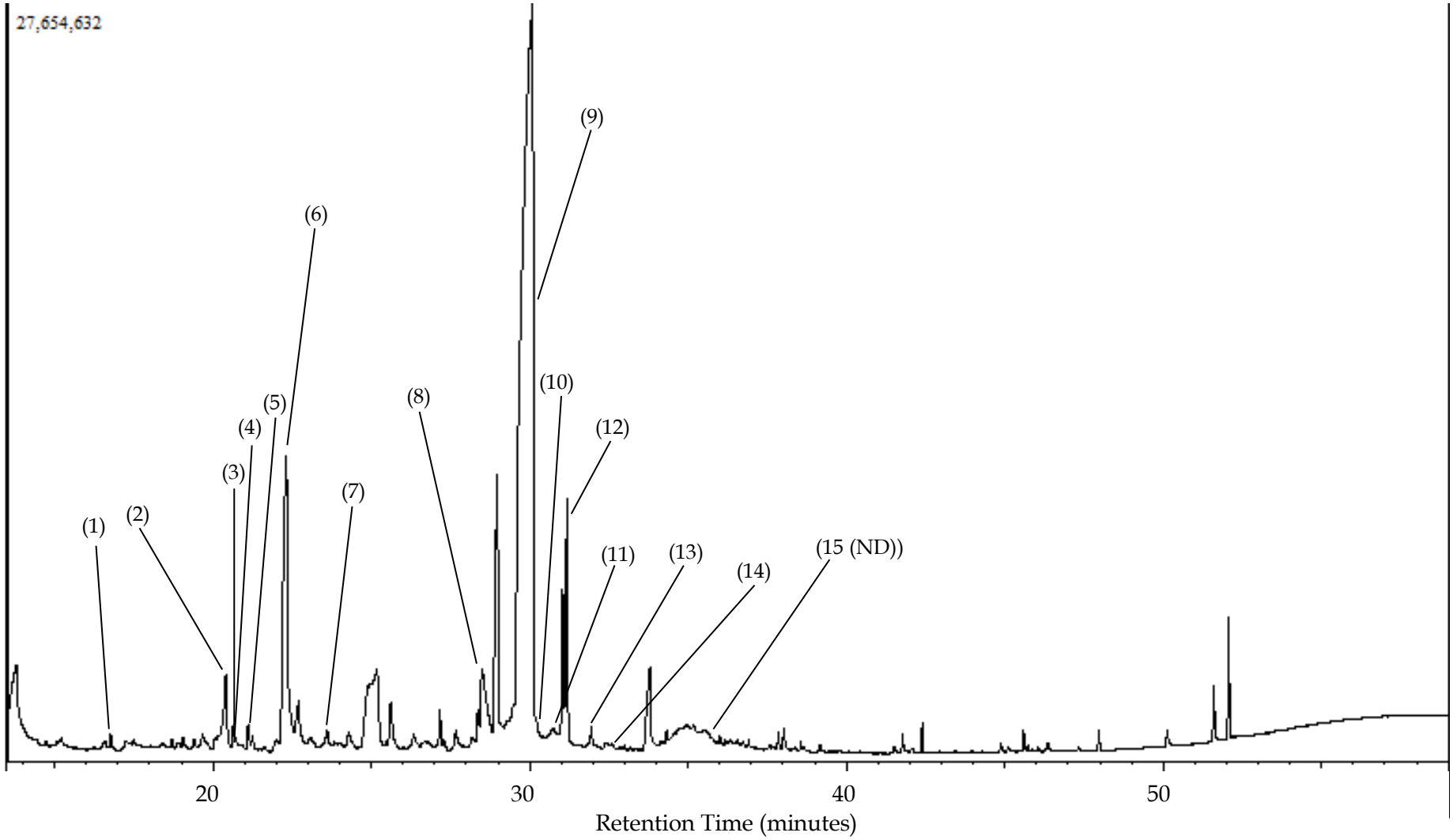


Figure 6.2: GCMS chromatogram of Heilala Five-Fold Extract (H5). Peaks corresponding to reference standards are identified using the numbering system from Table 6.2. Compound 15 was not detected (ND) in this sample, but its relative retention time is labelled for visualisation.

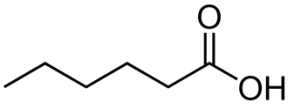
Table 6.4: Summary of countries of origin previously found to contain quantified compounds (Toth *et al.* 2010).

Compound Name	Country of Origin Identified in Previously
Hexanoic acid	M, Mex, U
p-cresol	M, Ja, Mex, U
Vanillic acid	All
Guaiacol	M, C, I, Io, Mex, PNG, T, U
Maltol	All
3-methyl-2-furoic acid	N/I
Creosol	M, Mex, U
4-hydroxybenzaldehyde	All
Vanillin	All
p-anisic acid	M, H, I, Mex, PNG, Ta
Vanillyl alcohol	M, I, Mex, PNG, Ta, T, U
Isovanillin	N/I
Acetovanillone	M
4-hydroxy benzoic acid	B, M, CR, J, Mex, PNG, Ta, T, U
Syringaldehyde	M, I, U

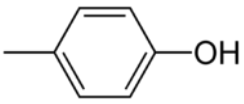
Abbreviations in the Table; B – Bali, M – Madagascar, C – Comoros, CR – Costa Rica, H – Hawaii, I – India, Io – Indonesia, J – Jamaica, Ja – Java, Mex – Mexico, PNG – Papua New Guinea, Ta – Tahiti, T – Tonga, U – Uganda.

As can be seen in Figure 6.3, 11 of the 15 compounds had molecular structures containing phenol rings as the main carbon component, and a range of functional groups attached around this central ring. Only hexanoic acid (1) had a linear carbon chain with six carbons.

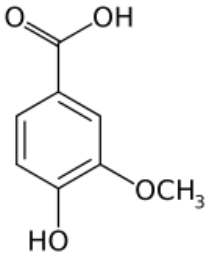
It was possible that the compounds with similar structures i.e. phenol rings, were formed concurrently in the biosynthetic pathway which produced vanillin during the curing process. A number of biosynthetic pathways have been proposed (Ranadive, 1992; Knorr *et al.*, 1993; Funk and Brodelius, 1994; Kanisawa *et al.*, 1994; Gallage *et al.*, 2014; Kundu, 2017; Yang *et al.*, 2017) which allowed for the formation of vanillin from precursors (glucovanillin, vanillic acid, ferulic acid and 3,4-dihydroxybenzaldehyde) identified in the green vanilla beans. The pathways for these are presented in *Section 2.5*. A range of reactions occurred in the proposed pathways, with over 20 enzymes reported in previous studies (Ranadive, 1992; Knorr *et al.*, 1993; Funk and Brodelius, 1994; Kanisawa *et al.*, 1994; Gallage *et al.*, 2014; Kundu, 2017; Yang *et al.*, 2017). Some studies concluded that the exact pathway was unclear, and further research was needed (Dignum *et al.*, 2001; Yang *et al.*, 2017).



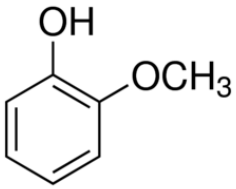
(1) Hexanoic Acid



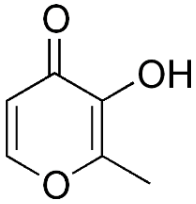
(2) p-cresol



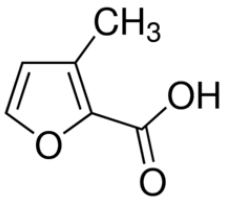
(3) Vanillic acid



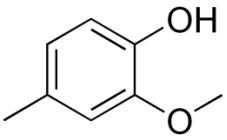
(4) Guaiacol



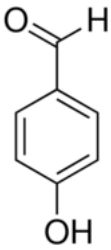
(5) Maltol



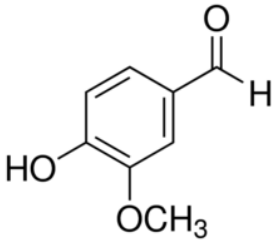
(6) 3-methyl-2-furoic acid



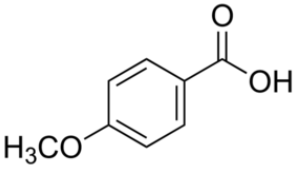
(7) Creosol



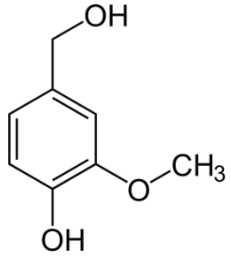
(8) 4-hydroxybenzaldhyde



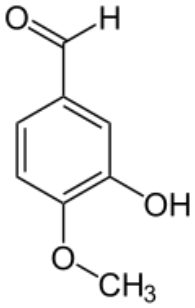
(9) Vanillin



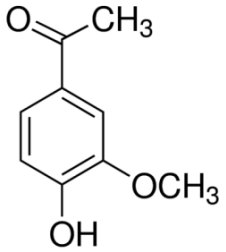
(10) p-anisic acid



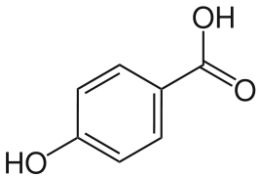
(11) Vanillyl alcohol



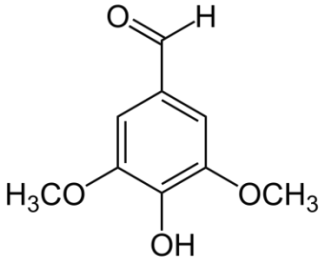
(12) Isovanillin



(13) Acetovanillone



(14) 4-hydroxybenzoic acid



(15) Syringaldehyde

Figure 6.3: Molecular structures of the confirmed reference compounds quantified in the vanilla extract samples. Numbers correspond to coding given in Table 6.3.

Of the 15 compounds identified in the vanilla extracts, 13 compounds had been found in vanilla extracts previously (Table A1, Appendix). Table 6.4 shows the regions that these compounds have been found in before, which confirms the findings found in this research.

With these differences both noted in the current study and in previous studies, there are clear differences in vanilla extracts and the chemical composition thereof based on where the vanilla beans were grown (Table 6.4). This same difference was noticed in Chapter 5, the sensory analysis of the vanilla extracts. Therefore, the logical next step in the data analysis was to compare the sensory data with the GCMS data to determine if there were any compounds that characterised each growing region and what sensory characteristics these introduced to the vanilla extract.

6.3.2 Correlation of Sensory Profiles and Concentration of Key Compounds in Vanilla Extracts

6.3.2.1 Aroma Description of Quantified Compounds

All of the reference chemical compounds were assessed by four of the sensory panel members to determine the aroma descriptors for each compound. Flavour was not investigated as the reference standards were analytical grade and not food grade. The panellists were asked to describe the aroma of each descriptor (Table 6.5). Descriptions of the reference standards had also been reported in literature; the details are listed in Table 6.6.

Table 6.5: Descriptions of the aroma of the 15 reference chemical compounds, as per the trained sensory panel.

	Compound Name	Aroma Description
(1)	Hexanoic acid	Ammonia, burnt, cat pee, very ripe soft cheese, concentrated dust, dirty clothes, rotting food waste, waxy.
(2)	p-cresol	Adhesive, chemical, chemical-based solvent, industrial cleaning agent, phenol, plastic, soapy, vinegar-like, vinyl.
(3)	Vanillic acid	Dusty, flowery, hot-cross buns, stale icing sugar, sweet, spicy, talc-type perfume, talc powder.
(4)	Guaiacol	Bitumen, chemical solvent, coal, tar, tree bark, woody (pine, resin).
(5)	Maltol	Anti-septic, artificial sweetener, burnt, chemical (slight), medicinal, phenol, sherbet, sweet, toffee.
(6)	3-methyl-2-furoic acid	Sharp acidic, caramel, candyfloss, lemon, concentrated meaty/bacon, sour, sherbet.
(7)	Creosol	Barbecue, coal, creosote, stale/rotting leaves, smoky, tar.
(8)	4-hydroxybenzaldehyde	Baking soda, chemical, dusty, medicinal, phenol, salt, slightly sweet, soapy, tea leaves.
(9)	Vanillin	Fruity sweet, generic floral perfume, sweet, vanilla.
(10)	p-anisic acid	Acidic, baking soda, sourdough bread, cardboard, cheese, milk, musty, parmesan, powdery, sweet.
(11)	Vanillyl alcohol	Candyfloss, old dried milk/coffee, floral, fudge, soapy, sweet, toffee.
(12)	Isovanillin	Candy, smoky with hint of bacon, soapy, sweet, woody.
(13)	Acetovanillone	Bacon, barbecue, spent barbecue charcoal, damp, lemon, lime, pepper, sharp, smoky, sweet, tangy, Thai food.
(14)	4-hydroxybenzoic acid	Ash, blue cheese, KCl (pool chemical), chocolate, cocoa, lemon, sweet, washing powder.
(15)	Syringaldehyde	Chalk dust, cheese, earthy, dry dust, powdery, savoury.

Table 6.6: Aroma descriptions and detection thresholds of compounds quantified by GCMS from a literature search.

	Compound Name	Aroma Description	Flavour Description	Detection Threshold (Burdock, 2009)	References
(1)	Hexanoic acid	Sickening, sweaty, rancid, sour, sharp, pungent, cheesy, fatty, unpleasant.	Acrid.	Aroma: 93 ppb to 10 ppm.	Burdock (2009); Palassarou *et al.* (2017); Sanchez-Palomo *et al.* (2017); Zhao *et al.* (2017)
(2)	p-cresol	Characteristic phenol-like odour. Horse stable-like.		Aroma: 55-100 ppb.	Kreissl and Schieberle (2017)
(3)	Vanillic acid	Vanilla-like. Undetectable. Sweet, creamy, vanilla, grape, wine.	Vanilla-like.		Pérez-Silva *et al.* (2006); Burdock (2009)
(4)	Guaiacol	Characteristic sweet odour, slightly phenolic. Phenolic, smoky, spicy, medicinal, vanilla, savoury meaty, woody with bourbon whiskey cask nuance. Chemical, sweet, spicy.	Woody, phenolic, bacon, savoury, smoky, medicinal.	Aroma: 3-31 ppb.	Pérez-Silva *et al.* (2006); Zhao *et al.* (2017)
(5)	Maltol	Caramel-butterscotch odour and in solution has a jam-like odour. Fruity, strawberry aroma in dilute solution.	At 100 ppm: Sweet, caramellic, cotton candy with jam, fruit and berry notes.	Aroma: 29 ppb.	Miyazawa *et al.* (2015); Maimone *et al.* (2017)
(6)	3-methyl-2-furoic acid				
(7)	Creosol	Sweet, spicy, slightly vanilla-like, smoky, phenolic, spicy-clove with floral carnation nuance. Chemical, medicine, leather. Sweaty, burnt.	Somewhat bitter taste, vanilla-like. Sweet, phenolic, spicy, vanilla, medicinal, clove-like, smoky, guaiacol-like with woody phenolic nuance.	Aroma: 90 ppb.	Shu and Shen (2008); Ross *et al.* (2010)

	Compound Name	Aroma Description	Flavour Description	Detection Threshold (Burdock, 2009)	References
(8)	4-hydroxybenz-aldehyde	Faint sweet, woody, balsamic, vanillic, nutty.	Sweet.		Burdock (2009); Ross *et al.* (2010)
(9)	Vanillin	Characteristic, creamy, vanilla-like. Sweet.	Very sweet, vanilla-like, marshmallow, creamy-coumarin, caramellic, with powdery nuance.	Aroma: 29ppb to 1.6 ppm.	Pérez-Silva *et al.* (2006);
(10)	p-anisic acid	Odourless.			(Burdock, 2009)
(11)	Vanillyl alcohol	Mild, sweet, balsamic, vanilla-like.	Sweet, creamy and milky with slightly powdery mouthfeel.		Palassarou *et al.* (2017)
(12)	Isovanillin	Vanilla-like			Burdock (2009)
(13)	Acetovanillone	Mild, balsamic, floral. Vanilla, sweet, honey. Sweet, vanilla notes.	Non-vanillin. Sweet, vanilla, creamy, powdery with balsamic nuance.		Pérez-Silva *et al.* (2006); Palassarou *et al.* (2017)
(14)	4-hydroxybenz-oic acid	Odourless. Phenolic, nutty.	Sweetish taste which becomes acrid and disagreeable.	Aroma: 5 ppm in water.	Palassarou *et al.* (2017)
(15)	Syringaldehyde	Aromatic aldehyde similar to vanillin.		Aroma: >50ppm.	Burdock (2009); Sanchez-Palomo *et al.* (2017)

A number of the compounds were described as sweet by the panellists including vanillic acid, maltol, vanillin, p-anisic acid, vanillyl alcohol, isovanillin, acetovanillone and 4-hydroxybenzoic acid (Table 6.5). Other common descriptors were chemical, bacon/meaty and smoky/barbecue/woody.

By combining the aroma descriptions of the compounds with the GCMS concentrations and the sensory profiles of the vanilla extracts, conclusions about the correlations between the data sets were determined.

6.3.2.2 Partial Least Squares Regression Analysis of GCMS data and Sensory correlations

To investigate how the individual volatile compounds were contributing to produce the aroma and flavour of the natural vanilla extracts, partial least squares regression (PLS) was used. For this regression, all 15 reference standards were compared to the sensory properties of the 15 vanilla extracts characterised with the aroma and flavour sensory profile determined in *Chapter 5*.

PLS analysis is a method by which predictor variables can be analysed to generate a linear regression equation to explain response variables. In the case of this study, the predictor variables are the chemical compounds quantified in the vanilla extracts and the response variables are the intensity scores for the different sensory attributes of the vanilla extracts. If successful, PLS would provide a regression equation which related the quantities of chemical compounds with the sensory attributes.

The regression was able to explain 64% of the variation in the GCMS data and 43.5% of the variation in the sensory data using three components. This is shown in Figure 6.4 with the red representing the sensory data (Y) and the green representing the GCMS data (X). As a breakdown of the different components identified, component 1 explained 33.0% of the variation in the sensory data (Y) and 24.4% of the variation in the GCMS data (X). Component 2 explained 17.2% of the variation in the sensory data and 9.8% of the variation in the GCMS data. Component 3 explained 13.8% of the variation in the sensory data and 9.7% of the variation in the GCMS data.

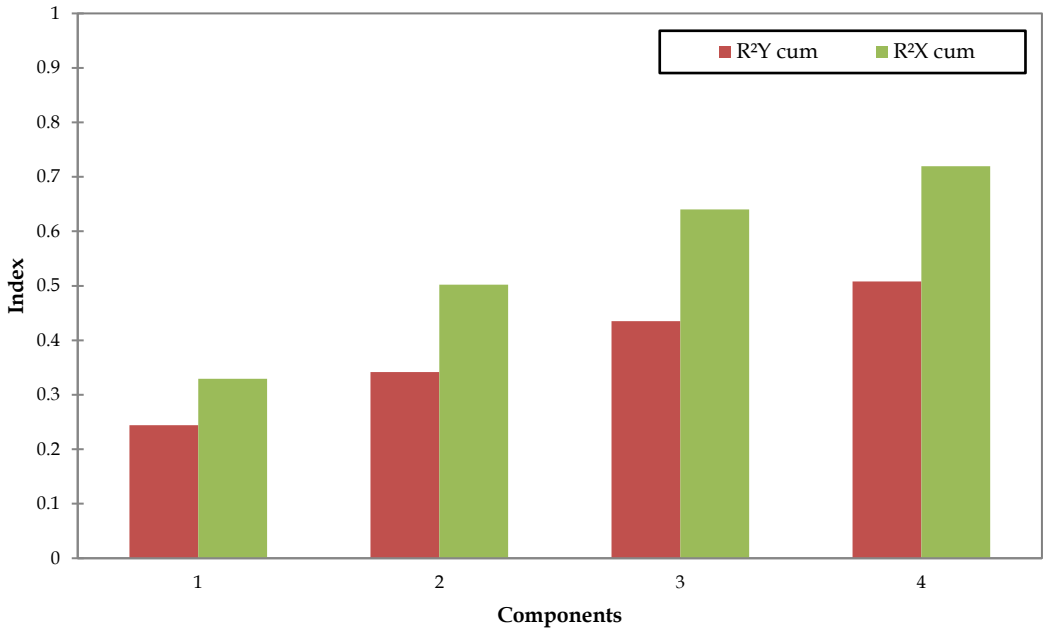


Figure 6.4: PLS model quality by number of components. The sensory data is in red, and the GCMS data is in green and each bar represents the cumulative proportion of variation explained by the increasing number of components (x-axis).

To simplify, each combination of the three dimensions was presented (Figures 6.5, 6.6 and 6.7). The correlations observed between the sensory data and the GCMS compounds were summarised into Table 6.7.

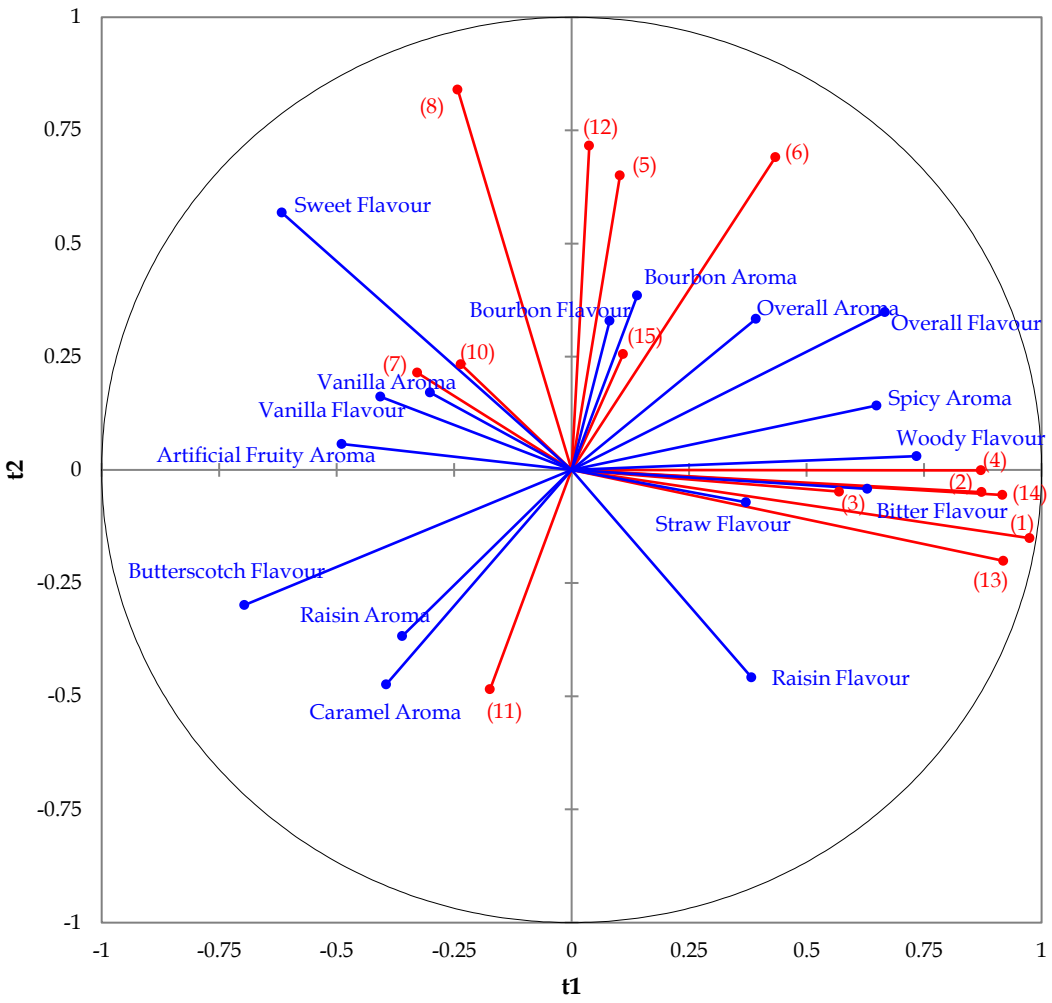


Figure 6.5: Bi-plot of components t1 and t2 for PLS of 15 reference chemical compounds (red) and 15 sensory characteristics (blue) used to describe 15 natural vanilla extracts. The combination of components 1 and 2 (t1 and t2) explain 50.2% of the variation in the sensory data and 34.2% of the variation in the GCMS data.

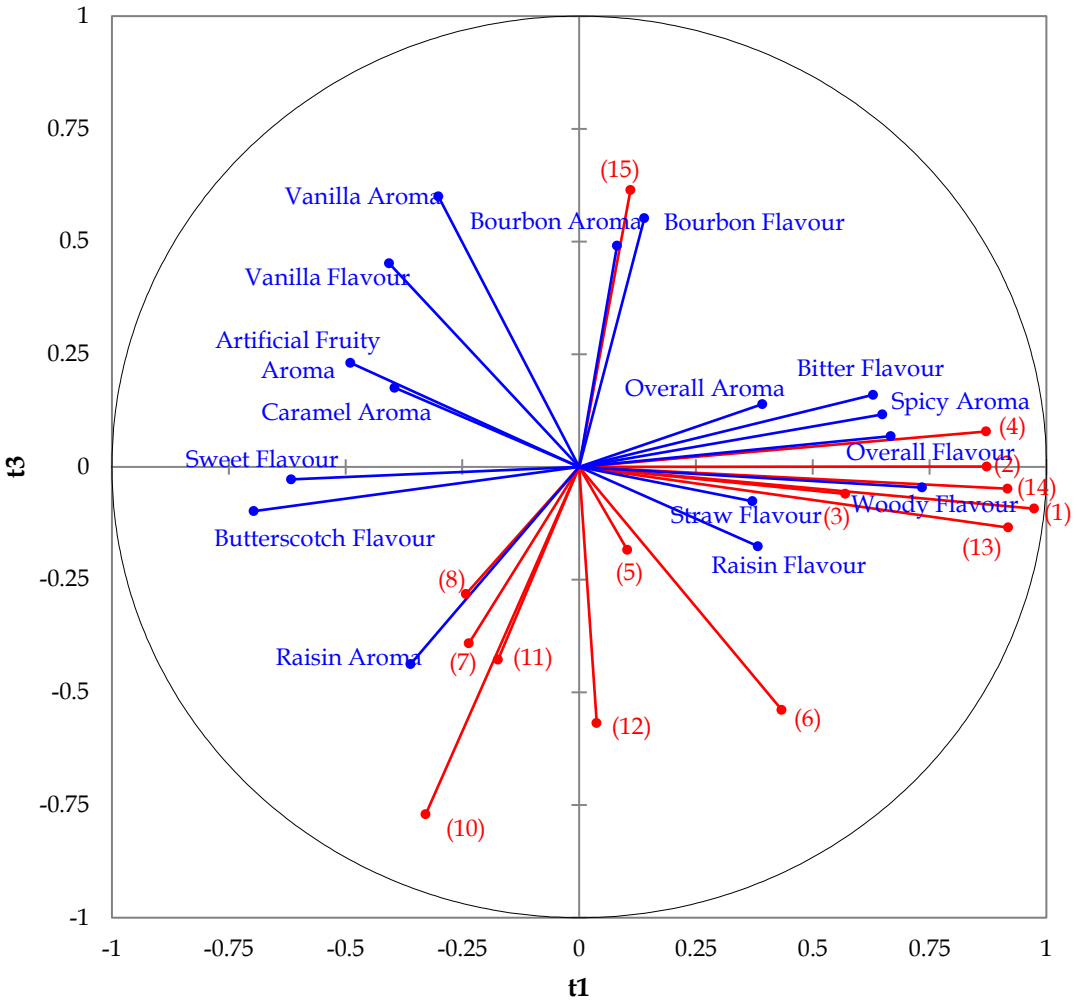


Figure 6.6: Bi-plot of components t1 and t3 for PLS of 15 reference chemical compounds (red) and 15 sensory characteristics (blue) used to describe 15 natural vanilla extracts. The combination of components 1 and 3 explained 46.8% of the variation in the sensory data and 34.1% of the variation in the GCMS data.

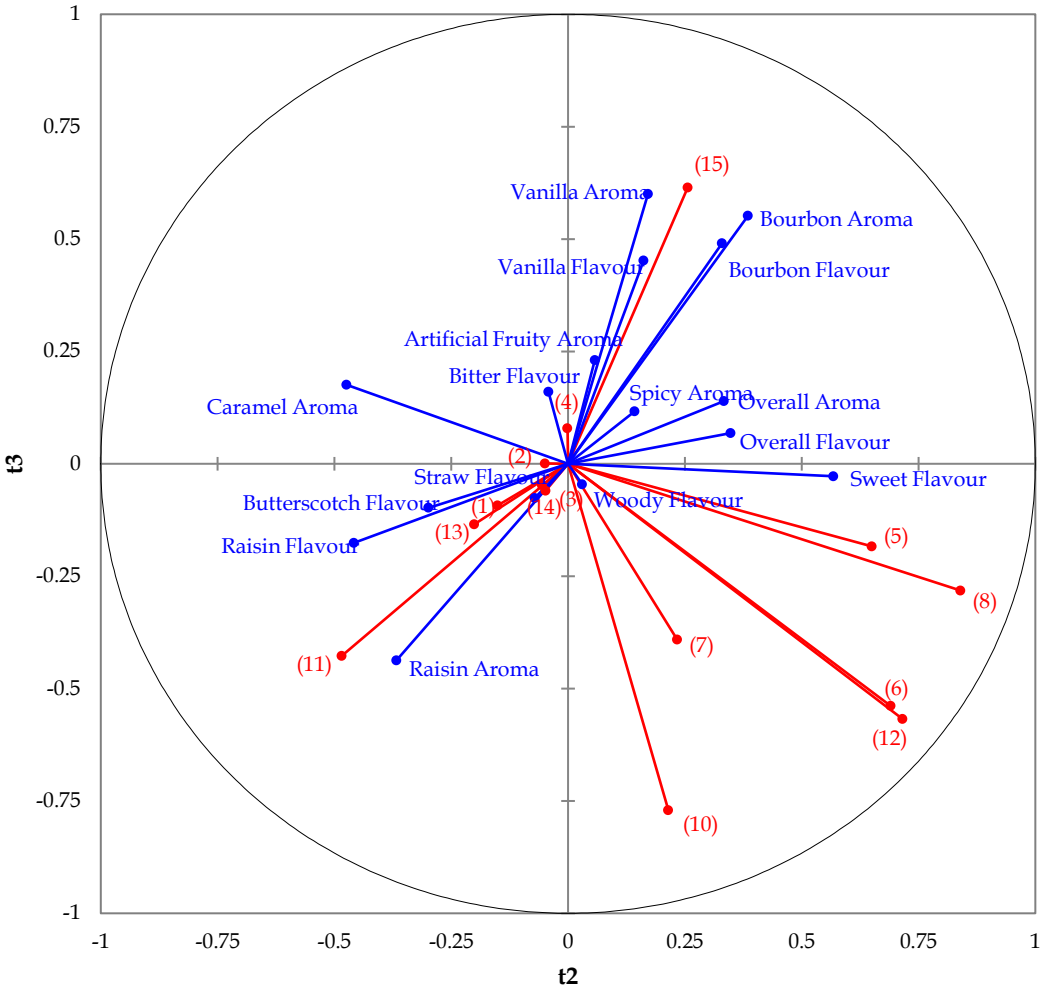


Figure 6.7: Bi-plot of components t2 and t3 for PLS of 15 reference chemical compounds (red) and 15 sensory characteristics (blue) used to describe 15 natural vanilla extracts. The combination of components 2 and 3 explained 31.0% of the variation in the sensory data and 19.5% of the variation in the GCMS data.

Vanillin (9) was not shown on the PLS bi-plots, as the data was standardised by vanillin concentration to allow for comparisons to the standardised sensory data, the samples were all presented at the same vanillin concentration, leading to very little differentiation between the samples based on vanillin (vanilla aroma and flavour). This was seen in Chapter 5, with only small differences between the samples based on vanilla aroma and flavour during sensory testing.

Attributes were considered to be correlated with chemical compounds if the correlations were greater than 0.7. These values can be found in Appendix 5. These

could also be seen by the close positioning (near parallel) of the red and blue lines on Figures 6.5, 6.6 and 6.7.

Vanilla flavour and vanilla aroma were correlated with creosol (7) on the bi-plot between components 1 and 2 (Figure 6.5). Creosol has been described as having a sweet, spicy, vanilla-like, smoky, clove, floral aroma, with a bitter taste that is also sweet, vanilla-like, spicy, clove, smoky and woody flavoured (Burdock, 2009) (Table 6.6). The aroma threshold for creosol is 90 ppb (0.09 ng/g), well below the concentrations detected in the vanilla extract samples with GCMS, from 2 µg/g to 50 µg/g. As creosol has been described to have vanilla-like aroma and flavour in literature (Shu and Shen, 2008; Ross et al., 2010), it was possible that this chemical contributed to the vanilla aroma and flavour notes in the vanilla extracts. The descriptions provided by the trained panel did not seem to match creosol with vanilla aroma and flavour, however the concentrations differed between the reference standard and the vanilla extract, suggesting that at low concentrations creosol provides a sweet, spicy, vanilla aroma but becomes more smoky as the concentration increases.

Creosol was also correlated with woody flavour (Figure 6.6). Creosol has been described as smoky and woody flavoured in literature (Shu and Shen, 2008; Ross et al., 2010) and by the trained panel. This smoky/woody description matches up well with the woody flavour attribute; therefore, it is likely that creosol is contributing to the woody flavour of the vanilla extracts and the vanilla aroma/flavour. Creosol has been found to correlate with phenolic, woody and smoky in vanilla extracts (Brunschwig *et al.* 2015), strongly supporting the relationships found here.

Bourbon aroma and bourbon flavour were correlated with syringaldehyde (15) in all three dimensions, indicating a strong correlation between these two variables. The vanilla extracts QO and NM were high in bourbon aroma and flavour as well as highest in concentration of syringaldehyde. Syringaldehyde was described as dusty, powdery, earthy, savoury and cheesy by the trained sensory panel and has been described as similar to vanillin in literature (Burdock, 2009; Sanchez-Palomo *et al.*, 2017). The detection threshold for syringaldehyde is 50 ppm (0.05 µg/g), and the concentrations of syringaldehyde in the samples ranged from 5 µg/g to 0.153 mg/g so the syringaldehyde was at sufficient concentration in the samples to be detected by the sensory panellists. Bourbon has been described by Poisson and Schieberle (2008) as malty, fatty, coconut-like, fruity, flowery, vanilla-like, phenolic (sweet) and smoky.

Table 6.7: List of positive correlations (>0.6) between sensory attributes and chemical compounds as observed from the PLS bi-plots, with the number of the bi-plot showing the correlation also reported.

Sensory Attribute	Number (Table 6.5)	Chemical Name
Vanilla aroma, vanilla flavour	7	Creosol
Sweet flavour	10	p-anisic acid
Bourbon aroma, bourbon flavour	5, 6, 12, 15	Maltol, 3-methyl-2-furoic acid, isovanillin, syringaldehyde
Bitter flavour, straw flavour, woody flavour	1, 2, 3, 4, 13, 14	Hexanoic acid, p-cresol, vanillic acid, guaiacol, acetovanillone, 4-hydroxybenzoic acid
Caramel aroma, raisin aroma	11	Vanillyl alcohol
Bourbon aroma, bourbon flavour	15	Syringaldehyde
Raisin aroma	7, 8	Creosol, 4-hydroxybenzaldehyde
Straw flavour, woody flavour	1, 2, 3, 13, 14	Hexanoic acid, p-cresol, vanillic acid, acetovanillone, 4-hydroxybenzoic acid
Bitter flavour, overall flavour, spicy aroma	2, 4	p-cresol, guaiacol
Raisin aroma, straw flavour	1, 11, 13, 14	Hexanoic acid, vanillyl alcohol, acetovanillone, 4-hydroxybenzoic acid
Butterscotch flavour, raisin flavour	1, 2, 13	Hexanoic acid, p-cresol, acetovanillone
Bitter flavour	4	Guaiacol
Bourbon aroma, bourbon flavour	15	Syringaldehyde
Woody flavour	7	Creosol
Sweet flavour	5, 8	Maltol, 4-hydroxybenzaldehyde

The sensory attributes bitter flavour, woody flavour, straw flavour and spicy aroma were found to be grouped together in all three bi-plots (Figures 6.5, 6.6 and 6.7). These attributes correlated with the compounds hexanoic acid (1), p-cresol (2), vanillic acid (3), guaiacol (4), isovanillin (13) and 4-hydroxybenzoic acid (14). The sensory attributes were found to be correlated with each other during PCA analysis of the sensory data (*Chapter 5*), and the compounds listed above (1, 2, 3, 4, 13 and 14) were correlated in a PCA analysis of the GCMS attributes (Table A28 *Appendix 5*). Of the compounds in this grouping, one compound that seemed to fit well with the attributes was guaiacol (4), described in literature to have a woody, phenolic, bacon, savoury, smoky, medicinal

flavour (Burdock, 2009), similar to the woody flavour attribute. Guaiacol was found to be highest in PNG at 0.35 mg/ml (after standardisation), and high in I, L and QO, which correlated well with the woody flavour attribute which was highest in PNG and I rated at 3.4 and 3.3 respectively by the trained sensory panel. A second compound that seemed to match up well with the sensory descriptor was 4-hydroxybenzoic acid, described as bitter in literature (Table 6.6), and bitter flavour was included in the descriptors in this grouping. 4-Hydroxybenzoic acid was measured at the highest concentration in PNG, and this sample rated the highest of all the vanilla extracts for bitter flavour. As the other compounds listed in Table 6.6 were described differently both by the trained panel and in literature to the sensory attributes, synergistic effects between the compounds may have caused the compounds to have different sensory profiles when combined rather than when presented as individual chemicals (Kemp *et al.*, 2009).

Sweet flavour was correlated with p-anisic acid (10), maltol (5) and 4-hydroxybenzaldehyde (8). P-anisic acid was described as having a sweet aroma by the trained panel however no description was found in literature. Maltol was described as artificial sweetener, sweet and toffee aroma by the trained panel, and sweet, caramel, cotton candy flavour by Burdock (2009), at 100ppm (0.1 μg/g). The concentration of maltol found in the vanilla extracts ranged from 1 μg/g to 38 μg/g. 4-hydroxybenzaldehyde was described to have a slightly sweet aroma by the trained panel and a sweet flavour by Burdock (2009). As all of these compounds have been described to have a sweet flavour they may have contributed to the rating for sweet flavour in the vanilla extract samples. As the trained panel only provided aroma descriptions, it was not possible to extrapolate to the flavour of the compounds.

Raisin flavour, raisin aroma and butterscotch flavour tended to group together on the PLS plots (Figures 6.5, 6.6 and 6.7), and they correlated with vanillyl alcohol (11). Vanillyl alcohol was described as candyfloss, floral, fudge, toffee and sweet by the trained panel and has been described as mild, sweet, balsamic and vanilla-like in aroma and sweet, creamy and milky in flavour by Burdock (2009). Vanillyl alcohol was also correlated with caramel aroma, matching well with the aroma descriptions from the trained panel, hence it was likely that vanillyl alcohol contributed to the caramel aroma of the vanilla extracts. It was also found that vanillyl alcohol was highest in T, but also high in NM, HI2 and I, and these vanilla extracts were also the highest in

caramel aroma rating in the sensory profiles. The descriptors raisin aroma and raisin flavour were described as dried fruity, sweet, slightly floral by the trained panel (*Chapter 5)* and raisins have been described with caramel, spice, sweet, sour, bitter and astringent flavour (Angulo *et al.*, 2007). All these attributes correlated well with the individual sensory descriptions of vanillyl alcohol (Table 6.5 and 6.6) therefore it was likely that the brown sweet note in the vanilla extracts was being introduced by the vanillyl alcohol. Butterscotch flavour also captured this brown sweet type flavour described in vanillyl alcohol and tended to be high in the vanilla extracts when caramel aroma was also high, with HI2 and H1 rating the highest for butterscotch flavour.

The attributes that did not seem to be correlated with any single chemical compound were artificial fruity aroma, overall aroma and overall flavour. Overall aroma and overall flavour were defined as being the total aroma or flavour impression when analysing the samples, and so were affected by the concentration of all the compounds within the samples. Artificial fruity aroma was not correlated with any compounds. The chemical compound providing the artificial fruity aroma may not have been quantified within the 15 chemicals chosen, so no correlations were observed

6.4 Conclusions

Fifteen volatile compounds were quantified in the vanilla extracts. Of these, 13 had previously been reported in vanilla. 3-methyl-2-furoic acid and isovanillin were the two newly identified compounds. Most of the 15 compounds identified in the vanilla extracts had a phenol type base to their molecular structures.

Comparing the quantified GCMS reference chemicals with the previously collected sensory profiles for each vanilla extract, a range of correlations were observed.

- Bourbon aroma and bourbon flavour correlated with syringaldehyde and was high in QO and NM.
- Vanilla aroma and flavour correlated with creosol
- Bitter flavour, woody flavour, straw flavour and spicy aroma were correlated with hexanoic acid, p-cresol, vanillic acid, guaiacol, acetovanillone and 4-hydroxybenzoic acid. This group of sensory attributes was correlated in PCA, as was the group of chemical compounds. In particular it was found that PNG was highest in both bitter and 4-hydroxybenzoic acid, as well as guaiacol and woody flavour.

- Sweet flavour was correlated with p-anisic acid, maltol and 4-hydroxybenzoic acid. All these chemicals were described as sweet by the trained panel.
- Raisin aroma, raisin flavour and butterscotch flavour were correlated with vanillyl alcohol and were found in HI2, H1 and NM at high concentrations and ratings.
- Overall flavour and overall aroma were not correlated with any specific compounds, as they were defined as a sum of all the components
- Artificial fruity aroma was not correlated with any compounds found by GCMS

7. Concentrated and Powdered Vanilla Extract

7.1 Introduction

The most common vanilla product is an ethanol vanilla extract (Cameron, 2011); glycerol extracts are also produced to provide an alcohol-free extract option for vanilla flavour. For a single fold vanilla extract, the concentration of vanilla within the typical ethanol extract is the equivalent of one vanilla bean per 5ml of solution (FDA, 1993). This concentration of vanilla is not ideal for some areas of food manufacture, where the addition of liquids is undesired (such as in chocolate making) or for large scale manufacture where the volumes required would be considerable using a single fold vanilla extract (such as in ice cream manufacture). In these applications, a vanilla powder or concentrated flavour would be preferable, so that the addition of water, ethanol and other solvents can be minimised.

Flavour companies are known to produce powders or concentrated flavours from vanilla beans. The powdered flavours are diluted with compounds called encapsulating agents, as they enable the flavour of the vanilla to be held or encapsulated as the solvent (ethanol and water) is removed. The encapsulating agent may result in a different sensory profile for the vanilla flavour. This raises the question, how does concentrating and drying the vanilla extract influence the sensory characteristics and how do they differ from the single fold ethanol extract?

There is very little information published about the methods used by flavour companies to create their concentrates and powders. The methods identified as possibilities for vanilla were supercritical carbon dioxide extraction, vacuum concentration, freeze drying and encapsulation. Spray drying and other methods that use either direct or indirect heat were not selected for trialling, as the ethanol present in the extract was a potential fire hazard. Glycerol has a boiling point of 290^{o}C, therefore any heat treatment used to remove the glycerol could lead to the loss of many of the volatile flavour and aroma compounds, leaving a poor-quality product, so this was not considered a viable option.

To fill the gaps in knowledge around this area of vanilla extracts, the aims of this research were:

- To investigate different methods for creating a concentrate or powder from vanilla beans or five-fold vanilla extract
- To compare the volatiles in the concentrates and powders to the original five-fold vanilla extract using analytical techniques
- To determine differences in the sensory profile of the concentrates and powders, using the trained sensory panel

7.2 Materials and Methods

The methods trialled were supercritical carbon dioxide extraction, freeze drying of ethanol extract, vacuum concentration and encapsulation of the vanilla flavour with maltodextrin.

7.2.1 Supercritical Fluid Extraction of Vanilla Beans with Carbon Dioxide

Tongan vanilla beans were sourced from Heilala Vanilla Ltd. (Year of harvest – 2014). To prepare the vanilla beans for extraction, the vanilla beans were hand cut to pieces 3-5 mm in length, frozen at -20 ^{o}C ± 2 ^{o}C for 24 hours, then freeze dried for 72 hours using a Labconco FreeZone6 Freeze drier (Labconco, U.S.A.). The vacuum during freeze drying averaged 0.1 mbar and the temperature of the sample trays went from -20 ^{o}C to 20 ^{o}C as the vanilla beans dried. The temperature of the collector coil was -50 ^{o}C ± 1 ^{o}C. The dried beans were then ground into a powder for 20 seconds using a 200W Breville coffee and spice grinder (BCG200BSS, Breville Pty Ltd, Australia). The vanilla powder was tested for water activity using a digital water activity meter (Decagon, pa$_w$kit, AES, South Africa) and moisture content before the supercritical extraction using the method outlined in Section *3.2.4*.

Two supercritical carbon dioxide trials were conducted on 12 January 2016, with two subsequent trials conducted on 26 October 2016 and 28 October 2016, with four total trials run.

A SFT- 100 XW (Supercritical Fluid Technologies, Inc) supercritical fluid extraction (SFE) system was used for extraction, located at MacDonald & Associates Ltd., Nelson, New Zealand. At the start of the extraction, the unit was turned on, and the fluid pump

left to cool for 20 mins. The vessel temperature was set at 40°C and the restrictor block (the outlet) temperature was set to 80°C. Fifty grams of dried vanilla bean powder was loaded into the 50ml capacity extraction vessel. At either end of the stainless steel extraction vessel there was a fine mesh and thirteen 2 mm glass beads, to prevent the vanilla powder from leaving the extraction vessel. Oxygen free carbon dioxide gas (BOC, New Zealand) was connected to the extraction vessel and the unit was left until it reached a pressure of 8500 ± 30 psi (58.6 ± 30 MPa) and was stable. A timer was started and the extraction continued under steady pressure conditions for 15 mins. The restrictor valve and static/dynamic valves were then opened slightly until the pressure dropped to between 7000 psi and 7500 psi (48.3 to 51.7 MPa). During this dynamic state period, CO_2 gas was slowly vented into a pre-weighed glass vial, carrying the extracted solution with it. The unit was left in this dynamic state for 5 min. At the end of these 5 min, the valves were closed, and the sample vessel weighed. This 15 min static – 5 min dynamic cycle was continued until less than 0.1g of extract was collected from one collection period. At this point, the CO_2 supply was closed off, and the pressure slowly released from the extraction vessel, to below 1000 psi (6.9 MPa). The spent vanilla bean powder (marc) was weighed, as well as the total extract collected. Samples were stored at -20 °C in glass jars with plastic lids and the spent powder stored in vacuum sealed foil pouches.

Extracts produced were analysed for vanillin content using HPLC (Section *3.2.2*) and for volatile content using GCMS (Section *3.2.1*). Samples were diluted to 0.020 ± 0.004g in 1.5ml in absolute ethanol (Analytical Grade, Fisher Scientific, New Zealand). The solvent cut time in the GCMS was increased to 20 minutes to compensate for the increased ethanol concentration. Marc (spent vanilla bean powder) was tested for final moisture content as per Section *3.2.4 Moisture Content*

7.2.2 Freeze Drying of Ethanol Vanilla Extract

Freeze drying was used to concentrate the vanilla extract. Heilala 5-fold Vanilla extract was frozen overnight to -25°C ± 2 °C as a 1 cm layer in plastic ziplock bags. The samples were then freeze dried with the plastic bags open using a Cuddon FD5 freeze dryer (Cuddon Freeze Dry Limited, New Zealand) at 1 mBar, with a shelf temperature of 40 °C. The samples were freeze dried for 24 hours.

7.2.3 Vacuum Concentration of Ethanol Vanilla Extract

To determine the effect of vacuum concentration on the volatiles in vanilla extract, Heilala 5-fold vanilla extract was concentrated by vacuum concentration to varying degrees of concentration. Samples were collected at different times and analysed to investigate the effect of the concentration process on the volatiles in the vanilla extract. Five different concentrations (in duplicate) were collected and analysed.

Figure 7.1: Buchi Rotavapor R-3 system for vacuum concentration of the ethanol vanilla extract.

Vacuum concentration was carried out using a Buchi Rotavapor R-3 system (Buchi Labortechnik AG, Switzerland) (Figure 7.1) at 40^{o}C ± 2^{o}C on rotation speed 6 and the condenser running tap water at 16^{o}C ± 2^{o}C. Each trial started with 50 ml of Heilala 5-fold extract, and was continued to different percentages of concentration, determined by both the mass of concentrated sample and the mass of collected condensate. All samples were stored in glass bottles with plastic lids at 4^{o}C ± 2^{o}C. Samples were diluted appropriately to ensure they were within the range of the standard curves before analysis on HPLC and GCMS (Section *3.2.1* and *3.2.2*).

7.2.4 Encapsulation of Concentrated Vanilla Extract to Produce a Vanilla Powder

One method used by flavour manufacturers to retain flavour during drying is to encapsulate the flavours with a carrier (Wang *et al.* 2015). The use of maltodextrin to encapsulate vanilla extract was trialled here to create a powdered vanilla flavour.

One litre of Heilala 5-fold vanilla extract was vacuum concentrated in two batches using the method outlined in Section *7.2.3* for 30 mins, until 43% $^{w}/_{w}$ of the original mass was removed. The vanilla extract's concentration increased from 7.3mg/ml to 16.8 mg/ml, an increase from 5-fold to 11.3-fold, with the vanillin concentration determined using the HPLC method in Section *3.2.2.*

Two maltodextrins – Avondex 30 Maltodextrin (DE 30, New Zealand Starch) and Avondex 10 Maltodextrin (DE 10, New Zealand Starch) were tested for encapsulation of vanilla extract. A range of flavour loadings were completed ranging from 15% $^{w}/_{w}$ (g vanilla concentrate/g maltodextrin powder) to 30% $^{w}/_{w}$ of 11.3-fold vanilla extract (16.8 mg/ml) concentrate (Table 7.1).

Table 7.1: Details of maltodextrin flavour loadings, vanilla concentrate weight and maltodextrin weight used in trials on encapsulation.

Maltodextrin Type	Target Concentration (% $^{w}/_{w}$* vanilla concentrate)	Weight of 16.8 mg/ml Vanilla Concentrate (g)	Weight of Maltodextrin (g)
Avondex 10	15	60.97	399.76
Avondex 10	20	79.96	401.43
Avondex 10	25	70.53	280.91
Avondex 10	30	60.24	200.89
Avondex 30	15	59.66	400.15
Avondex 30	20	60.36	299.98
Avondex 30	25	59.82	242.78
Avondex 30	30	59.75	201.03

*** g 16.8 mg/ml concentrate/100g maltodextrin**

At 20^{o}C, the maltodextrin and vanilla concentrate were mixed in a glass bowl using a spoon, until all of the powder had been dissolved. The mixtures were transferred into plastic ziplock bags in layers approximately 2 cm thick, laid flat on trays and frozen at -20^{o}C ± 2^{o}C for 12 hours. The samples were freeze dried on trays using a Labconco FreeZone6 Freeze Drier (Labconco, America). The vacuum averaged 0.1 mbar and the trays started at -20^{o}C and were increased to 20^{o}C gradually as the products dried. The collector coil was set to -50^{o}C ± 1 ^{o}C and the samples were left to dry for 51 hours.

The freeze-dried maltodextrin and vanilla concentrate samples were stored in glass jars, with plastic lids. The samples were tested for moisture content using the methods described in section *3.2.4.* The samples were also tested on GCMS for volatile content, as per the methods in *3.2.3* after dilution with RO water to 10% w/v, which was required to make a liquid product for direct injection into the GCMS.

7.2.5 Sensory Analysis of Vanilla Concentrates and Powders

Concentrated and powdered vanillas from each successful technique (vacuum concentration, maltodextrin powders and supercritical carbon dioxide extraction) were characterised using the trained sensory panel to investigate differences in the sensory profiles caused by the concentration methods.

All samples were standardised to 0.19 mg/ml vanillin concentration for aroma and 0.0225 mg/ml vanillin concentration for flavour, as in Section *3.1.* The samples (Table 7.2) were presented to the trained panel, after the panellists were given three hours of refresher training to ensure that they were familiarised with the vanilla products before starting the testing.

The Heilala single fold extract and 5-fold extracts were also presented to the panellists to allow for a comparison of the various concentrates to these original extracts. The 15 mg/ml and 30 mg/ml (vanillin concentration) vacuum concentrate samples (the medium concentration and the highest concentration achieved) were evaluated. The duplicate batches of vacuum concentrates at each concentration level were combined to provide one combined sampled for presentation to the trained sensory panel. The maltodextrin DE10 30% $^{w}/_{w}$ and DE30 30% $^{w}/_{w}$ were selected as they contained the highest flavour loadings of the encapsulated vanilla extracts. The SFE samples from 26/10/16 and 28/10/17 were chosen as they contained the highest concentration of vanillin of all SFE trials conducted. Vanillin (Rhovanil, Brenntag, New Zealand) was also presented to the trained panel for sensory evaluation to determine the flavour components of pure vanillin alone and how this compared to the concentrates produced.

Table 7.2: List of vanilla extracts, concentrates and powders presented to the trained panel for aroma and flavour evaluation.

Sample Name	Abbreviated Name
Heilala Single Fold Vanilla Extract	H1
Heilala Five-Fold Vanilla Extract	H5
Vacuum Concentrated sample at 30 mg/ml vanillin	VC30
Vacuum Concentrated sample at 15 mg/ml vanillin	VC15
Supercritical Carbon Dioxide Extract, created 26/10/2016	SFE1
Supercritical Carbon Dioxide Extract, created 28/10/2016	SFE2
Maltodextrin DE10, 30% $^{w}/_{w}$ vanilla concentrate	MD10
Maltodextrin DE30, 30% $^{w}/_{w}$ vanilla concentrate	MD30
Vanillin (Food Flavouring, Rhovanil, Brenntag, New Zealand)	V

7.3 Results and Discussion

The effects of the different concentration techniques and drying methods were investigated by analysing the composition of the extracts using GCMS as well as comparing the sensory profiles provided by the trained sensory panel. For this section, the mass balances and HPLC results will be presented, followed by GCMS results. This will be followed by the sensory results, presented for all the concentrates/powders in one section, to allow for comparison of the aroma and flavour profile of the final products created.

7.3.1 Supercritical Fluid Extraction of Vanilla Beans with Carbon Dioxide

For the supercritical fluid extraction of the vanilla beans, four trials were conducted. The first two were preliminary trials to check the suitability of the method for the application to vanilla extract, and the second two trials were carried out to improve the yield of extract.

7.3.1.1 SFE Trials 1 and 2

For the preliminary SFE trials, 50g of freeze dried, ground vanilla beans were used for each extraction trial. The water activity (a_w) and moisture content were 0.105 and 4.4% $^{w}/_{w}$ respectively. The first trial produced 5.03g of extract, a 10.1% $^{w}/_{w}$ (based on dry powder weight) yield and the second trial produced 4.71g of extract, a 9.7% $^{w}/_{w}$ (based on dry powder weight) yield. The vanilla bean powder changed from a dark brown to a light brown colour (Figure 7.2 and Figure 7.3) as the flavour was extracted. The extract produced was a pale brown/amber colour (Figure 7.4).

Figure 7.2: Photograph of freeze dried vanilla pods.

Figure 7.3: Photograph of spent vanilla pods, after supercritical carbon dioxide extraction.

The concentration of the vanillin, as well as other phenolics, was determined using HPLC. The vanillin was found to be 21.0 mg/ml in the SFE extract from Trial 1 and 24.2 mg/ml for Trial 2. Other phenolics monitored on HPLC (4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillic acid and vanillyl alcohol) were not detected as they were either below the detection limits or were not present. The concentration for the vanillin was lower than expected, as Fang *et al.* (2002b) found that they were able to extract 20.05 mg of vanillin from one gram of vanilla beans using SFE with carbon dioxide. This is compared to the approximately 2.3 mg of vanillin per 1 g of beans for Trials 1 and 2. A second set of trials was therefore required to check and/or improve the processing conditions.

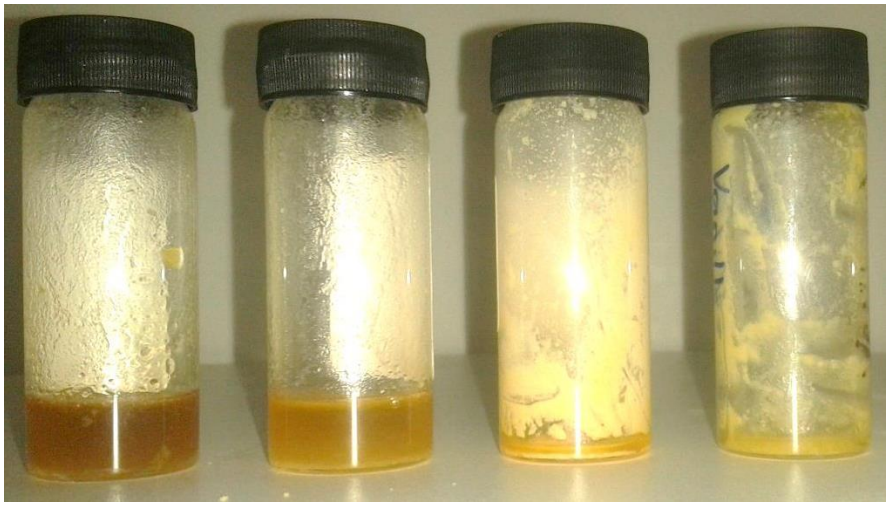


Figure 7.4: Photograph of vanilla extracts produced from freeze dried vanilla beans using supercritical carbon dioxide extraction. The samples, from left to right, are Trial 1, Trial 2, Trial 3 and Trial 4.

7.3.1.2 SFE Trials 3 and 4

For the second set of SFE trials (Trials 3 and 4), the weight of the vanilla bean powder used was 25.1 g and 39.7 g, respectively. The yield of extract from Trial 3 was 1.88 g of extract, a 7.5% $^{w}/_{w}$ (based on dry powder weight) yield. The yield for Trial 4 was 1.99 g of extract, a 5.0% $^{w}/_{w}$ (based on dry powder weight) yield. Figure 7.4 shows the extracts produced. Trials 1 and 2 had produced a clearer liquid, of greater volume.

It was found using HPLC that Trial 3 extracted 380.4 mg/ml of vanillin and Trial 4 extracted 362.2 mg/ml of vanillin. This equates to approximately 28.6 mg of vanillin and 18.1 mg vanillin per gram of vanilla beans. This is similar to the extraction yield obtained by Fang *et al.* (2002b), at 20.05 mg of vanillin per gram of vanilla bean. The conditions used by Fang *et al.* (2002) were 730K, 35MPa for 140 minutes, while samples collected for this research were extracted at 313K, 58MPa for 150 minutes. The temperature used by Fang *et al.* (2002) was much higher than used in this research and the pressure was also 40% lower, but the times used were similar. A lower temperature was chosen for this trial to reduce any potential thermal damage to the flavour compounds in the vanilla. No sensory trials were conducted by Fang *et al.* (2002) so the effect of this higher temperature on the quality of the extract was not reported.

7.3.2 Freeze Drying of Ethanol Vanilla Extract

Heilala 5-fold extract was freeze dried to investigate the use of freeze drying to create a concentrated extract. This sample was chosen as it had the highest concentration of volatiles (*Chapter 6*), although this did mean that it had a higher concentration of ethanol at 49.5 % v/v.

During the freeze-drying process, the extract foamed, resulting in the loss of the extract. This was caused by the high ethanol content of the sample preventing the sample from freezing fully, so the ethanol boiled off under vacuum, rather than slowly being drawn out as would happen in a lower ethanol content sample. No literature was found discussing the possibility of freeze drying an ethanol-based food flavour, although MacDonald and Associates had had success in similar applications and recommended trialling the process.

Previous studies that have used freeze drying to create a powder have used an encapsulating aid, such as maltodextrin (Desobry *et al.*, 1997; Madene *et al.*, 2005; Gharsallaoui *et al.*, 2007), which was carried out in section *7.3.4.*

7.3.3 Vacuum Concentration of Ethanol Vanilla Extract

From a range of different evaporation times starting with a 5-fold vanilla extract at 20^{o}C, it was found that after 15 minutes, with a 50 ml initial sample, 78% $^{w}/_{w}$ of the mass was removed (Table 7.3). At this point, the sample was very viscous and further evaporation made it difficult to remove the sample from the round bottom flask used for the concentration process. The highest concentration of vanilla achieved was 35.1 mg/ml vanillin, from a 5-fold vanilla extract containing 7.3 mg/ml vanillin. The condensate was analysed by HPLC; vanillin, vanillic acid, 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde were not detected. Therefore, these compounds were retained in the concentrate during the evaporation process.

Table 7.3: Vanillin concentration of concentrated extracts and % of evaporation for different times after vacuum concentration at 40°C.

Time (mins)	Initial vanillin concentration (mg/ml)	Final vanillin concentration (mg/ml)	% Evaporated ($^{w}/_{w}$) (g concentrate/ 100g 5-fold extract)
1	13.0	11.5	5.8
1	7.3	6.6	5.4
2	13.0	12.2	13
2	13.0	11.2	7.6
5	13.0	17.6	40.1
5	13.0	17.4	42
10	13.0	23.7	61.2
10	13.0	24.8	62.3
15	7.3	28.8	79.1
15	7.3	35.1	77.4

Figure 7.5 shows a comparison of the colours of the various extracts. As the samples were concentrated further with the vacuum concentration method, the colour darkened. The condensate was colourless.

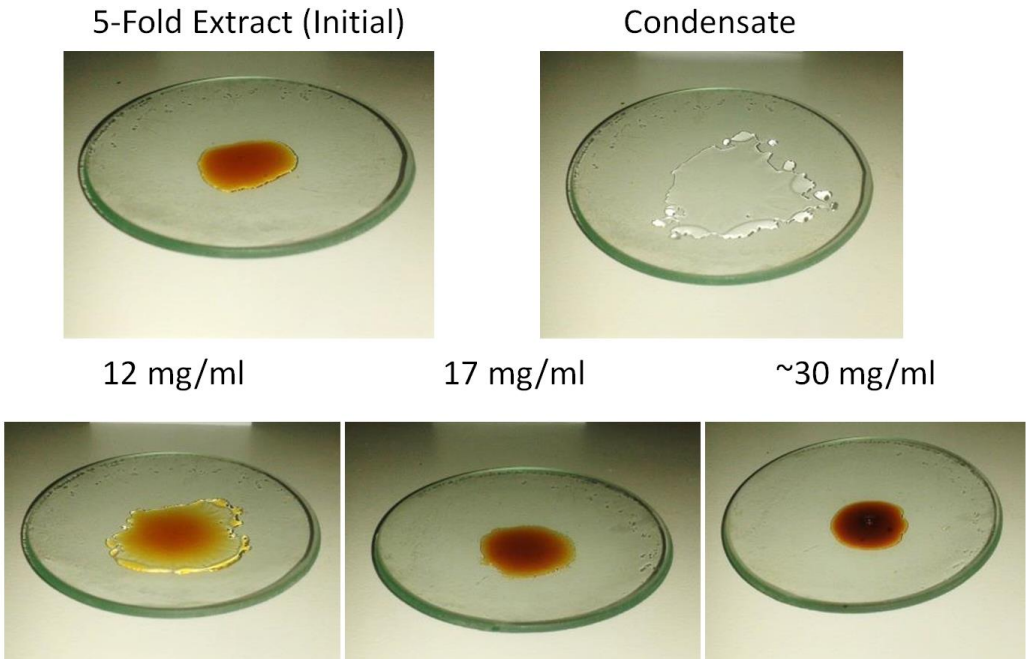


Figure 7.5: Photographs of the vacuum concentrated vanilla extract and condensate collected. Samples are labelled according to the vanillin concentration (mg/ml) achieved.

7.3.4 Encapsulation of Concentrated Vanilla Extract

A range of concentrations of vanilla extract concentrate, from 15% w/w to 30% w/w were dissolved into maltodextrin with two different dextrose equivalents (DE10 and DE30). It was found that when target concentrations of <25% w/w (Table 7.1) were used, the vanilla concentrate did not saturate the maltodextrin, leaving undissolved white maltodextrin powder after blending. Once the vanilla concentrate had been added to the maltodextrin, the mixture became highly viscous and sticky, making any form of mixing highly difficult. It would be recommended to trial spraying on the flavour in future experiments. The samples that did not have any unflavoured maltodextrin powder remaining were the DE10 30%, DE30 25% and DE30 30% (Figure 7.6).

During the freeze drying, the products foamed as the pressure was reduced. As a result, after freeze drying the final product was very porous with many air pockets, resembling a light foam that was easily crushed, so could be ground to a powder. This can be seen in Figure 7.6, in sample DE30 20%, where larger pieces of the aerated, light foam remain. The other samples were crushed more thoroughly during processing.

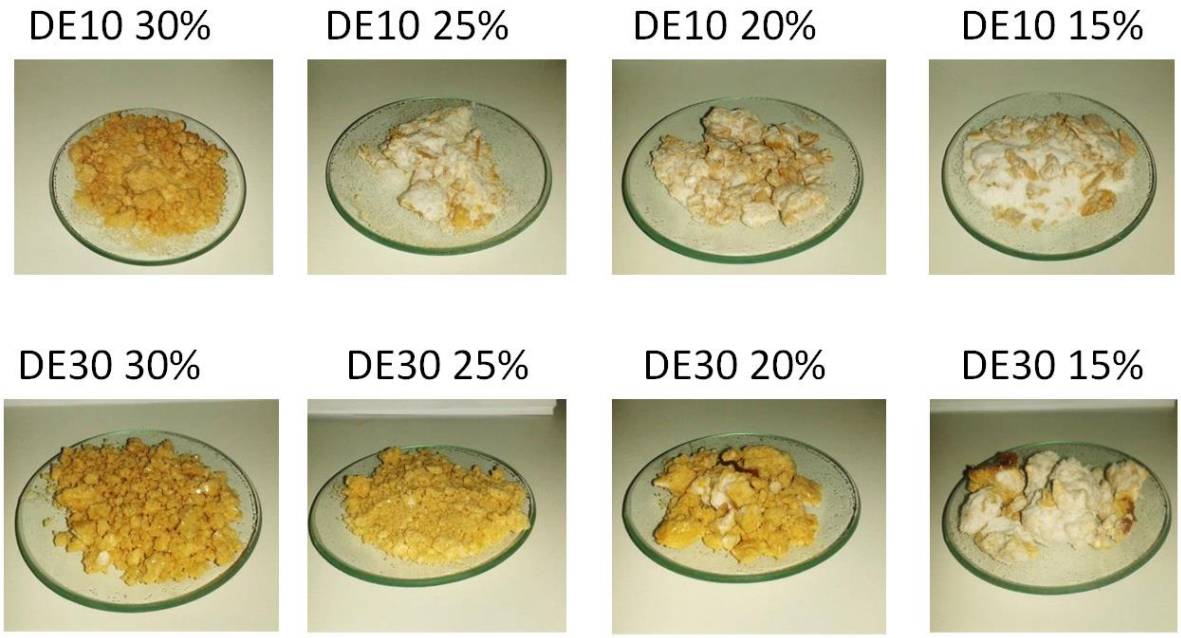


Figure 7.6: Photographs of the various maltodextrin vanilla powders produced after freeze drying. Samples are labelled according to the maltodextrin type (DE10 or DE30) and the target flavour concentration (%w/w).

The moisture content of the final powders was between 3.45 % and 8.45% w/w, compared to 6.0-6.7% w/w for the maltodextrin powder alone (Table 7.4). As up to

30% liquid was introduced to the samples when they were combined with the maltodextrin, this indicated that the water and any remaining ethanol after the vacuum concentration had been removed by the freeze drying, indicating complete drying occurred. There was no apparent relationship between the amount of vanilla concentrate added and the final moisture content of the samples.

Table 7.4: Moisture content of maltodextrin powders, before and after flavour encapsulation. Values are means ± standard error where n=3.

Maltodextrin Type	Target Vanilla Concentration (% w/w)	Moisture Content (% w/w)
DE10	0	6.67 ± 0.02
	15	3.51 ± 0.04
	20	4.23 ± 0.19
	25	5.32 ± 0.05
	30	3.64 ± 0.02
DE30	0	6.04 ± 0.00
	15	6.37 ± 0.07
	20	8.40 ± 0.05
	25	4.45 ± 0.02
	30	3.56 ± 0.08

The DE30 powder was found to be less water soluble than the DE10 powder. This was due to the nature of the maltodextrins used, with higher DE maltodextrins having a slightly lower solubility in water, although all maltodextrins are freely soluble in water with enough time (Kearsley and Dziedzic, 1995). If this is found to be an issue during food manufacture, an emulsifier could be used to facilitate dispersion, such as gum arabic or soy lecithin (Stauffer, 1999).

7.3.5 GCMS Analysis of Concentrates and Powders

To further investigate the effects of each concentration method, the concentrate and powder samples were analysed by GCMS and compared to the standard ethanol extract.

7.3.5.1 Supercritical Carbon Dioxide Extracts

For the supercritical carbon dioxide extracts, it was found that the majority of the compounds had longer retention times than vanillin (Figures 7.7, 7.8, 7.9 and 7.10). This indicated that the compounds either had longer carbon chains or higher boiling points than vanillin, using the RTX-5 column in this method. The compounds that were

quantified in Chapter 6 had retention times between 16.95 minutes and 35.75 minutes, with vanillin (the highest concentration compound) at 29.6 minutes). In comparison, the compounds found in the supercritical carbon dioxide extracts had retention times between 46 and 56 minutes, with only small peaks apparent at earlier retention times.

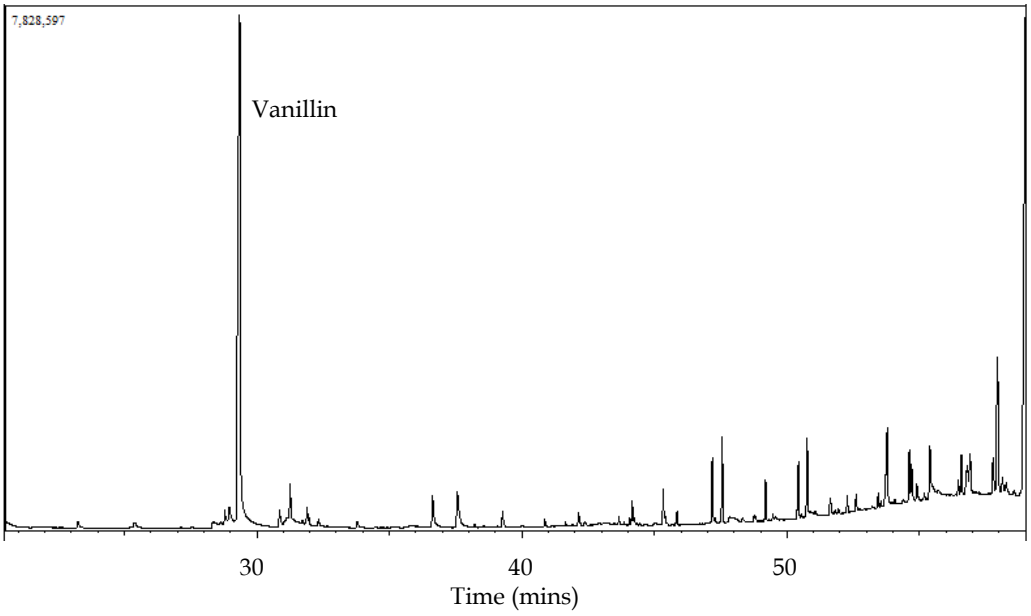


Figure 7.7: GCMS Chromatogram for supercritical carbon dioxide extraction of vanilla beans, SFE Trial 1. The graph is scaled on the y axis to a main peak height of 7,828,597.

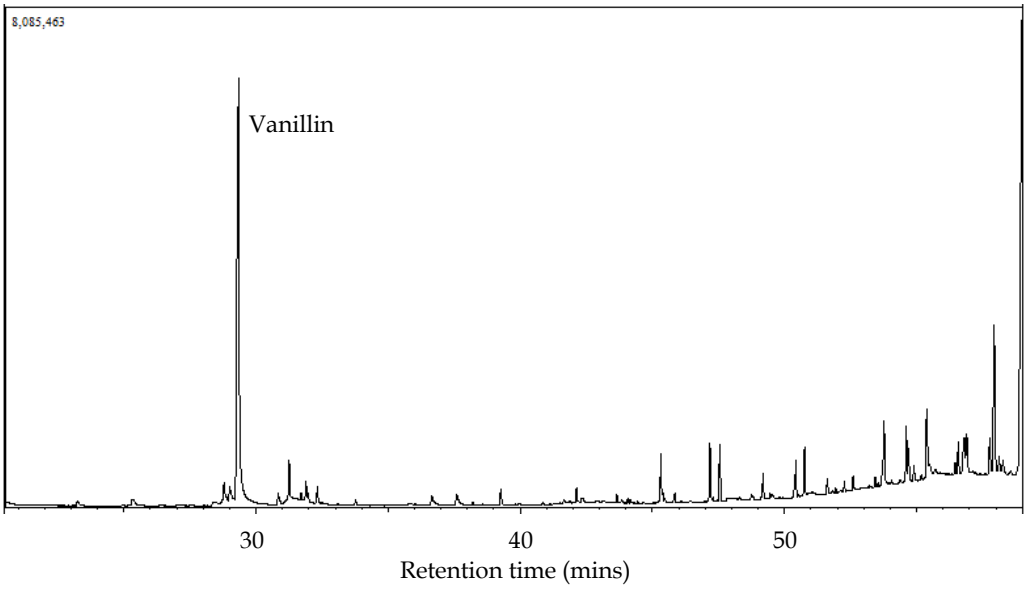


Figure 7.8: GCMS Chromatogram for supercritical carbon dioxide extraction of vanilla beans, SFE Trial 2. The graph is scaled on the y axis to a main peak height of 8,085,463.

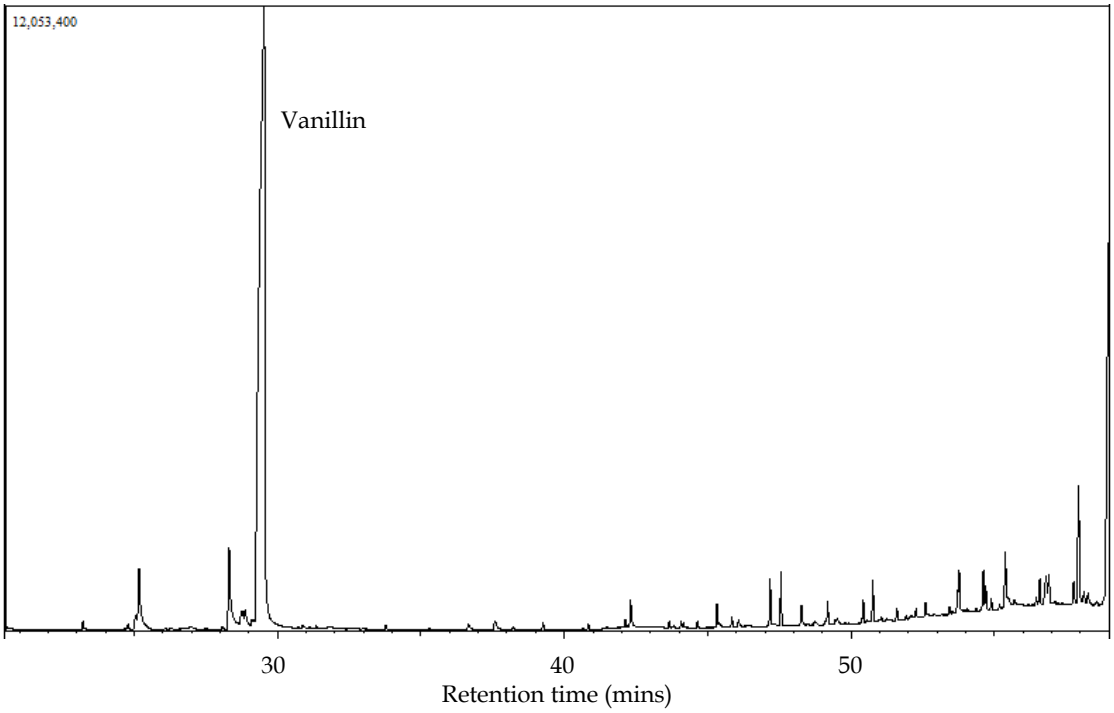


Figure 7.9: GCMS Chromatogram for supercritical carbon dioxide extraction of vanilla beans, SFE Trial 3. The graph is scaled on the y axis to a main peak height of 12,053,400.

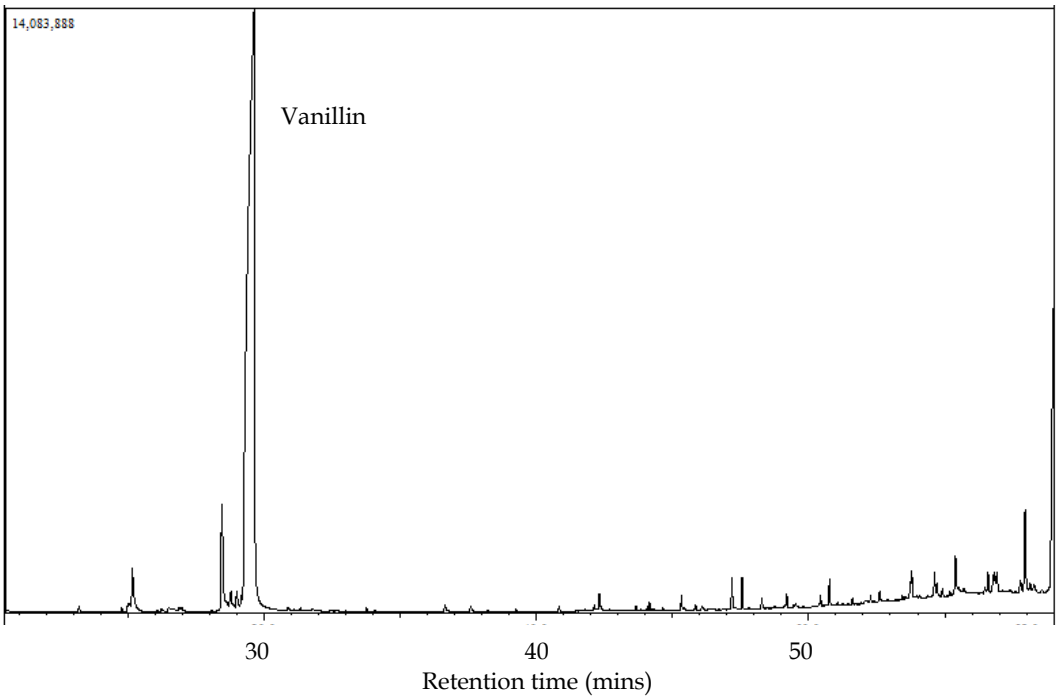


Figure 7.10: GCMS Chromatogram for supercritical carbon dioxide extraction of vanilla beans, SFE Trial 4. The graph is scaled on the y axis to a main peak height of 14,083,888.

Vanillin was not the base peak (largest peak) for the extracts from Trial 1 and 2, rather it was a compound identified (NIST 2008 MS Library) as nonadecane-2,4-dione, which had a retention time of 59 minutes. It was also found with HPLC that the vanillin concentration (21 mg/ml) was not as high as expected based on the average vanillin content of vanilla beans and other research (Section *7.3.1.1*) (Fang *et al.* 2002).

For Trials 3 and 4, the base peak in the chromatogram was vanillin (Figures 7.9 and Figure 7.10). This corresponded to the higher levels of vanillin measured with HPLC (380.4 mg/ml and 362.2 mg/ml, respectively).

Table 7.5: The 20 most concentrated compounds found in the supercritical carbon dioxide extract (Trial 3), as identified by mass spectrum (NIST 2008 Library).

Compound Name	Retention Time (mins)	Area (% Total)
Vanillin	29.6	20.7
1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene	31.5	1.06
5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene, [S-(R*,S*)]-	31.8	1.53
1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene, (S)-	32.1	0.93
3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene, [S-(R*,S*)]-	32.5	1.49
(Z)-9-tricosene	47.4	5.32
Eicosane	47.7	1.78
Hexatriacontane	50.9	1.61
Dihydro-5-tetradecyl-2(3H)-furanone	52.6	8.23
Tricosane-2,4-dione	52.8	1.02
17-Pentatriacontene	53.6	3.75
Dotriacontane	53.9	2.57
(Z)-14-Tricosenyl formate	54.0	1.79
9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	54.1	1.16
E,E-3,13-Octadecadien-1-ol	54.8	1.87
1,4-dimethyl-2-octadecyl-cyclohexane	54.9	1.65
dihydro-5-tetradecyl-2(3H)-furanone	55.6	6.78
1-triacontanol	56.8	1.7
octahydro-5-hydroxy-8a-methyl-1(2H)-naphthalenone,	58.0	1.02
E,E,Z-1,3,12-nonadecatriene-5,14-diol	58.2	4.71

The most concentrated compounds were then identified using the mass spectra from the GCMS, combined with the NIST MS library (2008). Table 7.5 shows the compounds identified in Trial 3. All the SFE trials contained similar compounds and are presented fully in Appendix 6.

The compounds found in the supercritical carbon dioxide vanilla extracts were mostly alkenes or cyclohexenes. The retention times were greater than vanillin (29.6 minutes), indicating that they had higher boiling points. The compounds identified were mostly lipophilic, as the solvent used - carbon dioxide - is non-polar and will extract these compounds in preference to the more polar compounds found in the ethanol extracts, such as 4-hydroxybenzaldehyde and hexanoic acid (Chapter 6). The SFE method was chosen as it had been found to produce a concentrated vanillin product (Fang *et al.*, 2002b); the product was more concentrated and had a different profile based on GCMS results hence the sensory profile of the extract must also be determined to compare it to an ethanol extract.

7.3.5.2 Vacuum Concentration of Vanilla Extract

For the vacuum concentrates, there was a clear progression in the products, with the concentration of the volatiles increasing as the sample was concentrated further. This was observable in the GCMS chromatograms (Figure 7.11).

In Figure 7.11, sample B, the condensate, it can be seen to contain two primary peaks, which had not been detected by HPLC analysis. These were identified using reference standards as guaiacol and vanillin. The concentration of vanillin in this sample was low compared to the other samples, at 3.2 mg/ml vanillin. This indicated that during vacuum concentration at 40^{o}C, many of the flavour volatiles in the ethanol extract were not evaporating and therefore most remained in the concentrated sample.

During vacuum concentration the vacuum reduces the boiling point of the solvents so that there was less potential thermal degradation of other compounds in the solution. The primary solvents present in vanilla extract are water and ethanol, which are commonly distilled in industry to purify the ethanol component and reduce the water content (Pouliot *et al.*, 2014). The boiling points of the compounds in Table 7.6 are much higher than those of water (100^{o}C) and ethanol (78^{o}C), and in theory they should be retained in the concentrate during the distillation process. However, some vanillin and guaiacol were identified in the condensate by GCMS. They were likely carried over physically in the evaporating ethanol and water vapours.

Table 7.6: Physical properties of main compounds identified in vanilla extract vacuum concentrates, with concentrations as determined by GCMS. 5-(hydroxymethyl)-furfural was not quantified (UQ) as the standard was unavailable. Concentrations are in mg/ml and are means where n=4. N/D means that the standard was not detected.

Retention time (mins)	Chemical Compound	Boiling Point (°C)	5-Fold Extract	Condensate	Vacuum Concentration Time (mins)			
					2	5	10	15
20.2	Guaiacol	204-206	N/D	0.29	0.12	0.21	0.23	0.47
23.4	3-methyl-2-furoic acid	236	0.90	N/D	0.71	0.69	1.03	0.48
24.7	5-(hydroxymethyl)-furfural	291	UQ	N/D	UQ	UQ	UQ	UQ
28.4	4-hydroxybenzaldehyde	310	0.28	N/D	0.25	0.21	0.25	0.24
29.6	Vanillin	285	7.3	3.2	12.2	17.6	23.7	28.8
30.8	Vanillyl alcohol	293	0.13	N/D	0.10	0.13	0.14	0.16

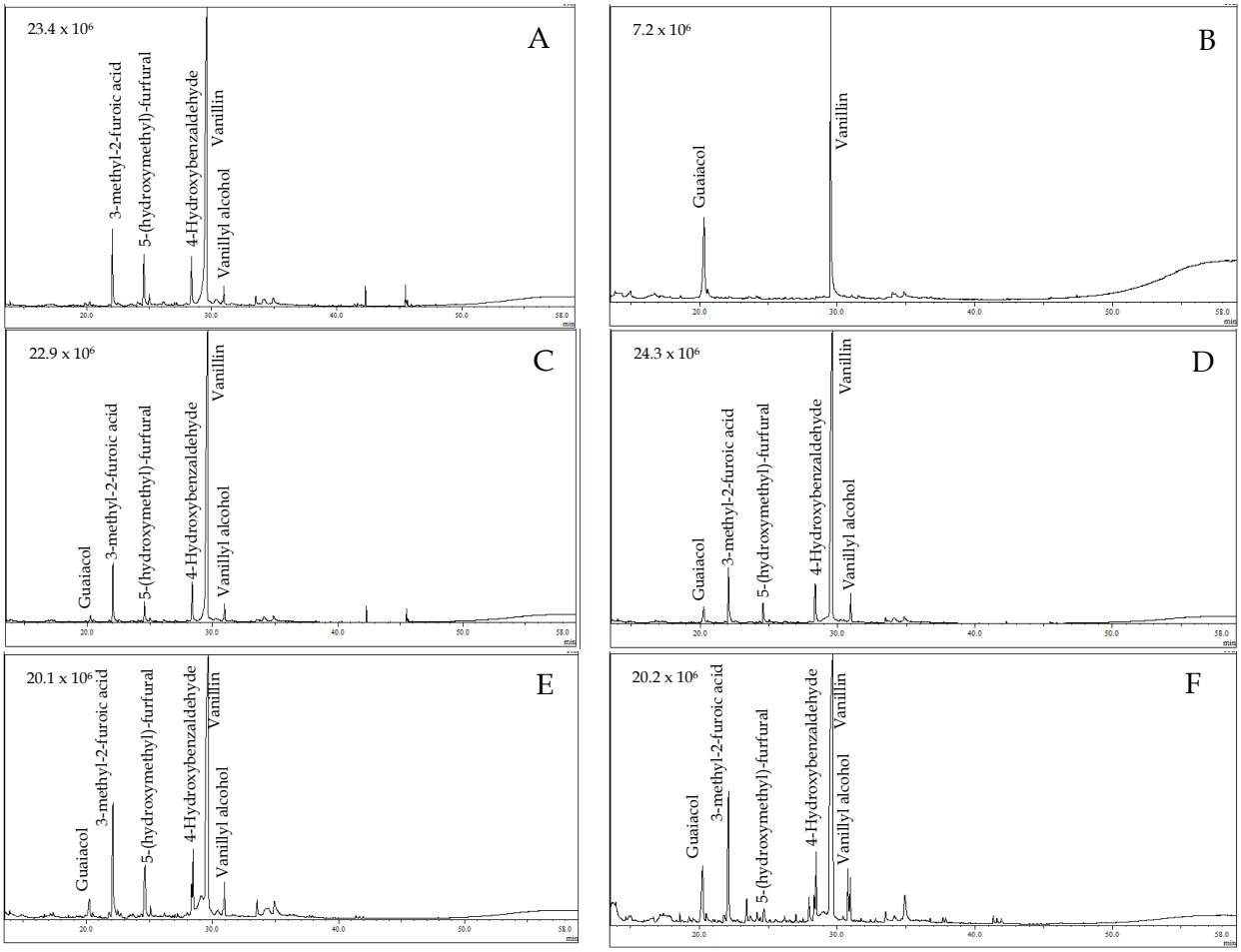


Figure 7.11: Chromatograms from GCMS for vacuum concentrates. A is the original, unconcentrated 5-fold extract. B is the condensate from vacuum concentration, C was concentrated 2 minutes, D was concentrated 5 minutes, E was concentrated 10 minutes and F was concentrated 15 minutes (different initial extract). The height of the base peak is labelled in the upper left corner and reflects the relative concentration of vanillin in the samples. Labelled peaks were compared to reference standards for identification by retention time, except 5-(hydroxymethyl)-furfural, identified by MS library (NIST, 2008).

7.3.5.3 Encapsulation of Vanilla Concentrate with Maltodextrin

Fewer peaks were detected in the encapsulated vanilla concentrate (Figures 7.12 and 7.13) compared to the standard ethanol extracts (Figure 7.11 A). Vanillin was the base peak, being at the highest concentration.

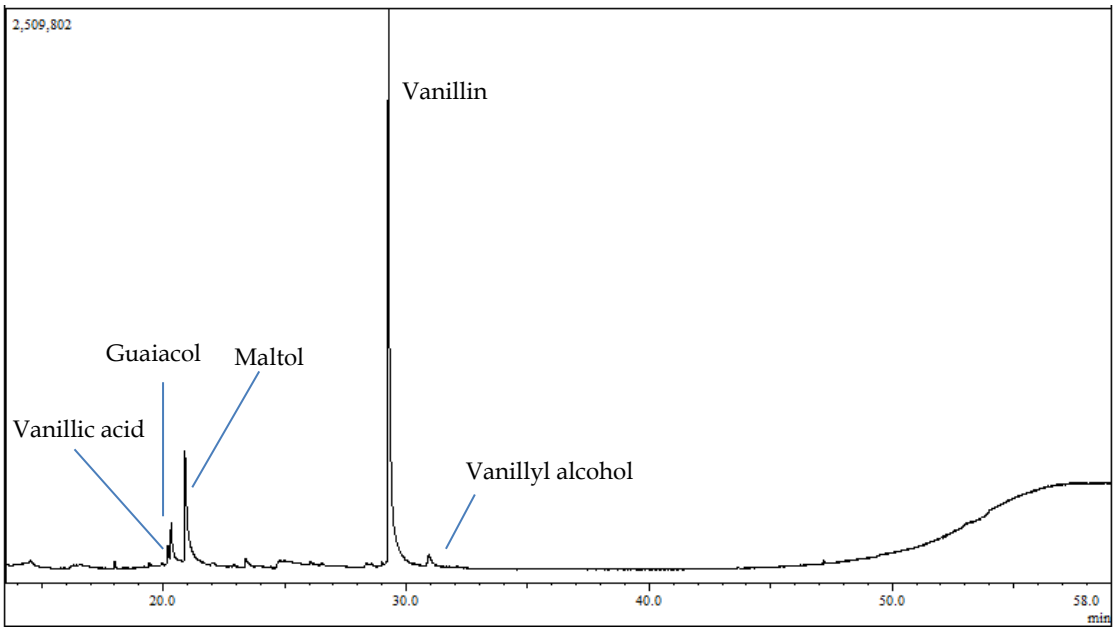


Figure 7.12: GCMS Chromatogram of vanilla concentrate encapsulated with maltodextrin (DE30), 30% w/w target vanilla concentration.

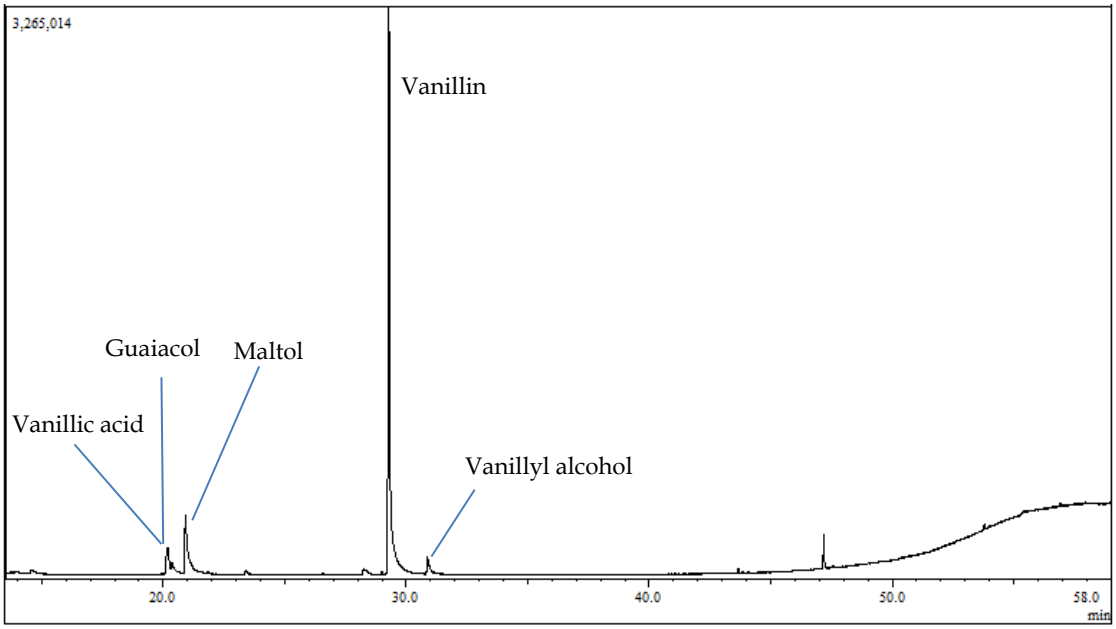


Figure 7.13: GCMS Chromatogram of vanilla concentrate encapsulated with maltodextrin (DE10), 30% w/w target vanilla concentration.

Of the main compounds present in the maltodextrin extracts, five were able to be quantified using the standard curves developed for *Chapter 6* (Table 7.7). The powders were diluted with maltodextrin during manufacture, leading to the lower concentrations of compounds compared to liquid extracts.

Table 7.7: Concentrations of five main peaks identified in maltodextrin extracts and standard ethanol extracts as determined by GCMS.

Retention Time (mins)	Compound Name	Heilala Single Fold (mg/ml)	Heilala 5-Fold (mg/ml)	MD 30% DE10 (mg/g powder)	MD 30% DE30 (mg/g powder)
20.3	Guaiacol	0.09	0.21	0.02	0.02
21.1	Maltol	0.00	0.04	0.18	0.25
23.4	Creosol	0.11	1.19	0.004	0.005
29.1	Vanillin	1.83	10.40	0.85	0.83
30.8	Vanillyl alcohol	0.06	0.04	0.01	0.02

The concentration of the compounds in the two powders was similar for all. However, the relative ratios of the compounds to each other changed from the standard ethanol extract, for example the powders lost 80% of the guaiacol yet lost >95% of the creosol. This indicated that there were uneven losses of volatiles during the processing, with some volatiles lost to a greater extent than others. This could be due to a number of factors, such as boiling point or binding capability with maltodextrin.

Based on these results, 2 g of the encapsulated vanilla was equivalent to 1 ml of single fold vanilla extract. This powder could be used in food systems where either alcohol and/or water cannot be added to the food.

7.3.6 Sensory Analysis of Concentrates and Powders

A selection of the concentrates and powders produced were presented to the trained sensory panel to evaluate the sensory characteristics and allow for comparison. The panellists were familiarised with the products for three hours of training before the testing was started.

7.3.6.1 Panel Performance for Sensory Testing

The panel performance was checked to ensure that they were rating the samples consistently, with no significant differences between their ratings across different sessions or for the same sample within each session.

A Student's t-test showed that of the 45 attribute-session combinations (15 attributes, 3 sessions), 43 were not different ($p \geq 0.05$). This showed that the participants were able to produce similar values for the samples during each testing session as a duplicate sample was presented at the end of each session. The results of the t-test can be seen in Table 7.8.

Analysis of variance (ANOVA) was also used to investigate the panel performance. It was found that the trained panellists were able to discriminate the concentrated samples based on all seven aroma attributes and six of the eight flavour attributes. The two flavour attributes that were not found to be significantly different were sweet flavour and vanilla flavour. As the samples were presented at the same vanillin concentration and had the same sugar concentration, this further demonstrated the reliability of the panel, rather than a lack of discrimination.

Table 7.8: Results from the Student's t-test comparing in-session samples with duplicates at the end of each testing session for aroma and flavour of vanilla extract concentrates. Significant values are in italics where p<0.05.

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma	
Session 1	0.666	0.305	0.477	0.374	*0.034*	0.070	0.512	
Session 2	0.778	0.486	1.000	0.587	0.426	0.749	0.587	
Session 3	0.394	0.178	1.000	0.374	0.178	0.648	0.108	
	Overall Flavour	**Sweet Flavour**	**Vanilla Flavour**	**Butterscotch Flavour**	**Raisin Flavour**	**Bitter Flavour**	**Woody Flavour**	**Bourbon Flavour**
Session 1	1.000	*0.033*	0.477	0.704	0.294	0.242	0.621	0.477
Session 2	0.075	0.187	0.529	0.374	0.573	0.189	0.358	0.206
Session 3	0.052	0.426	1.000	0.861	0.070	0.099	0.656	1.000

Table 7.9: ANOVA output comparing main effects and interaction terms of aroma and flavour using GLM for concentrated vanilla extracts. Values are p-values, with significant values in italics (p<0.05).

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma	
Product	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	
Participant	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.007*	*0.000*	
Session	0.065	0.182	0.444	0.698	0.365	0.005	0.038	
Product:Session	0.986	0.069	0.972	0.950	0.038	0.248	0.678	
	Overall Flavour	**Sweet Flavour**	**Vanilla Flavour**	**Butterscotch Flavour**	**Raisin Flavour**	**Bitter Flavour**	**Woody Flavour**	**Bourbon Flavour**
Product	*0.000*	0.560	0.165	*0.005*	*0.000*	*0.000*	*0.000*	*0.000*
Participant	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*
Session	*0.015*	0.110	0.109	0.824	*0.039*	*0.001*	*0.001*	0.238
Product:Session	0.978	0.105	0.320	0.366	0.212	0.787	0.754	0.624

7.3.6.2 Concentrated Vanilla Extracts - Mean Sensory Scores

After completing ANOVA to determine significance, the mean scores obtained for each of the concentrated vanilla extract samples were compared using a Tukey's HSD analysis. The results are shown in Table 7.10 for aroma and Table 7.11 for flavour.

Table 7.10: Comparison of mean values for aroma of concentrated vanilla extract using Tukey's HSD. Different letters in superscript denote values that are significantly different ($p < 0.05$).

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
H1	6.2^{a}	2.7^{a}	3.0^{a}	2.3^{a}	1.9^{ab}	2.8^{a}	3.7^{a}
H5	4.3^{b}	2.2^{abc}	2.3^{ab}	1.8^{abc}	2.1^{a}	2.4^{ab}	2.9^{bc}
VC15	3.6^{bc}	2.4^{ab}	1.8^{bc}	2.1^{a}	1.7^{abc}	2.4^{ab}	2.5^{bcd}
VC30	3.5^{bc}	1.7^{bcd}	2.5^{ab}	2.0^{ab}	2.0^{ab}	2.2^{abc}	3.2^{ab}
SFE1	2.2^{d}	1.7^{bcd}	1.2^{c}	1.2^{c}	1.4^{bc}	1.2^{d}	1.6^{e}
SFE2	2.1^{d}	1.6^{cd}	1.1^{c}	1.4^{bc}	1.4^{bc}	1.1^{d}	1.8^{de}
MD10	2.2^{d}	1.6^{bcd}	1.4^{c}	1.7^{abc}	1.6^{abc}	1.5^{cd}	2.1^{cde}
MD30	2.9^{cd}	2.0^{abcd}	1.4^{c}	2.0^{ab}	1.5^{abc}	1.8^{bcd}	2.8^{bc}
V	2.0^{d}	1.4^{d}	1.1^{c}	1.2^{c}	1.2^{c}	1.2^{d}	1.7^{de}

For almost all the aroma attributes, H1 and H5 rated the highest (Table 7.10). This may be due to the loss of aroma volatiles through the various processing methods used, which would decrease the intensity of the aroma and flavour of the products. The dilution required for sample preparation was determined by the vanillin concentration within the samples, as this was present in all the samples. However, vanillin concentration did not decrease during the concentration methods evaluated. Though other flavour volatile compounds appear to have been lost. The other volatiles present were not monitored quantitatively and may have been lost or concentrated depending on their volatility and solubility. The vanillin sample (V) was found to be rated the lowest for all aroma and flavour attributes as it was a single flavour compound whereas the other samples contained a number of compounds which contributed to the flavour (Tables 7.10 and 7.11). V was rated similarly to the SFE samples, indicating that the SFE samples did not contain many flavour compounds other than vanillin.

To produce the MD10 and MD30 concentrated extracts, the H5 extract was first vacuum concentrated, hence reducing the overall aroma, with loss of some volatiles, as was noted by the drop in overall aroma for VC15 and VC30. This concentrate was then added to the maltodextrin and freeze dried, which could have led to further losses in

the aroma and flavour compounds, resulting in the lower score seen for MD10 and MD30 compared to the H and VC extracts.

Table 7.11: Comparison of mean values for flavour of concentrated vanilla extract using Tukey's HSD. Different letters in superscript denote values that are significantly different ($p < 0.05$).

	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
H1	5.0^{a}	3.3^{a}	3.2^{a}	1.6^{b}	1.9^{a}	1.8^{ab}	2.5^{ab}	2.2^{a}
H5	3.9^{b}	3.5^{a}	3.4^{a}	1.5^{b}	1.3^{b}	1.7^{abc}	2.3^{abc}	1.9^{ab}
VC15	3.9^{b}	3.9^{a}	3.6^{a}	1.7^{ab}	1.5^{ab}	1.7^{abc}	1.9^{abc}	1.4^{bc}
VC30	3.3^{b}	3.3^{a}	3.0^{a}	1.7^{ab}	1.6^{ab}	1.5^{bc}	1.7^{bc}	1.3^{bc}
SFE1	3.7^{b}	3.9^{a}	3.1^{a}	2.2^{a}	1.1^{b}	1.9^{ab}	2.1^{abc}	1.2^{c}
SFE2	4.0^{b}	3.8^{a}	3.5^{a}	2.1^{ab}	1.4^{ab}	2.1^{a}	2.5^{a}	1.4^{bc}
MD10	3.4^{b}	3.8^{a}	3.0^{a}	1.7^{ab}	1.2^{b}	1.6^{bc}	1.5^{c}	1.4^{bc}
MD30	3.2^{b}	3.7^{a}	3.0^{a}	1.7^{ab}	1.3^{b}	1.5^{bc}	1.8^{abc}	1.3^{bc}
V	3.0^{b}	4.0^{a}	2.8^{a}	1.7^{ab}	1.2^{b}	1.3^{c}	1.6^{c}	1.2^{c}

Vanilla flavour was not significantly different in any sample (Table 7.11) as the samples were standardised to the same vanillin concentration for presentation to the trained panel. There was more variation in the flavour attributes than the aroma attributes, with no one sample rating highest or lowest for all attributes, as in aroma. Vanillin (V) tended to be the lowest and H1 and SFE2 the highest but overall no pattern emerged. H1 was rated the highest for overall flavour. This was likely due to the standardisation of the extracts and concentrates based on vanillin concentration, which would have made overall flavour tend to be rated the same. As H1 contained flavour compounds other than vanillin, these must have been causing the rating for overall flavour to increase, resulting in the higher rating observed.

7.3.6.3 Principal Component Analysis of Vanilla Concentrates

Principal component analysis (PCA) was used to visualise the patterns in the arrangement of the samples. The aroma and flavour attributes were analysed separately, to allow for more detail of the relationships to be observed.

a) Aroma

For PCA of the aroma attributes of H1, H5 and the concentrated vanilla extracts, the first component had an eigenvalue of 5.62, and explained 80.3% of the total variation in the data, and the second component had an eigenvalue of 0.84 and explained 12.0% of the total variation. When combined, they explained 92.3% of the total variation in the data set. The bi-plot of this is shown in Figure 7.14 PC1 was positively correlated with

overall aroma, bourbon aroma, caramel aroma, raisin aroma and spicy aroma and PC2 was positively correlated with artificial fruity aroma. Full details of the PCA are in Appendix 7.

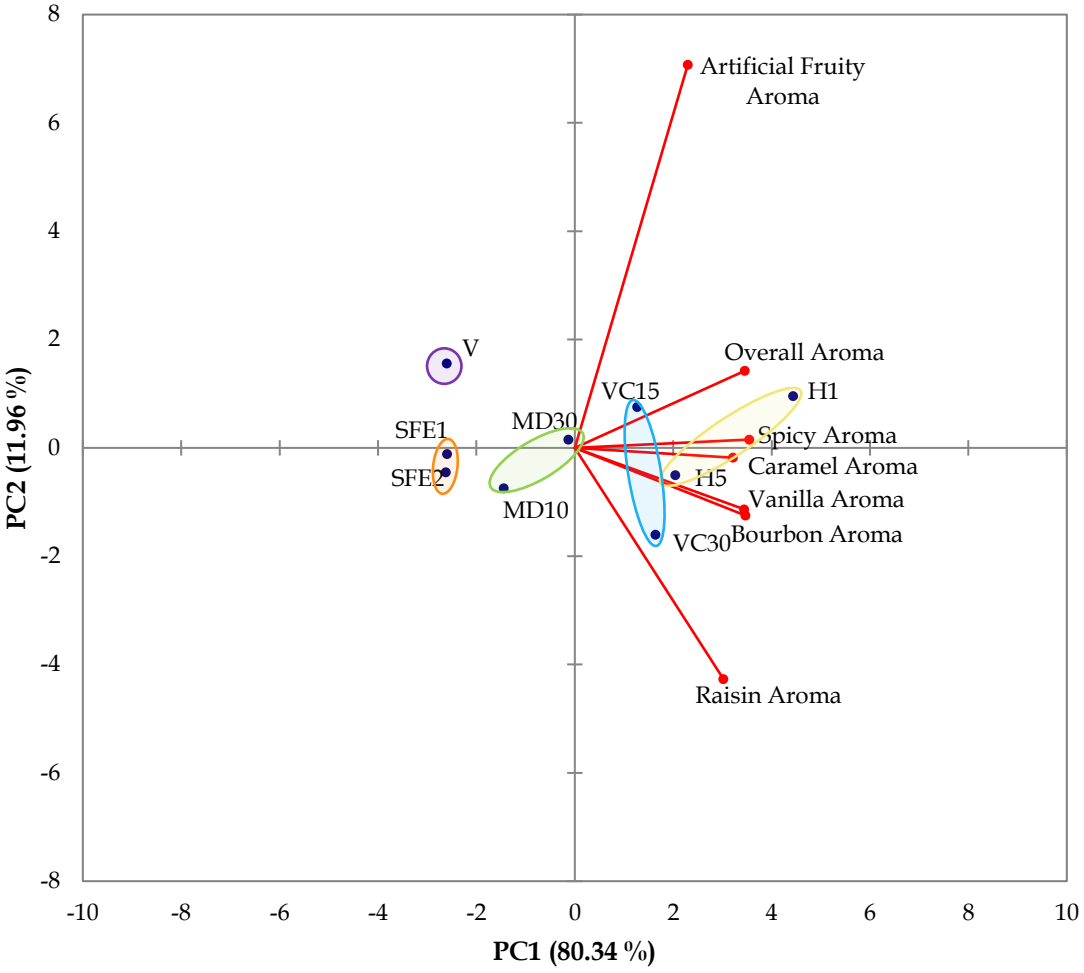


Figure 7.14: Bi-plot of the first two principal components identified through PCA of all attributes aroma for vanilla concentrate samples. The first component has an eigenvalue of 5.62 and the second component has an eigenvalue of 0.84. The cumulative proportion of variation explained by the components is 92.3%. H1 and H5 are circled in yellow, VC15 and VC30 are circled in blue, MD10 and MD30 are circled in green, SFE1 and SFE2 are circled in orange and V is circled in purple.

In the bi-plot, the samples of the same type tended to group together. The similar groups of samples have been circled in various colours in the plot. Table 7.12 shows the correlations between the attributes, with all attributes being positively correlated with overall aroma, meaning that a change in the rating of one of the attributes would be reflected in the overall aroma attribute. The only attribute that was not correlated with PC1 was artificial fruity aroma; this was correlated with PC2 instead.

As all the samples were standardised to the same vanillin concentration, changes in the overall aroma indicated that the other aroma compounds in the vanilla samples were also being affected by the processing methods used, leading to a reduction in the overall aroma. Therefore, overall aroma was based on the concentration of all the compounds, as was found in Chapter 6.

Table 7.12: Correlation matrix for aroma attributes of concentrated vanilla extracts in principal component analysis. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	**0.720**	**0.933**	**0.793**	**0.739**	**0.918**	**0.888**
Artificial Fruity Aroma	**0.720**	**1**	0.516	0.508	0.229	0.650	0.494
Bourbon Aroma	**0.933**	0.516	**1**	**0.781**	**0.870**	**0.920**	**0.935**
Caramel Aroma	**0.793**	0.508	**0.781**	**1**	**0.668**	**0.899**	**0.888**
Raisin Aroma	**0.739**	0.229	**0.870**	**0.668**	**1**	**0.841**	**0.794**
Spicy Aroma	**0.918**	0.650	**0.920**	**0.899**	**0.841**	**1**	**0.908**
Vanilla Aroma	**0.888**	0.494	**0.935**	**0.888**	**0.794**	**0.908**	**1**

For the arrangement of the samples on the bi-plot in Figure 7.14, H1 and H5, the original extracts were positioned on the right-hand side of the plot, with the highest rating for all attributes and highest overall aroma. To the left of these on the bi-plot were VC15 and VC30, in the blue circle, followed by MD10 and MD30 in the green circle. Based on PC1, SFE1 and SFE2 were about the same as V, therefore these samples were similar in aroma profile to vanillin. The pattern seen was that the samples tended to decrease in PC1 as they underwent more processing before reaching the final product. The H extracts were the original extracts for the experiments; the VC samples underwent one processing step, the MD samples underwent two processing steps and the SFE samples underwent an entirely different process that used CO_2 as the solvent instead of ethanol on the vanilla beans and included a drying step to remove all water from the ground beans As the samples went through more processing they lost more of the aroma volatiles, while retaining vanillin, therefore they were more similar to the vanillin sample, V, than to the H1, H5, VC15 or VC30. VC15 and VC30 were equally distant from H1 and H5 based on PC1. As these samples were concentrated for different lengths of time with vacuum concentration, and yet they had a similar aroma profile indicating that the volatiles that differentiated them from the H1 and H5 were

lost near the beginning of the processing and were likely more volatile, with lower boiling points.

b) Flavour

PCA analysis of the flavour attributes found that the first component had an eigenvalue of 4.24, and explained 53.0% of the total variation, and the second component had an eigenvalue of 2.30 and explained 28.8% of the total variation, combining to explain 81.8% of the total variation in the data. PC1 was positively correlated with overall flavour, vanilla flavour, raisin flavour, woody flavour and bourbon flavour and negatively correlated with sweet flavour. PC2 was positively correlated with butterscotch flavour and bitter flavour (Figure 7.16). Full details of PCA are in Appendix 7.

The overall flavour attribute had the most effect on the other attributes, being positively correlated with raisin flavour, bitter flavour, woody flavour and bourbon flavour (Table 7.13). Sweet flavour was negatively correlated with raisin flavour and bourbon flavour. There were positive correlations between bitter flavour and vanilla flavour as well as bitter flavour and woody flavour. This maps onto the patterns seen in the eigenvectors on Figure 7.16.

Table 7.13: Correlation matrix for flavour attributes in principal component analysis. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	-0.525	0.538	-0.076	**0.714**	**0.681**	**0.804**	**0.814**
Sweet Flavour	-0.525	**1**	0.024	0.468	**-0.725**	-0.131	-0.386	**-0.728**
Vanilla Flavour	0.538	0.024	**1**	0.105	0.288	**0.672**	0.629	0.303
Butterscotch Flavour	-0.076	0.468	0.105	**1**	-0.351	0.567	0.208	-0.582
Raisin Flavour	**0.714**	**-0.725**	0.288	-0.351	**1**	0.247	0.458	0.655
Bitter Flavour	**0.681**	-0.131	**0.672**	0.567	0.247	**1**	**0.853**	0.276
Woody Flavour	**0.804**	-0.386	0.629	0.208	0.458	**0.853**	**1**	0.578
Bourbon Flavour	**0.814**	**-0.728**	0.303	-0.582	0.655	0.276	0.578	**1**

The patterns seen in the PCA bi-plot (Figure 7.15) differ from those seen for the aroma attributes (Figure 7.14). The samples were again grouped based on their type, but the arrangement of the groups relative to each other differed. H1 and H5 were positioned in the lower right-hand side of the plot, indicating that they had high raisin flavour, bourbon flavour and overall flavour, and low sweet flavour and butterscotch flavour. The next closest samples to H1 and H5 were the VC samples, which were positioned near the centre of the plot - they were moderate in rating for all the attributes. MD10 and MD 30 were the next samples in the line of samples along the x axis, being slightly negative for PC1 – they were low in raisin flavour, bourbon flavour and overall flavour and higher in sweet flavour. At the end of this line of samples was V, the pure vanillin sample. As it was positioned on the lower left-hand side of the plot, it was far from all the eigenvectors on the plot, so was low in all attributes. This agreed with the values seen in Table 7.11, the mean values, where V was low in all attribute ratings. This was very similar to the pattern seen in the aroma PCA, where the samples were arranged in order from most to least processed, ending with vanillin.

The difference between the aroma analysis and the flavour analysis however lies in the positioning of the SFE extracts. For aroma, they followed the same pattern as the other samples. For flavour the SFE extracts differed in flavour profile the most from any of the other samples, being positioned in the upper centre of the plot (Figure 7.15), indicating that they were high in sweet flavour, butterscotch flavour, bitter flavour and vanilla flavour.

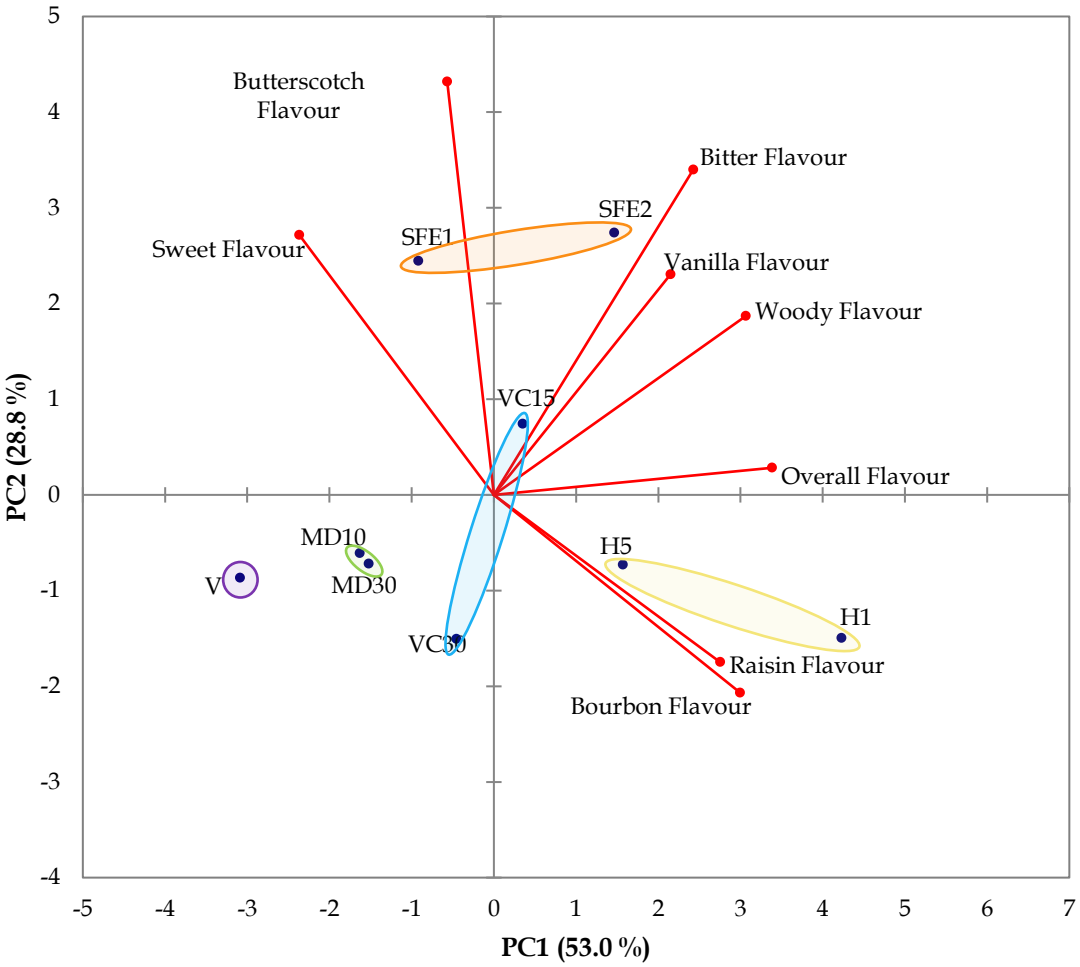


Figure 7.15: Bi-plot of the first two principal components identified through PCA of all attributes for vanilla concentrate samples. The first component had an eigenvalue of 4.24 and the second component had an eigenvalue of 2.30. The cumulative proportion of variation explained by the components is 81.8%. H1 and H5 are circled in yellow, VC15 and VC30 are circled in blue, MD10 and MD30 are circled in green, SFE1 and SFE2 are circled in orange and V is circled in purple.

The reason that these SFE samples would have differed from the other samples was the process used. All the other samples started with the ethanol extracts – H1 and H5 – whereas SFE1 and SFE2 started from the vanilla beans and used the non-polar solvent of carbon dioxide compared to the more polar solvent of ethanol used for the other extracts. As vanillin is non-polar, this makes supercritical carbon dioxide ideal for extracting vanillin (Castillo-Ruz *et al.*, 2011) The difference in the flavour profile indicated that much of the characteristic flavour profile of an ethanol extract was coming from the smaller concentration compounds, which are likely to be more polar, and so were not extracted in the supercritical carbon dioxide process.

From both the sensory results and the GCMS results, it was seen that the vacuum concentrates were the most similar to the standard ethanol extract. The maltodextrin powders were next, containing some of the same volatile compounds as the ethanol extract, but many had been lost in the concentration process. The supercritical carbon dioxide extract was the most different compared to the ethanol extract, containing only vanillin in common with the ethanol extracts, although it was more concentrated and did contain many other non-polar compounds.

The vacuum concentrates were most similar to the ethanol extract, as the distillation process used was gentle, and did not cause noticeable loss of the volatiles. This was a result of the lower temperature of 40^{o}C used; most of the flavour compounds had boiling points much higher than ethanol and water, so would not be removed in the vacuum concentration process. This was seen for both the GCMS, where the chromatograms did not change as the samples were concentrated. In the sensory analysis, with this set of samples was the most similar to the standard ethanol extract (H1 and H5) of the various concentrates produced. The vacuum concentrated samples, as the most similar to the standard ethanol extract would be most suitable for applying to foods, where a concentrated vanilla flavour extract is required.

The maltodextrin powders were less similar to the ethanol extracts and had lost many of the volatile compounds identified in Chapter 6, with just five main peaks seen on the GCMS chromatograms. The volatiles would have been lost during both the vacuum concentration and the freeze-drying process. In the freeze drying, the processing resulted in the loss of more volatiles than vacuum concentration, as there was a greater reduction in pressure, which could have allowed more volatiles to evaporate and be lost from the vanilla concentrate. This combination of conditions may have allowed more of the flavour compounds in the vanilla concentrate to evaporate during the drying. This would have resulted in the different GCMS profile for the maltodextrin vanilla powders, and the greater degree of difference in the sensory profile of the original ethanol vanilla extract. These powdered forms of vanilla would be useful in industry were the application requires no moisture present, such as in powdered cake mixes, as the flavour of the natural vanilla extract is still preserved.

The final method trialled, supercritical carbon dioxide extraction produced the extract that differed the most from the standard ethanol extract, both in terms of GCMS profile and sensory profile. Carbon dioxide was the solvent used in the supercritical

carbon dioxide extraction, any extraction using this solvent would primarily remove non-polar volatiles. In Chapter 6, it was found that the majority of the volatiles compounds in vanilla extract are polar. Vanillin is lipophilic and so was the volatile extracted most efficiently by the carbon doixide, seen both in the GCMS profile and the sensory profile.

The supercritical extracts were more similar to pure vanillin than the standard ethanol extract. The non-polar, long chain compounds also extracted in this process did not contribute to any of the aroma or flavour attributes normally found in vanilla extracts and as vanillin was the main compound extracted these concentrates had a similar sensory profile to vanillin. The application of supercritical carbon dioxide extracts to food products should be carefully considered as the method itself is expensive and the sensory profile was different from an ethanol vanilla extract. However, if a natural vanilla extract with minimal moisture content is required, this extract could be useful. Vanillin can also be produced from lignin (Arabi *et al.,* 2016*),* a highly cost-effective method of producing the flavour from a natural source.

7.4 Conclusions

During initial trials, it was found that the vacuum concentration was limited by viscosity to approximately 35 mg/ml vanillin concentration. The maltodextrin powders were successful with a flavour loading of 30% w/w, although in future trials a higher flavour loading could be tested. Supercritical carbon dioxide was able to produce extracts up to 380 mg/ml concentration, with yields of 7.5% w/w from freeze dried vanilla beans.

Based on GCMS chromatogram profiles the vacuum concentrates were most similar to the ethanol extract, containing many of the same compounds. Some vanillin and guaiacol were detected in the condensate and therefore were lost from the concentrate during processing. The maltodextrin powders were less similar to the ethanol extracts, containing only five main peaks compared to the approximately 15 main peaks in the ethanol extract. The supercritical carbon dioxide extracts were least similar, containing only vanillin in common with the ethanol extracts and being primarily comprised of long chain, non-polar compounds extracted by the non-polar CO_2 solvent.

Using sensory analysis, it was found that the vacuum concentrates were most similar to the original ethanol extracts. The supercritical carbon dioxide extracts were

more similar to pure vanillin than an ethanol extract, and the maltodextrin powders were intermediate – having characteristics like both vanillin and the ethanol extracts.

Overall, the vacuum concentrates were the most viable extracts produced, being simplest to produce and most similar to ethanol extracts for both sensory characteristics and GCMS profile. The maltodextrin powders would be useful for applications were the addition of moisture is undesirable, and other drying methods could be explored to reduce the cost of production. The supercritical carbon dioxide extract will be expensive to produce and has sensory characteristics more like vanillin than the ethanol extract.

8. Effect of Milk Fat and Sucrose on the Aroma and Flavour Profile of a Natural Vanilla Extract

8.1 Introduction

Although vanilla extract is used in a wide range of food products, little research has been carried out to investigate the effects that the food matrix components have on the aroma and flavour of natural vanilla. Using a range of different sensory analysis methods, including quantitative descriptive analysis and the sensory spectrum method, Stampanoni Koeferli *et al.* (1996) investigated the effects of changing the fat, sugar and solids non-fat (SNF) content in a typical vanilla ice cream formulation, flavoured with natural vanilla extract, on a range of attributes chosen by the participants. It was found that fat, sugar and protein all affected the flavour of the ice cream, but interactions between the components made it hard to draw definite conclusions on which component was causing the effect.

Other studies have investigated vanillin rather than vanilla extract. These include investigations into proteins (Li *et al.*, 2000; Reiners *et al.*, 2000) and fats (Li *et al.*, 1997; Hyvönen *et al.*, 2003; Carrapiso *et al.*, 2004; Frøst *et al.*, 2005; Tomaschunas *et al.*, 2013), carried out in ice cream, custard or with a milk base. Proteins were found to decrease the perception of vanillin, due to cysteine-aldehyde condensation or Schiff base formation (Hansen and Heinis, 1991), also supported by Li *et al.* (2000).

Fats were found to not affect the perception of vanillin (Carrapiso *et al.*, 2004; Tomaschunas *et al.*, 2013), although Li *et al.* (1997) found that higher fat content increased the time taken for the vanillin flavour to reach maximum intensity and Carrapiso *et al.* (2004) found that fat increased the sweetness perceived. Frøst *et al.* (2005) in contrast found that a range of flavourings in ice cream, including vanillin, b-ionone, d-nonalactone and isopentyl acetate tended to have their flavour enhanced by the increase in fat content.

There have been no previous studies looking at the effects of sugar on the sensory profile of vanilla extract or vanillin, however sugar can increase the perception of sweet tastes, mask bitter flavour and can cause a 'salting out' effect at concentrations over 20% (Van Ruth and Roozen, 2002; Reineccius, 2006; Guichard, 2012).

The conflicting or missing data reported on the impact of different components on vanilla flavour emphasises the lack of information about the interactions between flavours and food components. Hence the aims of this chapter were:

- to investigate the effect of milk fat on the aroma and flavour of natural vanilla extract in a model system
- To investigate the effect of sugar on the aroma and flavour of natural vanilla extract in an aqueous solution

8.1.1 Background Information on Mixture Designs

A mixture design is an experimental method that allows for a range of components to be investigated – any number of components can be included in the design and the sum of the components is always one (Box and Draper, 2007). By varying the relative proportions of the components of a mixture, it is possible to determine the effects of each component on the factor under investigation such as the aroma and flavour sensory profile of vanilla extract. The proportions of each component required to achieve a maximum or minimum response for that factor can be determined, without having to run all the possible combinations of components.

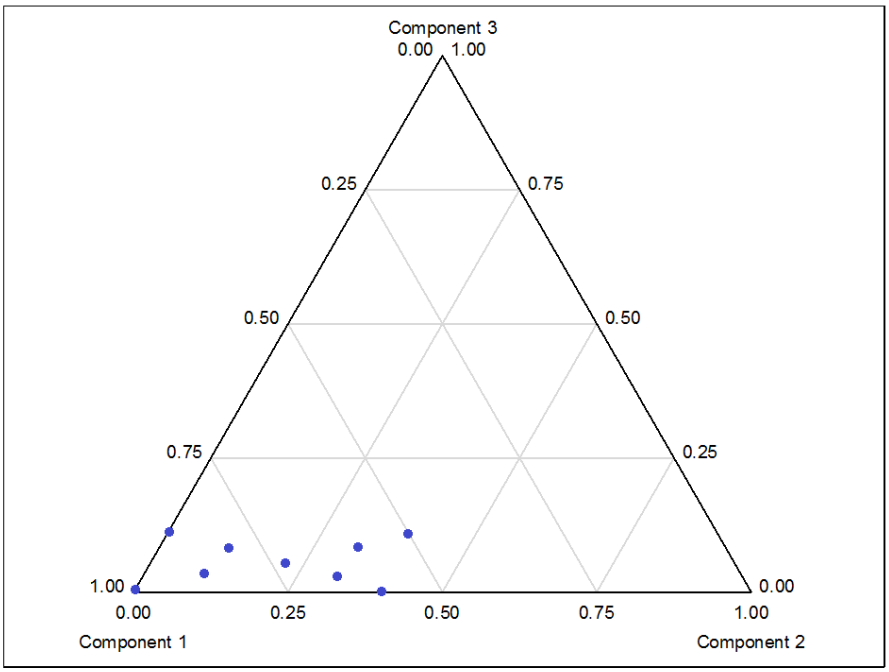


Figure 8.1: Example contour plot showing the placement of the samples used in the mixture design for the investigation in to the effect of fat on vanilla aroma and flavour. For the example experiment Component 1 is Skim Milk, Component 2 is Cream and Component 3 is Vanilla Extract. The blue dots represent the proportions of each component chosen.

For the investigation into the effect of fat on the aroma and flavour of vanilla extract, a milk base was used, as vanilla is often used in milk products, such as ice cream, custard and chocolate. Using a milk base also meant that the proportions of fat and vanilla could be varied by using cream, skim milk and vanilla extract as the three components of the mixture.

Sucrose, water and vanilla extract were used as the three components in the mixture design for the investigation into the effect of sugar on vanilla. As the aim was to study the relationship between the sugar and the vanilla extract, a water base was used rather than milk for the investigation into the effects of sugar to reduce confounding factors such as milk flavour.

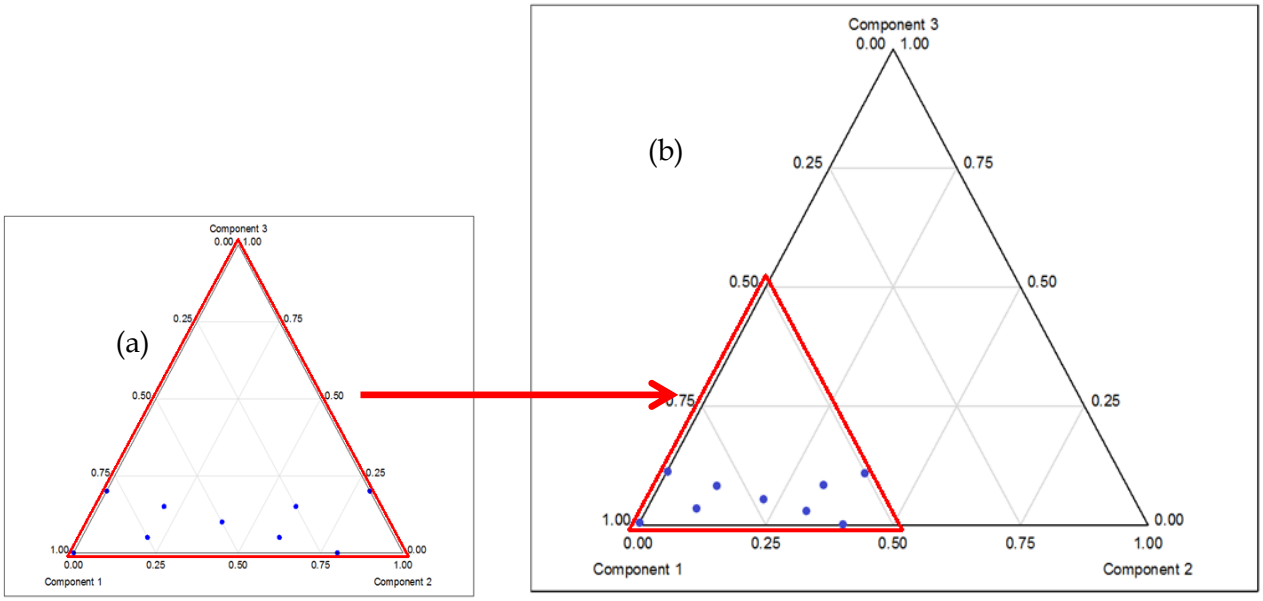


Figure 8.2: Diagram demonstrating how pseudo components relate to the original components in a mixture method design. Plot (a) shows the arrangement of the samples on the pseudo component plot, and Plot (b) shows the arrangement of the samples on the full plot. The red triangle shows how plot (a) fits into plot (b).

In choosing the concentrations for the experiment, the vanilla ranged from 0% to 10% v/v for both experiments. Although this is a higher concentration than would be expected in any typical food product, it would increase the range of the results, allowing for a clearer picture of the effect of the vanilla on the given attribute. The fat content ranged from 0 to 15%, as this encompasses ranges normally found in foods, such as ice cream. The sugar ranged from 0% to 20% as sugars in food products can range up to 20%. For example, ice cream can contain 21% sugar, soft drinks range up

to 12% sugar and custard up to 15% sugar. Skim milk and water were chosen to fill in the remaining proportion in the mixture designs, as they both represented 'not present' for the chosen food component, that is, fat and sugar, respectively.

The mixture design used is shown in Figure 8.1. It can be seen that the points are clustered in the bottom left corner of the plot. This is because the concentrations of the vanilla and cream were chosen to be similar to real food systems – 100% vanilla extract or 100% cream would not often be found in a food product.

For the analysis of the results, pseudo-components were used, to focus on the area of interest in the plot. Pseudo components are used when the range being investigated does not span the total of the available mixture space. Figure 8.2 shows how pseudo components relate to the original components of the contour plot. It can be seen that although the pseudo components are given proportions from 0 to 1 in (a) of Figure 8.2 they are in fact only relating to a small area of the original plot from 0 to 0.5 in (b) of Figure 8.2. This stands true for all contour plots in this chapter. After the regression analysis is completed and a contour plot generated, the effects of each component on each attribute will be determined.

One final point regarding the interpretation of the contour plots concerns the legend on the plots. The legend reflects the predicted response for each attribute, and so for some of the attributes the scale exceeded nine, as within the area of the contour plot, according to the regression analysis the sample would have been rated higher than nine. The legend for each plot is different and reflects the regression equation determined for each individual attribute.

8.2 Materials and Methods

8.2.1 Materials

The materials used for this experiment were:

- Anchor skim milk (<0.1% milk fat) (Fonterra Brands, Auckland, New Zealand)
- Anchor cream (37.7% milk fat) (Fonterra Brands, Auckland, New Zealand)
- Chelsea white sugar (Sucrose) (NZ Sugar, Auckland, New Zealand)
- Heilala Single fold natural vanilla extract (1.65 mg/ml vanillin concentration) (Heilala Vanilla Ltd., Tauranga, New Zealand)
- Reverse Osmosis (RO) water (Merck Millipore, Darmstadt, Germany)

The composition of each material used in the mixture designs, based on fat, protein and sugar content can be found in Table 8.1.

Table 8.1: Concentration of fat, protein and sugars for ingredients used for mixture method designs. Information was sourced from the nutritional panel of each products.

	Fat (g/100g)	Protein (g/100g)	Sugars (g/100g)
Skim Milk	0.1	4	5
Cream	37.3	2.4	3
Sucrose	0	0	100
RO water	0	0	0
Vanilla Extract	0	0	0

For sensory testing, all milks were used at one week before their expiration date, having been purchased at two weeks to expiry and stored at 4^{o}C ± 1^{o}C before use to control storage conditions as best as possible.

8.2.2 Methods

8.2.2.1 Sensory Analysis

The sensory analysis of samples was conducted using the method outlined in *Section 3.1.4,* using eight previously trained panellists; however there were some modifications in the methods, to accommodate the differing solution types.

Two new attributes were introduced to encompass all the characteristics of the vanilla extract in the milk – creamy aroma and creamy flavour. Using a nine-point categorical scale, with one as barely detected and nine as strongest imaginable, the references used were skim milk rated as a two, 20% cream in skim milk rated as a five and 40% cream in skim milk rated as a nine for both aroma and flavour. The straw attribute was removed from the list of flavour attributes, as the panellists were not able to differentiate between the products with this attribute in Chapter 5, so it was not aiding in the understanding of the relationships between the variables.

For the samples in sugar water, overall flavour, sweet flavour and bitter flavour were rated on a scale from one to 17. The reasons for this were that the vanilla extract was up to 10% concentration, whereas the previous analyses had vanilla extract at approximately 1.5% concentration. Sugar was also added at up to 20% concentration, whereas previous analyses had sugar at 3% concentration.

Table 8.4: Full list of references required for the testing of the effects of fat and sugar on the aroma and flavour of the natural Tongan vanilla extract. RO water is defined as Reverse Osmosis.

Category	Attribute	Reference	Low	Medium	High	Modified Scale, Very High	Modified Scale, Extremely High	Preparation
Aroma	Creamy (Milk based samples only)	Anchor Skim Milk (0.1% Fat) and Anchor Pure Cream (37% Fat)	0% (2)	20% (5)	40% (9)			Mix cream, at stated percentages, with skim milk at 16°C. Serve within 6 hours.
	Overall Aroma	Heilala single-fold vanilla extract	5% v/v (2)	10% v/v (4)	15% v/v (7)			Diluted with RO water at 20°C.
	Artificial Fruity	Sensient artificial banana flavour No. N26	0.001 g/L (2)	0.005g/L (5)	0.01g/L (9)			Diluted with RO water at 20°C.
	Bourbon	Jim Beam bourbon whiskey	0.5% v/v (3)	1.5% v/v (6)	3% v/v (9)			Diluted with RO water at 20°C.
	Caramel	Chelsea brown sugar		10 g/kg (4)	50g/kg (8)			Mix given weight of Chelsea brown sugar with Chelsea white sugar. (g brown sugar/kg white sugar)
	Raisin	Sunmaid Californian raisins		50 g/L (4)	100g/L (8)			Soak raisins in 20°C RO water overnight (12-15 hours), then strain. Use strained liquid as reference.
	Spicy	Gregg's ground spices: 5g cinnamon, 9.4g allspice, 1.1g nutmeg		2 g/kg (4)	8 g/kg (8)			Mix spices together. Mix this combination with Chelsea white sugar to strength specified. (g spices/kg white sugar)
	Vanilla	Brenntag Rhovanil® Vanillin		25 g/L (4)	50 g/L (8)			Dissolve into fresh sunflower oil at 20°C.
Flavour	Creamy (Milk based samples only)	Anchor Skim Milk (0.1% Fat) and Anchor Pure Cream (37% Fat)	0% (2)	20% (5)	40% (9)			Mix cream, at stated percentages, with skim milk at 16°C. Serve within 6 hours.
	Overall Flavour	Heilala single-fold vanilla extract	1% v/v in 3% w/v sugar (3)	1.5% v/v in 3% w/v sugar (6)	2% v/v in 3% w/v sugar (8)	0% in 20% w/v sugar (15)	10% v/v, no sugar (17)	Diluted with RO water at 20°C.

Category	Attribute	Reference	Low	Medium	High	Modified Scale, Very High	Modified Scale, Extremely High	Preparation
	Vanilla	Brenntag Rhovanil® Vanillin	0.16 g/L (4)	0.24 g/L (6)	0.32 g/L (8)			Diluted with RO water at 20°C. Add 3% w/v Chelsea white sugar.
	Sweet	Chelsea white sugar	1.5% w/v (2)	3% w/v (5)	4.5% w/v (8)	12.5% w/v (13)	17.5% w/v (16)	Diluted with RO water at 20°C.
	Butterscotch	Kiwiland butterscotch sweets	5 g/L (3)	7.5 g/L (5)	10 g /L (7)			Diluted with RO water at 20°C until the sweets were fully dissolved.
	Raisin	Sunmaid Californian raisins	30 g/L (3)	45 g/L (5)	60g/L (7)			Diluted with RO water at 20°C with 3% (w/v) Chelsea white sugar. Leave overnight at 20°C (12-15 hours). Strain out raisins and use liquid as reference.
	Bitter	Caffeine (Invita, pure caffeine)	0.25g/L (3)	0.40g/L (5)	0.54 g/L (8)	1.0 g/L (12)		Diluted with RO water at 20°C.
	Straw	Morlife oat straw tea leaves	0.8g/L (3)	1.25g/L (5)	1.6 g/L (8)			Soak tea leaves in boiling RO water for 5 minutes, then strain. Add 1.5% w/v Chelsea white sugar.
	Woody	Vintner's Harvest French toasted oak chips	1:20 dilution of stock solution (3)	1:15 dilution of stock solution (5)	1:10 dilution of stock solution (8)			Stock solution is 1g of wood chips into 250 mL of boiling RO water for 5 minutes, then strain.
	Bourbon	Jim Beam bourbon whiskey	0.1% v/v (3)	0.3% v/v (5)	0.5% (7)			Diluted with RO water at 20°C.

These higher concentrations of vanilla extract and sugar exceeded the upper limits of the original 9-point scale, so it was extended to accommodate the new attribute ratings. New references were chosen by the panel members accordingly to fit the adjusted scales and can be seen in Table 8.2.

Each sample was assessed in triplicate by each of the eight trained panellists, over three testing sessions. Samples were presented monadically and no more than five samples were presented to the panellists in any one session to reduce fatigue. All references for aroma and flavour were provided during the testing session to refer to.

8.2.2.2 Experimental Design

The experimental design was a mixture design with three components: skim milk, cream and vanilla extract for the fat testing, and sucrose, water and vanilla extract for the sugar testing. The final experimental designs used are in Table 8.3 and Table 8.4.

Table 8.3: Mixture design for investigation into effect of milk fat on aroma and flavour of natural vanilla extract. Values are proportions of the total.

Sample	Skim milk	Cream	Vanilla
A	1	0	0
B	0.6	0.4	0
C	0.875	0.1	0.025
D	0.675	0.3	0.025
E	0.75	0.2	0.05
F	0.825	0.1	0.075
G	0.625	0.3	0.075
H	0.9	0	0.1
I	0.5	0.4	0.1

Table 8.4: Mixture design for investigation into the effect of sucrose on aroma and flavour of natural vanilla extract. Values are proportions of the total.

Sample	Water	Sucrose	Vanilla
1	1	0	0
2	0.8	0.2	0
3	0.925	0.05	0.025
4	0.825	0.15	0.025
5	0.85	0.1	0.05
6	0.875	0.05	0.075
7	0.775	0.15	0.075
8	0.9	0	0.1
9	0.7	0.2	0.1

Samples were prepared within four hours of the testing time and stored at the required temperature (16°C ± 2°C for milk solutions (ISO, 2012) and 20°C ± 2°C for water solutions (ISO, 1991)). For the milk solutions, the skim milk and cream were combined to the required proportions by volume, then stirred vigorously for 30 seconds to ensure homogeneity of the samples without the formation of foam. The vanilla extract was then added by volume and the samples stirred vigorously for 15 seconds. For the sugar solutions, the water and sugar were combined to the required proportions by weight, then stirred vigorously for 30 seconds. The sugar solutions were left for five minutes to dissolve the sugar fully before being stirred vigorously for a further 30 seconds. The vanilla extract was then added by weight to the solution and the samples stored at the required temperature.

8.2.2.3 Data Analysis

SPSS (Version 21, IBM, USA) was used to check the performance of the panel with a Student's t-test and the significance of the results with ANOVA. Statistica (Version 13, Dell Inc., USA) was used to generate regression analysis and contour plots to illustrate the relationships between the components in the mixture design. During the regression analysis using the Statistica program, to select either the linear regression or the quadratic regression equation, the R^2 values were compared, as well as the significance of the model in ANOVA. If the linear R^2 was below 0.8, the quadratic regression was selected, as long as it was also significant ($p<0.05$). Singh *et al.* (2015) found that an R^2 of 0.69 was sufficient to analyse results relating to the sensory attributes of chicken and Zheng *et al.* (2011) used the significance of the model, as per ANOVA, to select the appropriate model, setting significance at $p=0.10$ for attributes of rough rice.

8.3 Results and Discussion – Effect of Milk Fat on Aroma and Flavour Perception of Natural Vanilla Extract

For the experiment investigating the effect of milk fat on the aroma and flavour of natural vanilla extract, there were several stages in the results that needed investigation. Firstly, the panel performance had to be assessed to make sure that they were performing reliably as the panel members had taken a break from training while the experiment was being designed and prepared. Secondly, the regression equation for each of the attributes had to be selected from either a linear regression or quadratic regression, and finally the results had to be looked at for significance, with discussion of patterns and relationships discovered by the regression analysis as well as comparison to literature.

8.3.1 Assessment of Panel Performance

As the sensory testing for the aroma and flavour was conducted at separate times, the panel performance for each set of testing was evaluated separately.

The panel performance was evaluated using a duplicate sample at the end of each session, which was compared to the same sample within the same testing session using a Student's t-test. If the two values were not significantly different ($p \geq 0.05$), this indicated that the panellists were able to reliably assess and rate the samples repeatedly.

8.3.1.1 Aroma Panel Performance

A Student's t-test (Table 8.5) showed that the panel was performing reliably. Only one of the 24 attribute-session groupings (eight attributes in each of three sessions) had a significant difference ($p < 0.05$) between the in-session sample and the repeat sample at the end of the session.

Table 8.5: Student's t-test comparing the in-session responses with those of the repeat sample at the end of the session, for all sessions and all attributes, during the analysis of the effects of milk fat on the aroma of natural vanilla extracts. Values shown are p-values. Significant values (p<0.05) are in italics.

Session	Overall Aroma	Creamy Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
1	0.084	0.414	0.235	0.363	0.465	1.000	0.185	1.000
2	*0.006*	0.374	0.296	0.394	0.695	0.175	0.203	0.394
3	0.215	0.690	0.102	0.636	0.771	1.000	0.809	0.611

Table 8.6: Results from ANOVA, looking at all main effects and secondary interaction effects. Values shown are p-values. Significant values (p<0.05) are in italics.

	Overall Aroma	Creamy Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Participant	*0.000*	0.600	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Session	0.271	0.018	0.204	0.438	0.636	0.815	0.664	0.715
Product	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Participant: Session	0.275	0.082	0.464	0.110	0.102	0.623	0.861	0.171
Participant: Product	*0.010*	0.411	*0.001*	*0.002*	0.156	*0.001*	*0.009*	*0.000*

ANOVA showed that the participants were able to differentiate between the products ($p<0.05$) for all of the attributes (Table 8.6). Only one of the eight attributes had a significantly different result between sessions – creamy aroma. This indicated that the participants were rating the samples similarly in each session.

The participant:session interaction was not significant for any of the attributes. This is another indication that the panel was performing well during the testing, as this showed that the results for each participant did not differ between sessions.

8.3.1.2 Flavour Panel Performance

For the flavour attributes, the panel performed well, as can be seen by the Student's t-test, which looked at the duplicate samples tested at the end of each session, compared to the same sample within the session (Table 8.7). For all of the attributes, there was no significant difference ($p < 0.05$), indicating that the panellists were rating the replicate samples in each session the same, and therefore were performing reliably.

Table 8.7: Results from Student's t-test to determine the difference between in-session samples and duplicates at the end of each session. Values are p-values, with a significance of p<0.05. Significant values (p<0.05) are in italics.

Session	Creamy	Overall	Sweet	Vanilla	Butterscotch	Raisin	Bitter	Woody	Bourbon
1	0.98	0.74	0.71	0.91	0.16	0.08	0.88	0.77	0.39
2	0.52	0.92	0.82	0.97	0.43	0.23	0.65	0.70	0.16
3	0.93	0.98	0.32	0.74	0.051	0.89	0.89	0.87	0.93

Table 8.8: Summary of ANOVA for the effect of fat on flavour of natural vanilla extract. Significant values (p<0.05) are in italics.

	Creamy Flavour	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
Participant	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*
Session	0.263	0.323	0.816	0.054	0.129	0.358	0.327	*0.001*	0.185
Product	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Participant:Session	0.236	*0.008*	*0.031*	0.205	0.204	0.865	0.071	*0.013*	0.313
Participant:Product	0.612	*0.000*	*0.000*	*0.000*	0.115	0.127	*0.000*	*0.000*	*0.007*

A further indication of the performance of the panel was the ANOVA (Table 8.8). Participant was significant for all attributes but session was not. This showed that although the panellists were rating the samples differently to each other, they were consistent across the sessions, apart from woody flavour. For woody flavour, the session was significant, which indicated that the ratings for woody flavour were different depending on which session it was rated in. As the reference material for woody flavour is a natural product (wood chips), there would be some natural variation in the product, which may have affected the references, and therefore the ratings for woody flavour in the sessions, although every effort was made to reduce this type of effect.

8.3.2 Effect of Milk Fat on Aroma Perception of Natural Vanilla Extract

8.3.2.1 Multiple pair comparison results: mean and differences

For the investigation into the effect of milk fat on the aroma of natural vanilla extract, all samples were separated into significantly different groups based on the attributes (Table 8.9). For all attributes, there were either four or five groups from the nine samples (letters a-d/e), indicating that there was a good spread in the data. However, for some of the attributes, there was only a small range in the ratings, such as artificial fruity, which was rated between 1.1 and 3.0 (Table 8.9). As the panel was found to be performing reliably (Section 8.3.1.1), this indicated that there was little variation in artificial fruity in the samples.

Table 8.9: Multiple pair comparison results for all attributes tested during investigation into effects of milk fat on aroma profile of natural vanilla extracts. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test.

Sample	Overall Aroma	Creamy Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
A	2.2^{e}	2.0^{e}	1.1^{e}	1.0^{d}	1.3^{cd}	1.0^{d}	1.0^{d}	1.2^{e}
B	3.1^{d}	7.3^{a}	1.2^{de}	1.0^{d}	1.2^{d}	1.1^{d}	1.0^{d}	1.1^{e}
C	3.4^{cd}	4.3^{d}	1.5^{de}	1.6^{cd}	2.0^{bc}	1.4^{cd}	1.5^{cd}	2.3^{d}
D	4.0^{bc}	6.3^{abc}	1.7^{cd}	1.6^{cd}	2.1^{b}	1.4^{cd}	1.7^{c}	2.8^{cd}
E	4.1^{bc}	5.1^{cd}	2.2^{bc}	2.0^{bc}	2.4^{ab}	1.9^{bc}	2.1^{bc}	3.1^{bc}
F	4.8^{b}	4.2^{d}	2.1^{bc}	2.5^{b}	2.4^{ab}	1.9^{b}	2.4^{b}	3.4^{bc}
G	4.6^{b}	6.0^{bc}	2.1^{bc}	2.4^{b}	2.6^{ab}	1.7^{bc}	2.0^{bc}	3.5^{bc}
H	6.5^{a}	2.0^{e}	3.0^{a}	3.9^{a}	2.9^{a}	2.8^{a}	3.5^{a}	4.9^{a}
I	4.5^{b}	6.7^{ab}	2.3^{b}	2.3^{b}	2.6^{ab}	1.9^{b}	2.3^{b}	3.9^{b}

The samples that did not contain vanilla extract (A and B), were also rated to contain some artificial fruity aroma, caramel aroma, raisin aroma and vanilla aroma, attributes which described vanilla extract. These attributes may have been provided by the milk. Attributes that have been described in milk include caramelised, soapy, fruity and barny (Wolf *et al.*, 2013).

8.3.2.2 Regression Equations and Contour Plots

Both a linear regression and quadratic regression were carried out for each attribute. They were compared for R^2 value, with the linear regression selected if the R^2 was over 0.8, with the appearance of the contour plot also considered.

Table 8.10: Summary of regression analysis looking at the effect of milk fat on the aroma of vanilla extract.

			Regression Coefficients				
Attribute	**Quadratic or Linear**	**R^2**	**Skim Milk**	**Cream**	**Vanilla Extract**	**Skim Milk -Cream**	**Skim Milk -Vanilla**
Overall Aroma	Q	0.97	2.2	3.7	7.7	-0.6	19.2
Creamy Aroma	L	0.96	2.6	8.6	1.2		
Artificial Fruity Aroma	L	0.89	1.3	1.0	8.6		
Bourbon Aroma	L	0.87	1.4	0.6	11.2		
Caramel Aroma	L	0.89	1.5	1.4	8.4		
Raisin Aroma	L	0.86	1.2	0.8	7.3		
Spicy Aroma	L	0.88	1.3	0.7	10.2		
Vanilla Aroma	L	0.91	1.6	1.2	16.4		

Most of the attributes were best described with linear regression, as can be seen by the R^2 values (Table 8.10). Overall aroma was better described with a quadratic regression, having an R^2 of 0.77 for the linear regression compared to an R^2 of 0.97 for the quadratic regression. Artificial fruity aroma, bourbon aroma, caramel aroma, raisin aroma, spicy aroma and vanilla aroma are all affected the most by the vanilla extract component, having the largest regression coefficient of the three components (Table

8.10). These regression equations were then plotted out into contour plots to visualise the patterns between the variables (skim milk, cream and vanilla extract).

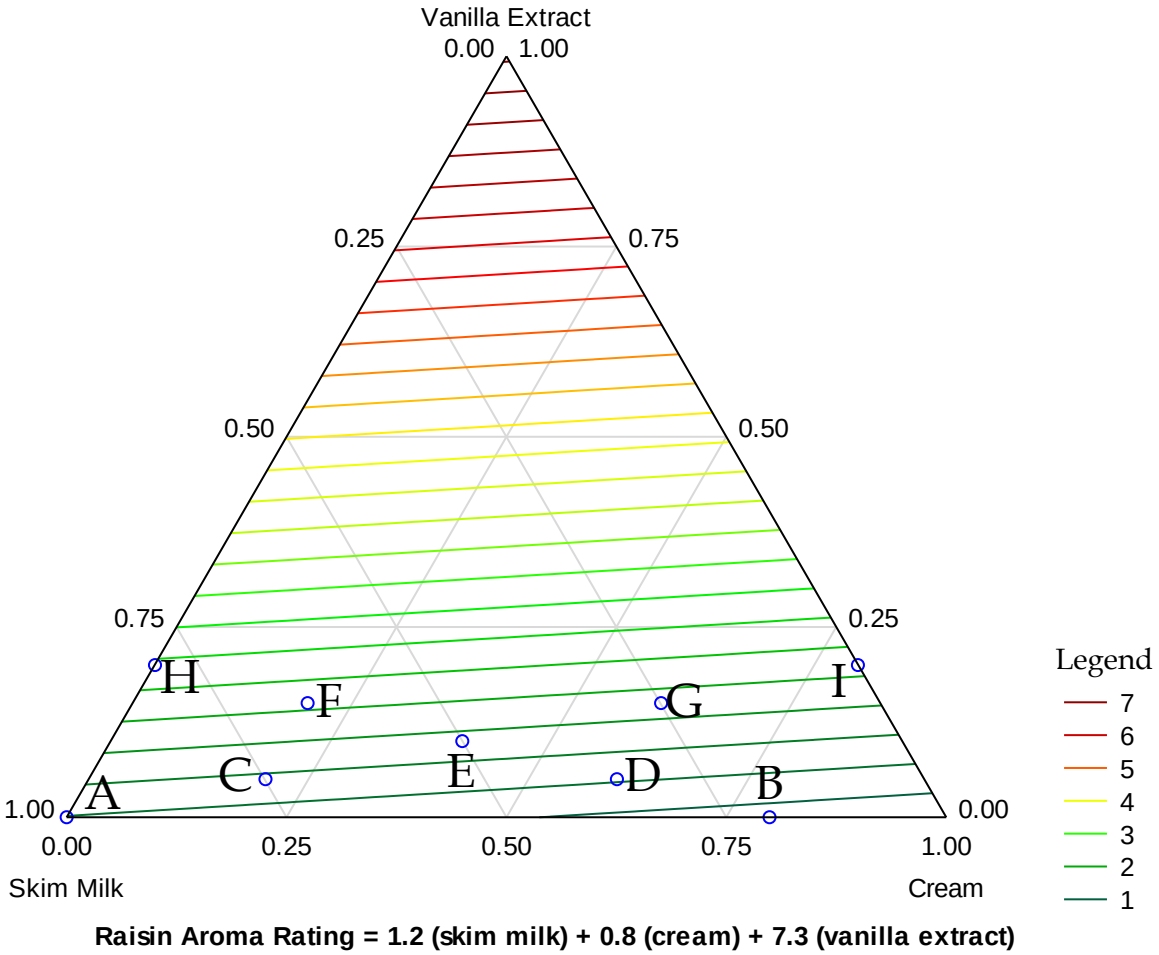


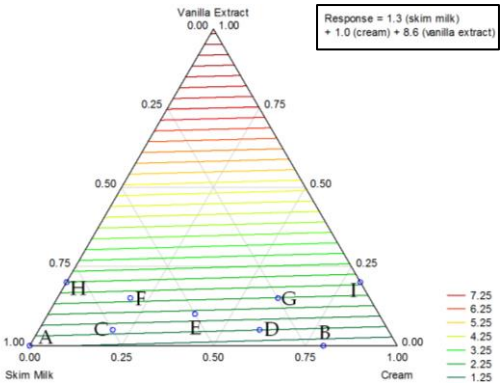
Figure 8.3: Contour plot for raisin aroma for mixture design investigating effects of fat on aroma of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.86, adjusted value 0.82.

Figure 8.3 shows the contour plot for raisin aroma, to illustrate the patterns seen between the regression coefficients for most of the attributes (all aroma attributes except overall aroma and creamy aroma). All contour plots are presented in Figure 8.4. The letters for each of the samples are labelled on the plot, to show the relative positions of each and the legend on Figure 8.3 is only applicable to raisin aroma, the scale of the legend differed for the other attributes, but they followed the same trend. The legend represents the rating given to the given sample by the trained panel. The highest response was seen when the vanilla extract proportion was the highest (sample H), and the lowest response was when the cream proportion is the highest (sample B). This indicated that the cream was reducing the rating, which was reflected in the regression coefficients, with the cream having the lowest value of the three components for all of the attributes (Table 8.10).

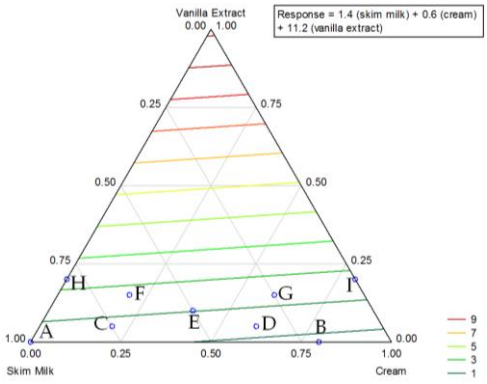
There are several possible explanations for why the cream was able to mask the various aromas. Firstly, the increased fat content in the solution could be dissolving some fat soluble compounds in the mixture from the vanilla extract, making them unavailable to enter the headspace and therefore could not be detected by the panellists. These fat soluble compounds are attracted to the fat globules in the milk and can be either incorporated into the fat globule, or attached to the outside, depending on the specific nature of the compound (lyophilic or amphiphilic respectively). This would reduce the amount of aroma compound in solution, and hence there are less free molecules detected during the sensory testing (Van Ruth and Roozen, 2002; Reineccius, 2006; Guichard, 2012). This effect is dependent on the melting point of the milk fat, as aroma compounds are released when milk fat is liquid but not when solid (Relkin *et al.*, 2004). Milk fat melts over a range of temperatures, due to its complex composition of triglycerides, and so contains both liquid and solid fat at temperatures from -10^{o}C and 70^{o}C (Cant *et al.*, 2017). Therefore, the temperature of the solution would have an effect on the sensory ratings for aroma.

A decrease in aroma with an increase in fat has also been observed in strawberry custards. Martuscelli *et al.* (2008) found that less aroma compounds were released for detection by headspace gas chromatography when the fat content was increased, and the effect was dependent on the hydrophobicity of the compound. The more hydrophobic the compound, the less it was released as the fat content was increased. Akiyama *et al.* (2016) also found that increasing milk fat levels decreased the release of headspace volatiles in coffee and Bayarri *et al.* (2006) found using rapeseed oil that the GCMS measured headspace concentration of lipophilic compounds decreased according to hydrophobicity of the compounds. A study by Arancibia *et al.* (2015) found that upon increase of fat content of a lemon flavoured dairy dessert, the release of linalool, a lipophilic compound, was decreased. In comparison, they found that the release of cis-3-hexen-1-ol, a more hydrophilic compound, was not affected as much. Hyvönen *et al.* (2003) also found that the intensity of a generic strawberry aroma in ice cream decreased with increasing fat content.

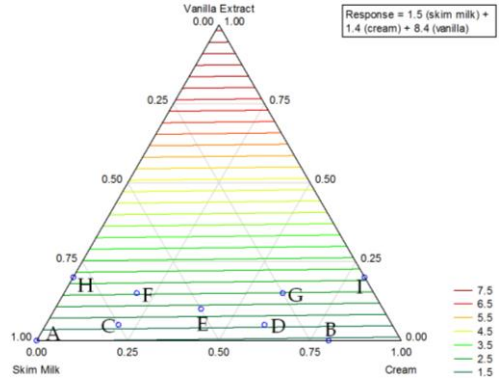
Artificial Fruity Aroma



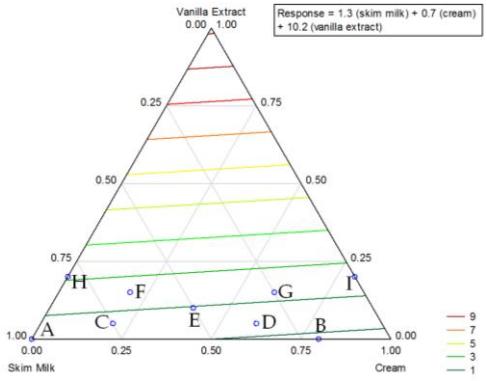
Bourbon Aroma



Caramel Aroma



Spicy Aroma



Vanilla Aroma

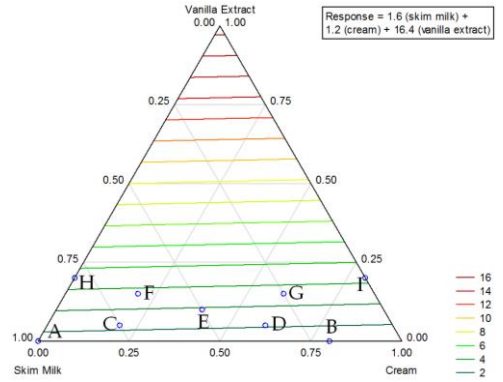


Figure 8.4: Contour plots for the effect of milk fat on artificial fruity aroma, bourbon aroma, caramel aroma, spicy aroma and vanilla aroma.

The increased solids content and viscosity of the solution could also have decreased the rate of migration of aroma particles to the surface of the milk, and therefore decreased the amount of volatiles in the headspace. According to the Stokes-Einstein law (equation 8.1), the diffusion coefficient of a molecule in a given medium is inversely proportional to its viscosity.

$$D_i = \frac{kT}{6\pi\eta r}$$ **(Equation 8.1)**

Where:
D_i is the diffusion coefficient (m^2/s)
k is the Boltzman constant ($1.38 \times 10^{-23} J\ K^{-1}$)
T is the temperature (K)
η is the dynamic viscosity (Pa s)
r is the radius of the molecule (m)

The result of this is that the increased fat and total solids introduced by the cream would have caused a decrease in the migration of the aroma particles to the surface, so they were detected at lower strengths. What was not clear however was whether it was the solubilisation of the aroma compounds with the fat, or the increased viscosity of the solution that was the main cause of the decrease in aroma responses seen.

van Ruth *et al.* (2002) found that the aroma profile of 20 aromas in a sunflower oil in water emulsion, stabilised with Tween 20, was affected by changes in the ratios of the different components. They found that the release of the aromas decreased with an increase in the fat proportion, an increase in the emulsifier proportion or a decrease in the particle diameter. This showed that the interactions between the different components of a food matrix are complex and a range of different factors could be causing the effects seen.

It was noted that the contour plot for vanilla aroma (Figure 8.4) had a wider range of values in the contours on the plot itself. This was due to the wider range of responses received during the testing and is reflected in the vanilla aroma having the largest regression coefficient for vanilla extract, at 16.4 compared to the next highest at 11.2 for bourbon aroma and the lowest at 7.3 for raisin aroma. Therefore, a small change in the concentration of vanilla extract in the formulation would have a large effect on the overall aroma profile.

The attributes that did not follow this same pattern were overall aroma and creamy aroma. Overall aroma was best described with a quadratic regression, and the corresponding contour plot can be seen in Figure 8.5.

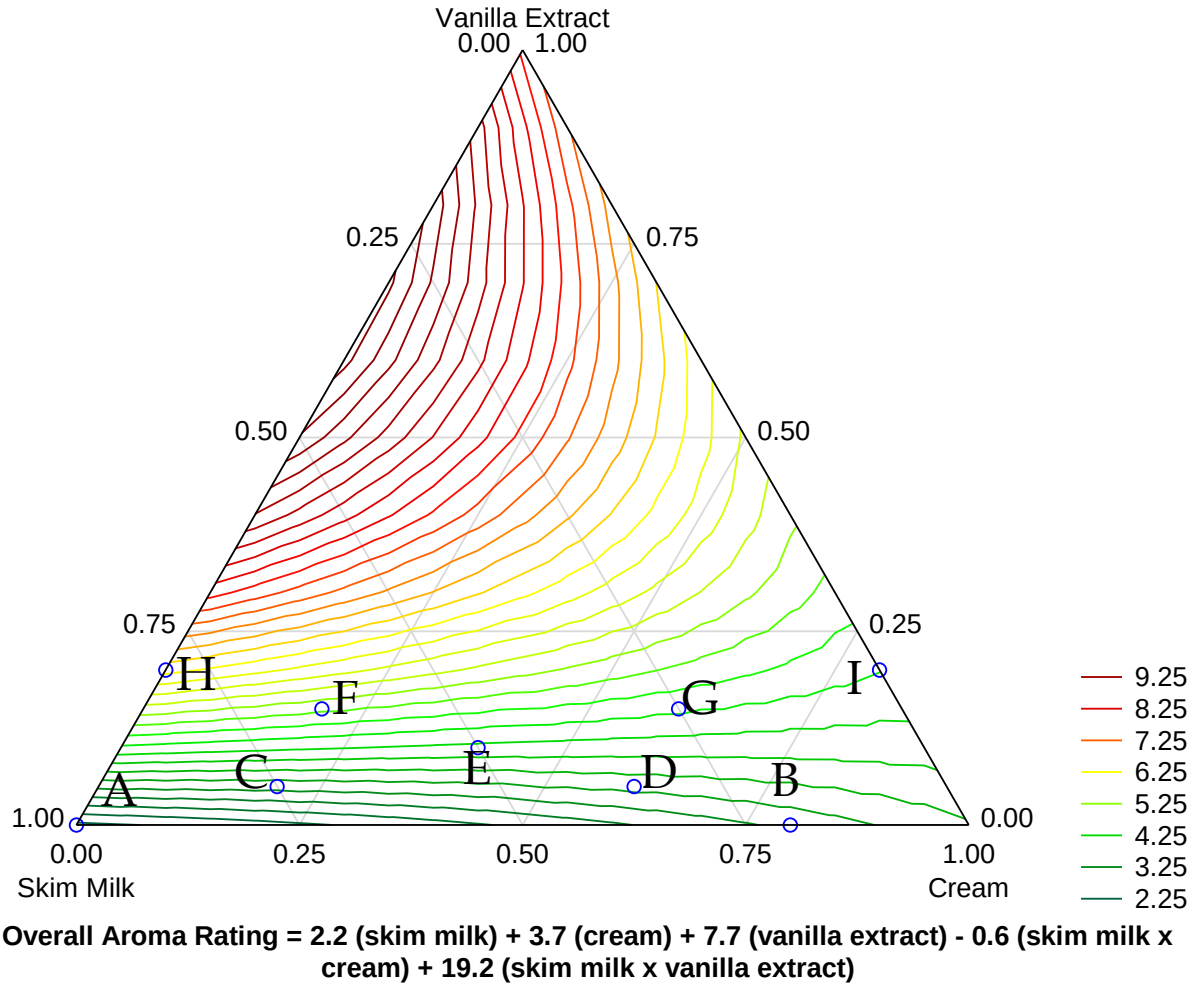


Figure 8.5: Contour plot for overall aroma for mixture design investigating effects of fat on aroma of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.97, adjusted value 0.94.

For overall aroma, the model predicted that the maximum response would occur at 0.35 vanilla extract, 0.65 skim milk (upper left hand side). It would be expected that the highest rating would occur with 100% vanilla extract, as it is a food flavouring ingredient, whereas skim milk and cream are both food ingredients so have a lower aroma intensity. The reason for this difference from the expected was that the experimental design was limited to 10% vanilla and 40% cream.

Within the experimental range, the component that had the most effect on the overall aroma response was the vanilla extract, with a regression coefficient of 7.7 compared to 3.7 for cream and 2.2 for skim milk (Table 8.10). As the regression coefficients for the individual components were positive, this indicated that they all added to the overall aroma rating when increased, however the regression coefficient for the skim milk-cream interaction was -0.6. When both skim milk and cream were increased, the overall aroma rating decreased. The lowest overall aroma response was

recorded when the mixture contained only skim milk (sample A), and the highest response was when the mixture contained only skim milk and vanilla extract (sample H). The samples that contained more cream (sample B) rated higher than skim milk alone (sample A), yet lower than the high vanilla in high cream (sample I). This difference between the skim milk and the cream was caused by cream having more aroma than skim milk (Frøst *et al.*, 2001). However, when the vanilla content was increased, the same effects as were seen in the other attributes were seen, with the higher cream samples (B, G and I) having a lower overall aroma than the higher skim milk samples (A, C and H).

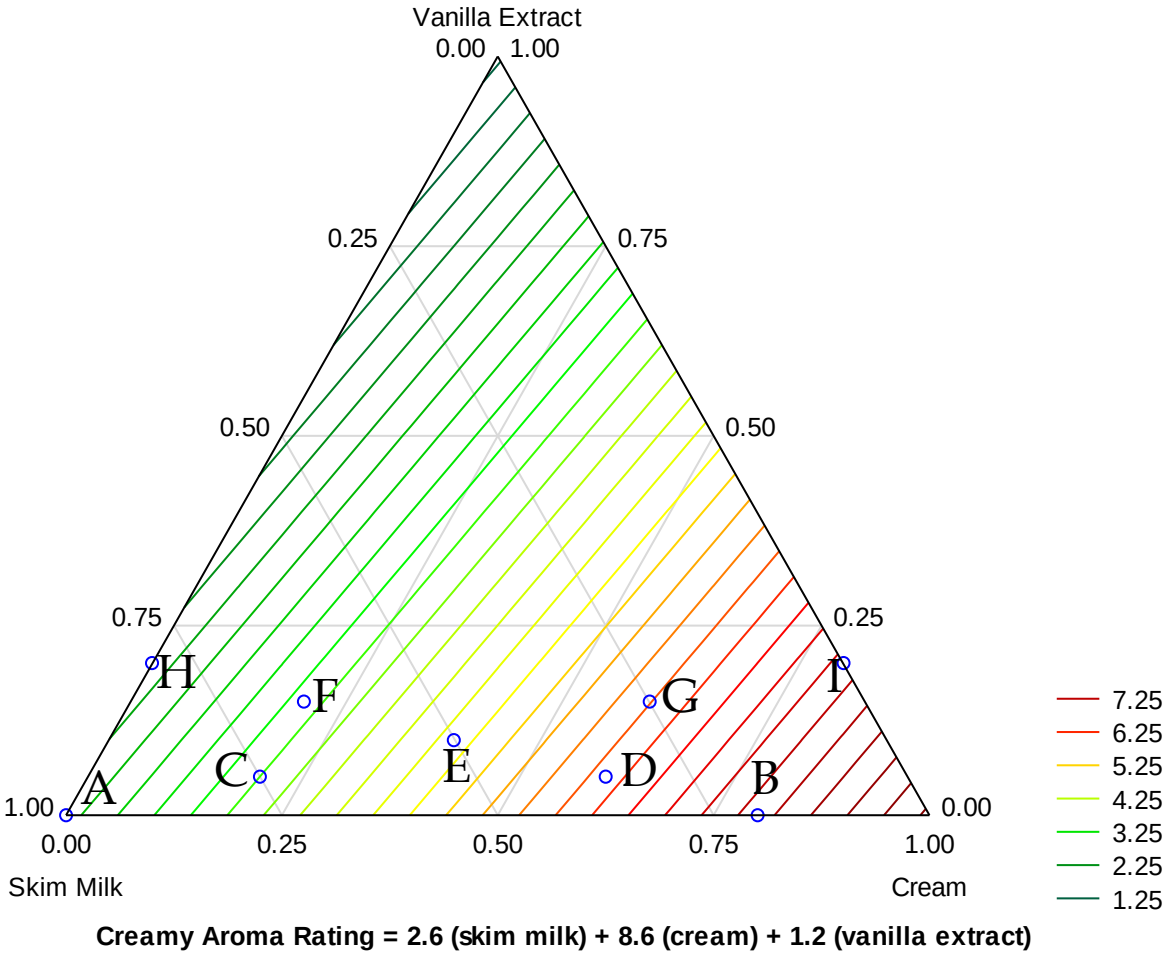


Figure 8.6: Contour plot for creamy aroma for mixture design investigating effects of fat on aroma of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.96, adjusted value 0.94.

The other attribute that did not follow the trend seen for the majority of the aroma attributes was creamy aroma. The regression analysis found a linear relationship in the data, with an R^2 of 0.96 (Table 8.10). For creamy aroma, the cream component had the

most effect on the response (Figure 8.6) and it also had the largest regression coefficient. As this attribute was defined as the characteristic aroma of cream or butter, it was expected that the response would be primarily based on the cream concentration. However, skim milk also increased the rating of creamy aroma, with a regression coefficient of 2.6, which was attributed to the nature of the skim milk as a milk product and so it would contain some of the characteristic creamy aroma associated with milk. Frøst *et al.* (2001) showed that cream had a higher characteristic creamy aroma than skim milk, owing to the increased fat content. The vanilla extract component also had a positive regression coefficient and increased the creamy aroma response. Milk has been described as sweet, fruity and floral (Wolf *et al.*, 2013) The addition of vanilla extract to the milk may have caused an increase in these aroma notes, and thus increased the perceived creaminess.

8.3.3 Effect of Milk Fat on Flavour Perception of Natural Vanilla Extract

8.3.3.1 Multiple pair comparison results: mean and differences

The products were differentiated by the attributes (Table 8.11). Within the range of the mixture design, the attributes were able to separate the products into either three or four significantly different groups.

Sample A and sample B did not contain any vanilla extract, and so any ratings for the attributes were provided by the milk. Milk should have a "neutral flavour profile that is pleasantly sweet, with no distinct aftertaste" (Alvarez, 2009). This was reflected in the rating of the sweet flavour as 2.0 for sample A, skim milk alone, and 2.4 for sample B, 40% cream and 60% skim milk. Although the difference between the sweet flavour in sample A and B was not significant, it was found that this did not reflect the sugar content of the samples. Skim milk contained more total sugars, at 5.0 g/100g, than cream, at 3.0 g/100g ((Anchor, 2017b; Anchor, 2017a). This effect had been seen before, with Frøst *et al.* (2001) noting that milk samples tended to rate higher in sweetness with increased fat rather than increased sugars.

Table 8.11: Multiple paired comparison results for all flavour attributes tested to determine the effect of milk fat on flavour profile of natural vanilla extracts. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test.

Sample	Creamy Flavour	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
A	2.1^{e}	2.0^{e}	2.0^{d}	1.0^{d}	1.1^{c}	1.0^{c}	1.1^{d}	1.0^{d}	1.0^{d}
B	6.5^{a}	2.6^{e}	2.4^{cd}	1.1^{d}	1.2^{c}	1.0^{c}	1.1^{d}	1.0^{d}	1.0^{d}
C	3.8^{cd}	2.8^{de}	2.4^{bcd}	2.2^{c}	1.5^{bc}	1.2^{c}	1.5^{d}	1.4^{d}	1.4^{d}
D	5.9^{ab}	2.9^{d}	2.7^{abc}	2.1^{c}	1.5^{bc}	1.2^{c}	1.3^{d}	1.3^{d}	1.3^{d}
E	5.3^{ab}	4.4^{c}	3.0^{a}	3.2^{b}	1.8^{ab}	1.5^{bc}	2.2^{c}	2.2^{c}	2.3^{c}
F	3.2^{de}	4.9^{c}	2.6^{abc}	3.9^{ab}	1.9^{ab}	1.8^{b}	3.1^{b}	3.4^{b}	3.3^{b}
G	4.9^{bc}	5.2^{c}	2.8^{ab}	4.0^{ab}	2.3^{a}	1.8^{b}	3.3^{b}	2.9^{b}	3.3^{b}
H	2.5^{e}	7.2^{a}	2.7^{abc}	4.4^{a}	2.2^{a}	2.6^{a}	4.8^{a}	4.8^{a}	5.2^{a}
I	6.0^{ab}	6.1^{b}	2.8^{abc}	4.5^{a}	2.3^{a}	1.8^{b}	4.3^{a}	3.3^{b}	4.1^{b}

However, there were some flavour defects that could occur in milk which might have affected the responses of the attributes of the vanilla extract. A scorecard created by the ADSA (American Dairy Science Association) included the following list of attributes that could appear in poor quality milk: acid, bitter, cooked, feed, fermented/fruity, flat, foreign, garlic/onion, lacks freshness, malty, oxidised – light, oxidised – metal, rancid, salty and unclean. Of these, the attributes that overlap with those of the natural vanilla extract are bitter, cooked and malty.

8.3.3.2 Regression Equations and Contour Plots

After regression analysis, the relationship between skim milk, cream and vanilla extract was found to be linear for most of the flavour attributes, except sweet flavour (Table 8.12). For sweet flavour the quadratic regression was selected, as it had an R^2 of 0.88 compared to 0.60 for the linear regression.

Table 8.12: Summary of regression analysis looking at the effect of milk fat on the flavour of vanilla extract.

			Regression Coefficients				
Attribute	**Quadratic or Linear**	**R^2**	**Skim Milk**	**Cream**	**Vanilla Extract**	**Skim Milk -Cream**	**Skim Milk-Vanilla**
Creamy Flavour	L	0.93	2.7	7.6	0.9		
Overall Flavour	L	0.95	2.1	1.9	24.0		
Sweet Flavour	Q	0.88	2.0	2.1	5.6	2.1	-0.6
Vanilla Flavour	L	0.98	1.8	1.3	18.3		
Butterscotch Flavour	L	0.96	1.1	1.3	6.7		
Raisin Flavour	L	0.91	1.1	0.7	6.9		
Bitter Flavour	L	0.95	0.9	0.6	18.3		
Woody Flavour	L	0.94	1.2	0.3	16.9		
Bourbon Flavour	L	0.95	0.9	0.4	19.5		

Overall flavour, vanilla flavour, butterscotch flavour, raisin flavour, bitter flavour, woody flavour and bourbon flavour all followed the same linear trend in the results (Table 8.12). The vanilla extract component had the largest regression coefficient for all

the attributes, indicating that the vanilla extract concentration had the largest effect on the response of these attributes. This was also observed in the contour plots (Figure 8.7 and Figure 8.8), where the highest response was at the top of the graph, where the vanilla extract concentration was highest.

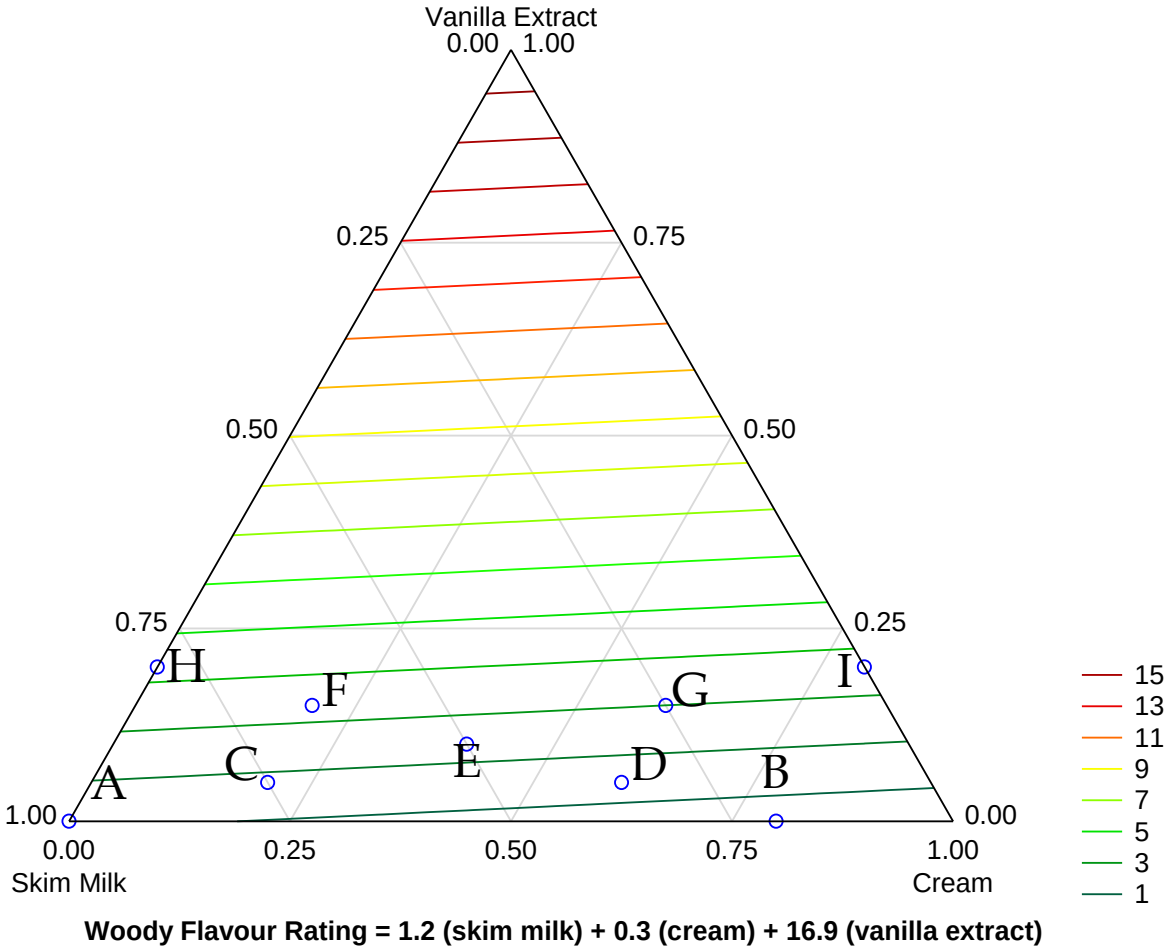
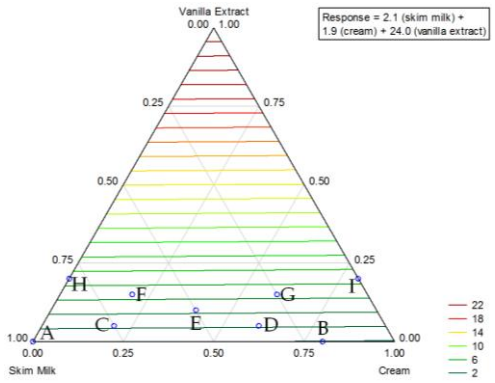


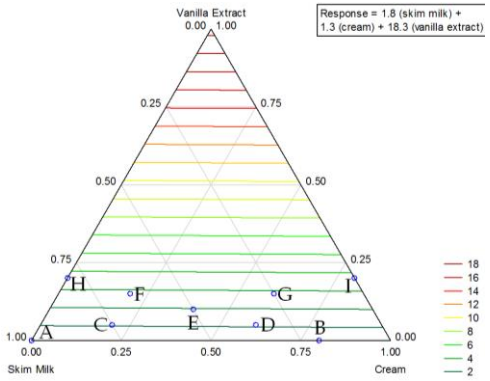
Figure 8.7: Contour plot for woody flavour for mixture design investigating effects of fat on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.94, adjusted value 0.92.

The cream component had the lowest regression coefficient for most of the linear attributes (except creamy flavour and butterscotch flavour). This indicated that the cream was having a reducing effect on the response, with the lowest rating for the attributes being in Sample B – high cream and no vanilla extract (Figure 8.8).

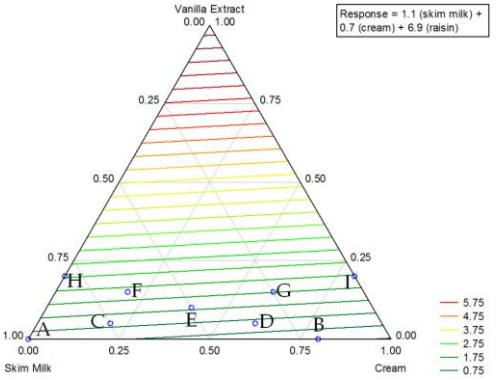
Overall Flavour



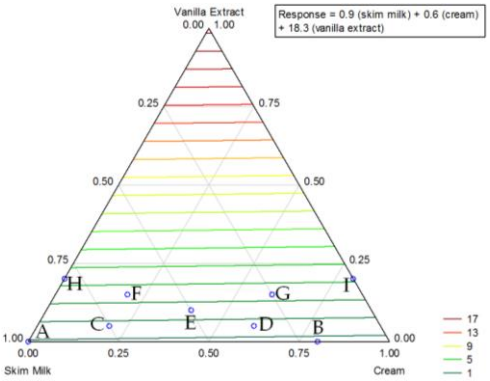
Vanilla Flavour



Raisin Flavour



Bitter Flavour



Bourbon Flavour

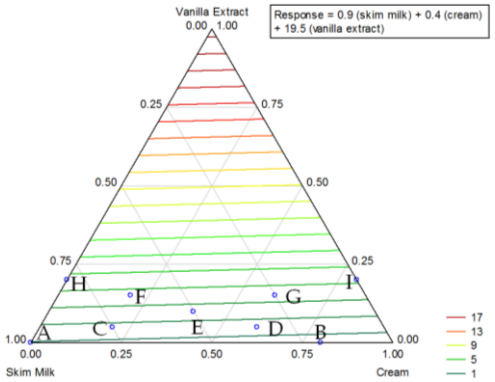


Figure 8.8: Contour plots for the effect of milk fat on overall flavour, vanilla flavour, raisin flavour, bitter flavour and bourbon flavour.

Higher viscosity of the samples could have led to a reduction in the perceived flavour of the mixtures (Hollowood *et al.*, 2002). A review by de Roos (2003) reported that with increased viscosity, the intensity of flavours would decrease. However, the exact cause for this was unclear, with some studies suggesting that it was due to binding of the flavour compounds to the thickening agent (cream in this case)

(Pangborn and Szczesniak, 1974; Yven *et al.*, 1998). Other studies suggested that the reduction in the flavour perceived was due to the reduction in the transport of flavour molecules to the surface (Baines and Morris, 1987; Baek *et al.*, 1999; Rega *et al.*, 2002).

As the cream contained more fat than the skim milk, the fat soluble compounds may have been trapped in the fat phase making them less available to be perceived. A review by Guichard (2002) found that fat was able to retain hydrophobic flavour compounds reducing the headspace concentration and the flavour perceived. The reduction in flavour release was also found in an oil in water emulsion, where the flavour release linearly decreased with the addition of oil (Jo and Ahn, 1999).

In order for flavour compounds to enter the headspace above a food and be detected they must first be released from the lipid phase. The release of the flavour is affected by the melting point of the fat, the solubility of the flavour compound in both water and lipids and the particle size of the fat droplets (Guichard, 2002; Relkin *et al.*, 2004). In particular, vanillin is soluble in fats, slightly soluble in water and freely soluble in ethanol (Burdock, 2009d). As ethanol is used as the solvent to extract the flavours from the vanilla beans during processing (Havkin-Frenkel and Belanger, 2011), the flavour compounds in the vanilla extract that contributed to the various flavour attributes are more fat soluble than water soluble. The polarity of ethanol is 0.654 compared to fat at 0 and water at 1.000. The chemical compounds extracted from the vanilla beans will contain a mix of polar and non-polar compounds with the non-polar compounds dissolving into the fat when mixed with the cream. As vanillin is fat soluble it is likely that there are other flavour compounds in vanilla extract that are also fat soluble. These compounds would also be dissolved by the fat in the cream, and therefore their detection would be reduced during tasting, which would explain the lower ratings obtained when the fat content was higher.

For bitter flavour, the response was highly driven by the vanilla extract component, with a regression coefficient of 18.3 compared to 0.9 for skim milk and 0.6 for cream (Table 8.10). This could be due to the skim milk and cream reducing the response for bitter flavour. Both skim milk and cream contain a range of different food components – fat, protein, sugars. Milk fat has been found to reduce the bitter taste of ibuprofen (Bennett *et al.*, 2012), an increase in cocoa butter in chocolate reduces the bitter flavour from the cocoa beans (Guinard and Mazzucchelli, 1999) and the addition of whole milk rather than skim milk decreased the bitterness of tomato soup (Rosett *et al.*, 1997).

Protein compounds are also able to mask bitter taste, with a range of bitter substances being masked by lipoproteins in a study by Katsuragi *et al.* (1995) and the addition of sodium caseinate to olive oil was able to mask the bitter taste of the phenolics (Pripp *et al.*, 2004). Although milk only contains a small amount of sugars, at around 4% lactose (Siddique *et al.*, 2010), this sugar can still have an effect on the bitter taste of the vanilla extract. Walters (1996) found that bitter taste and sweet taste are linked, so when one is increased the perception of the other is decreased. This relationship has been supported since. An investigation by Clark *et al.* (2011) found that sugar added to beer decreased the perception of the bitter from the hops. Beck *et al.* (2014) found that sucrose was able to mask the bitter taste in model cabbage systems.

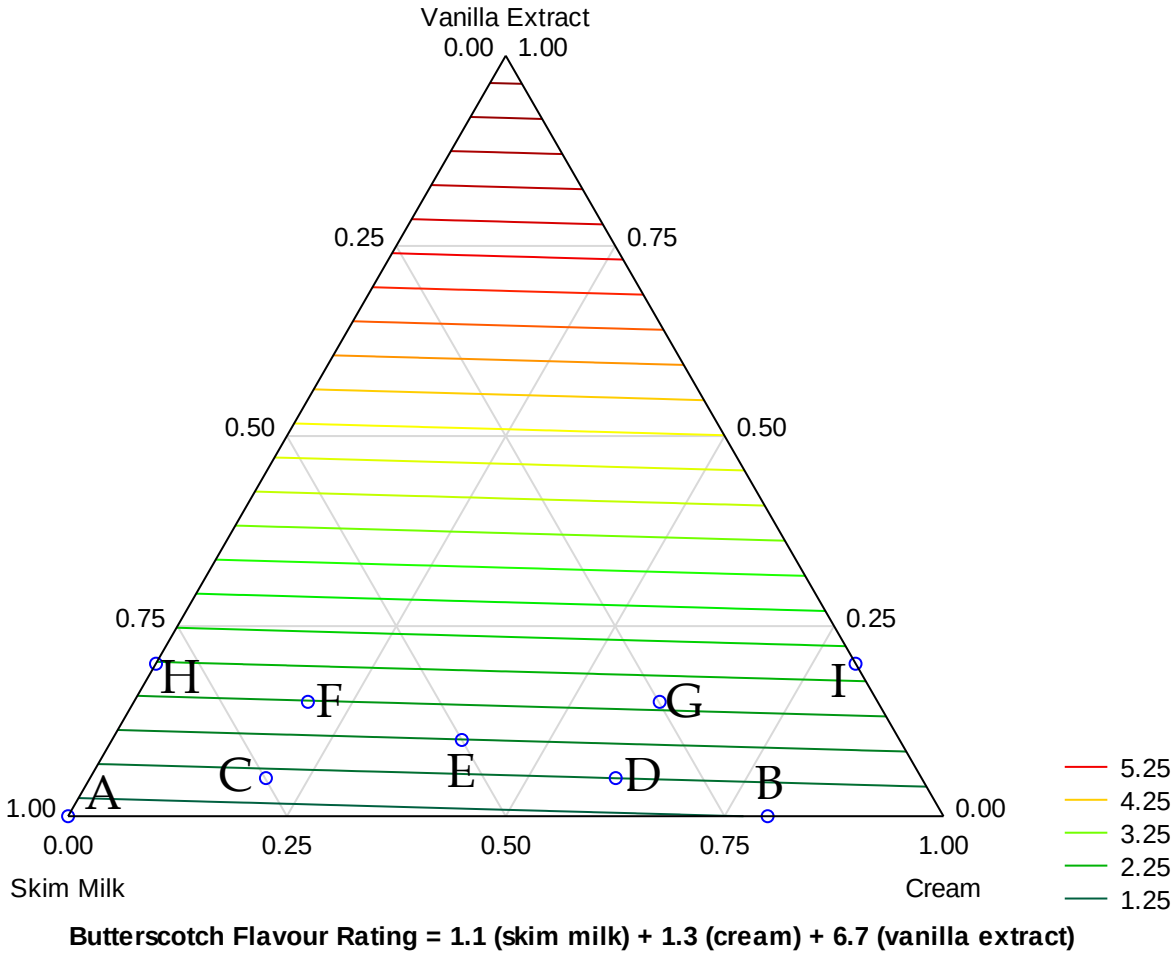


Figure 8.9: Contour plot for butterscotch flavour for mixture design investigating effects of fat on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.96, adjusted value 0.95.

The presence of milk fats, proteins and sugar could have caused the reduction in the bitter flavour that was perceived by the panellists during tasting. As the fat content was varied the most of the three milk components (Table 8.1), it was likely to have

been the primary cause of the effects seen, but the effects of the protein and the lactose should not be ignored. The presence of cream though ultimately was not found to be significant in the results, as the samples with the same vanilla extract concentration but different cream content (and therefore different fat, protein and lactose content) were found to be not significantly different in terms of bitter flavour (Table 8.8). The sample with the higher cream content rated lower in bitter flavour for all pairings of the same vanilla concentration (C-D, F-G, H-I), although it was not significant (Table 8.11).

Butterscotch flavour was found to differ from the other attributes, as it had a higher regression coefficient for cream than skim milk, the opposite to the rest of the attributes (Figure 8.9) thus the lowest response was with skim milk rather than cream. Butterscotch flavour was defined as the characteristic flavour of a butterscotch sweet, including descriptors such as caramel and buttery (*Section 3.1.3.2*). As there was a caramel note included in the definition of this attribute, it was possible that the skim milk and cream also contained some butterscotch. With a mean score of 1.1 for the solution containing skim milk alone (Sample A), and 1.2 for the solution containing 40% cream and 60% skim milk (Sample B), there was possibly some butterscotch flavours coming from the milk as well as the vanilla extract. During processing, all milks are heat treated to 72^{o}C for 15 seconds (Cant *et al.*, 2017). This heat can cause the browning of some of the natural sugars in the milk, which leads to a caramel type note in the milk (Gould and Sommer, 1939). With the low range in the mean scores for butterscotch, from 1.1 to 2.3, the cream and skim milk only had a slight influence on the response, but the primary influencer was vanilla extract.

The other attributes that differed were creamy flavour and sweet flavour. Creamy flavour was found to be mostly affected by the cream component (Figure 8.10), as expected, with a regression coefficient of 7.6 compared to 2.7 for skim milk and 0.9 for vanilla extract (Table 8.10).

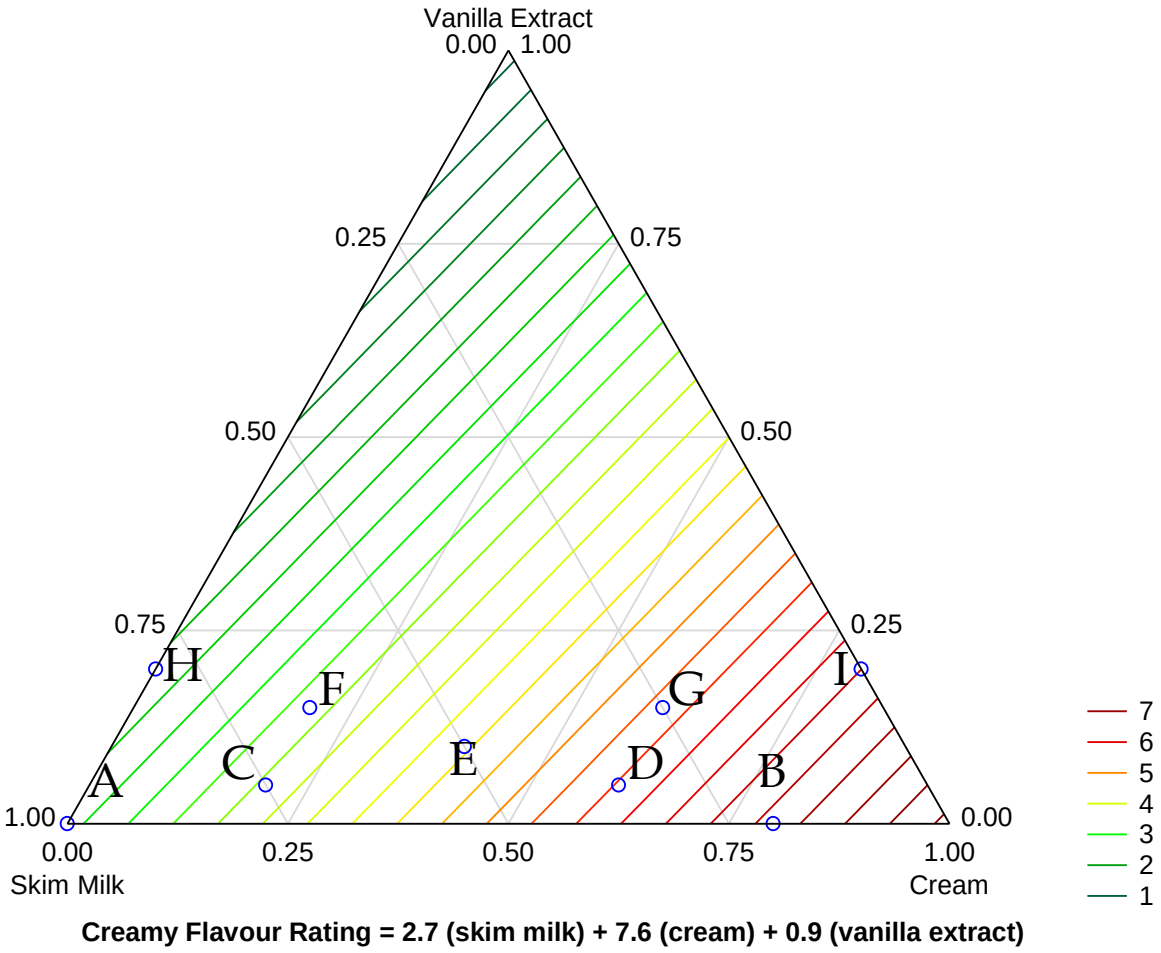


Figure 8.10: Contour plot for creamy flavour for mixture design investigating effects of fat on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.93, adjusted value 0.90.

Creamy flavour was defined as being the characteristic flavour of cream hence the response was largely determined by the proportion of cream in the mixture as it has the most intense creamy flavour of the three components (Frøst *et al.*, 2001). Skim milk also had a slight creamy flavour and the vanilla extract had no creamy flavour.

Sweet flavour was best described with a quadratic regression equation (Figure 8.11). The highest response for sweet flavour was predicted to be when the proportion of vanilla extract was highest and the lowest response was when there was either 100% skim milk or 100% cream. Food products are not often high in both sweet and bitter (Walters, 1997) but in Figure 8.11 and Figure 8.8 the highest response for both sweet flavour and bitter flavour was when there was the most vanilla extract present, in the same sample (H and I).

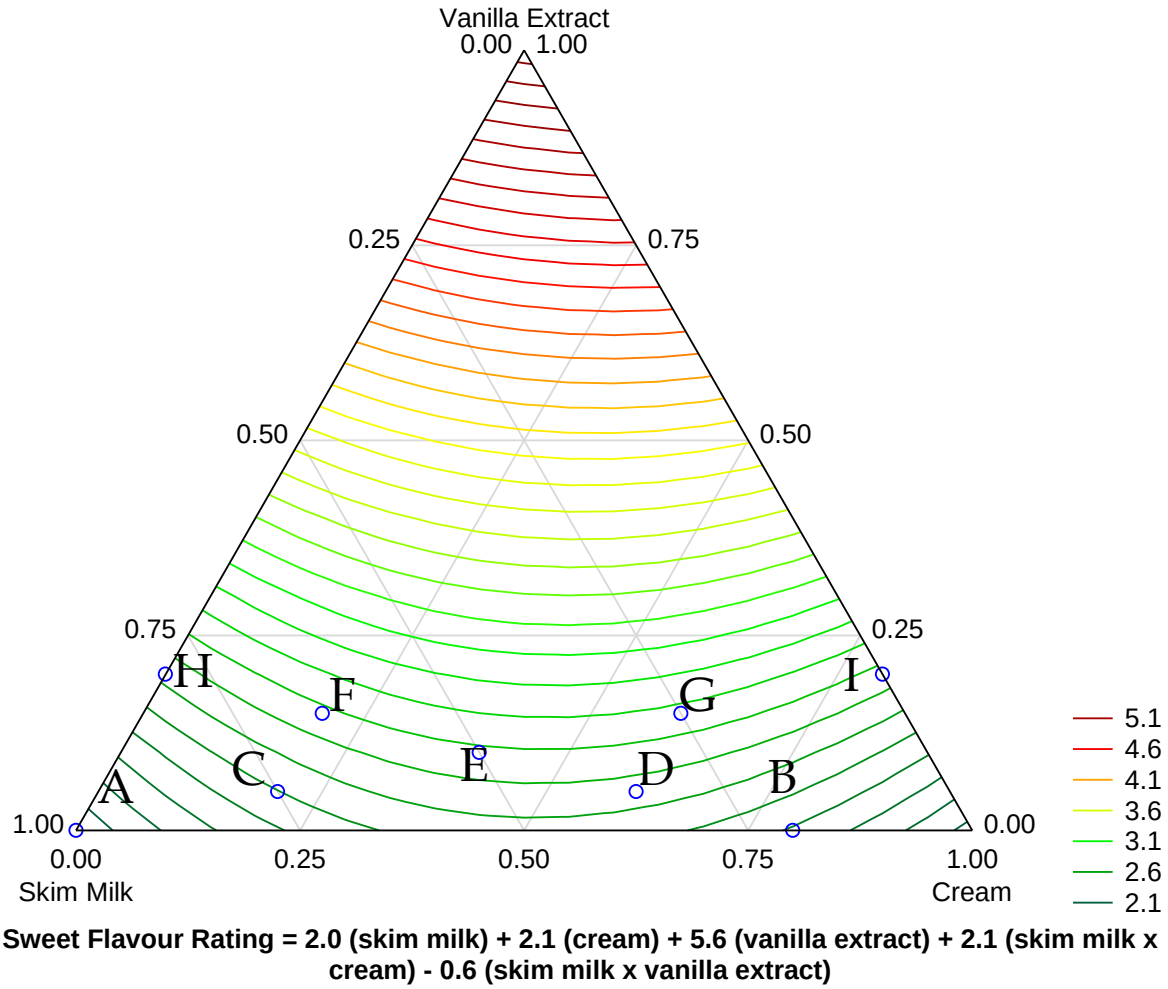


Figure 8.11: Contour plot for sweet flavour for mixture design investigating effects of fat on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.88, adjusted value 0.76.

The range of values for sweet flavour were low, from 2.0 to 3.0 (Table 8.7), suggesting that mixtures of skim milk, cream and vanilla extract do not have much sweetness, and the components did not have much effect on the response rating. It is possible that the cream and the skim milk were adding to the sweet flavour, as both contain some lactose. Lactose has a sweetness of 16 relative to sucrose with a value of 100 (Biester *et al.*, 1925). Vanilla extract has also been reported to contribute a sweet flavour (Hariom *et al.*, 2006), and was found to be sweet by the panellists in Chapter 5, and may have also contributed somewhat to the sweet flavour perceived by the panellists.

8.4 Results and Discussion - Effect of Sucrose on Aroma and Flavour Perception of Natural Vanilla Extract

To investigate the effect of sucrose on the aroma and flavour of natural vanilla extract, several results will be presented. Firstly, the panel performance was assessed to make sure they were performing reliably. Secondly, the regression equation for each of the attributes, compared to each of the components of the mixture design (vanilla extract, sugar and water) was selected as either linear regression or quadratic regression. Finally, the results were analysed for significance, patterns and relationships, discovered from the regression analysis.

8.4.1 Assessment of Panel Performance

In order to check that the panel members were producing reliable results, a duplicate sample was presented at the end of each sensory testing session. The ratings for this were compared to that of the same sample within the session using a Student's t-test. If they were not significantly different ($p \geq 0.05$), the panel was considered to be performing reliably and consistently.

8.4.1.1 Aroma Panel Performance

The t-test found that for 40 of the 42 session attribute combinations (six sessions, seven attributes), the trained panel results found that the in-session sample and the duplicate sample run at the end of the session were not significantly different (Table 8.13). The panellists were able to reproduce their results and therefore were considered to be performing reliably.

Table 8.13: Results from Student's t-test to determine the difference between in-session samples and duplicates at the end of each session. Values are p-values, with a significance of $p<0.05$. Significant values ($p<0.05$) are in italics.

Session	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
1	0.054	0.175	0.203	0.465	*0.033*	0.203	0.524
2	0.783	0.576	0.363	0.102	0.656	0.275	0.765
3	0.341	0.415	0.332	1.000	0.102	0.111	0.695
4	0.393	0.034	0.580	0.490	0.203	0.045	0.880
5	0.054	0.611	0.363	0.259	0.175	0.611	0.465
6	0.363	0.465	1.000	0.203	0.415	*0.041*	0.286

A second analysis of panel performance, looking at the ANOVA of the responses in Table 8.14 found that although participant was significant, session was not, indicating that the panellists were scoring the samples differently from one another, but they scored the samples the same from one session to the next relative to their own ratings. This indicated that the panellists were performing consistently across the sessions.

Also shown in the ANOVA (Table 8.14), all of the products were significantly different ($p<0.05$), meaning that the panellists were able to differentiate between the products. The participant: session interaction was not significant for any of the attributes; another indication that the panel was performing well, as the ratings from each panellist was not affected by the session that they were testing the product in.

8.4.1.2 Flavour Panel Performance

Using a Student's t-test, it was found that for flavour, the panellists did not score the in-session samples significantly different to the duplicate sample at the end of the session, for all session-attribute combinations (Table 8.15). This indicated that the panellists were performing reliably, able to reproduce their values within each session.

Table 8.16 – the ANOVA found that although the panellists were scoring differently to each other ($p<0.05$) for all but one of the attributes (bitter flavour), the scoring for the sessions was not significantly different, and the products were rated significantly different. This meant that the panellists were able to determine differences in the products, and gave the same responses for each attribute regardless of the session that they were testing the product in.

Table 8.14: Summary of ANOVA for the effect of sugar on the aroma of natural vanilla extract. Significant values (p<0.05) are in italics.

	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Participant	*0.007*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Session	0.470	0.189	0.459	0.299	0.091	0.262	0.709
Product	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Participant:Session	0.499	0.096	0.176	0.156	0.945	0.530	0.320
Participant:Product	*0.041*	*0.012*	*0.003*	*0.006*	0.003	0.520	*0.000*
Session:Product	0.865	0.916	0.611	0.124	0.356	0.853	0.808

Table 8.15: Results from Student's t-test to determine the difference between in-session samples and duplicates at the end of each session. Values are p-values, with a significance of p<0.05. Significant values (p<0.05) are in italics.

Session	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
1	0.592	0.797	0.187	0.465	0.175	0.328	0.394	0.454
2	0.194	0.722	0.053	0.069	0.191	0.636	0.845	0.377
3	0.396	0.120	0.275	0.611	0.394	1.000	0.175	0.302
4	0.456	0.499	0.203	0.363	0.175	0.679	0.771	0.363
5	0.728	0.911	0.695	0.363	0.363	0.809	1.000	0.185
6	0.751	0.907	0.611	0.175	1.000	0.758	0.328	0.695

Table 8.16: Summary of ANOVA for the effect of sugar on the flavour of natural vanilla extract. Significant values (p<0.05) are in italics.

	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
Participant	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.104*	*0.000*	*0.000*
Session	0.415	0.991	0.215	0.855	0.810	0.179	0.973	0.859
Product	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Participant:Session	0.282	0.157	0.343	0.133	*0.035*	0.276	0.511	0.637
Participant:Product	*0.002*	*0.004*	*0.000*	*0.000*	*0.000*	*0.141*	*0.000*	*0.000*
Session:Product	0.585	0.146	0.263	*0.035*	0.495	0.073	0.638	0.282

8.4.2 Effect of Sucrose on Aroma Perception of Natural Vanilla Extracts

8.4.2.1 Multiple pair comparison results: mean and differences

Using a multiple paired comparison it was found that there was little variation in the aroma responses based on the concentration of sugar in the solution (Table 8.17). For all attributes, the samples with the same vanilla extract concentration were rated the same ($p \geq 0.05$). The samples that were the same vanilla concentration were 1 and 2, 3 and 4, 6 and 7, and 8 and 9. The latter of each pair was the sample with the higher sugar concentration.

Table 8.17: Multiple pair comparison results for all attributes tested during investigation into effects of sugar on aroma profile of natural vanilla extracts. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test.

Sample	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
1	1.1^{d}	1.0^{b}	1.0^{c}	1.0^{b}	1.0^{b}	1.0^{c}	1.0^{c}
2	1.2^{d}	1.0^{b}	1.0^{c}	1.1^{b}	1.0^{c}	1.0^{c}	1.1^{c}
3	2.6^{c}	1.5^{ab}	1.5^{bc}	1.9^{a}	1.6^{bc}	1.5^{bc}	1.9^{b}
4	3.0^{bc}	1.5^{ab}	1.7^{bc}	2.1^{a}	1.7^{ab}	1.9^{ab}	2.6^{ab}
5	3.8^{ab}	1.8^{a}	1.8^{ab}	2.2^{a}	1.7^{ab}	2.0^{ab}	2.7^{a}
6	3.9^{ab}	1.8^{a}	2.0^{ab}	2.2^{a}	2.0^{ab}	2.0^{ab}	2.6^{a}
7	4.1^{ab}	1.8^{a}	2.2^{ab}	2.0^{a}	2.2^{a}	2.1^{ab}	2.8^{a}
8	4.8^{a}	1.8^{a}	2.1^{ab}	2.3^{a}	2.2^{ab}	2.3^{a}	3.2^{a}
9	4.6^{a}	1.8^{a}	2.5^{a}	2.2^{a}	2.1^{ab}	2.4^{a}	3.2^{a}

There were differences in the ratings for most attributes based on vanilla extract concentration, indicating that the vanilla extract was the component affecting the aroma response. However, many of these differences were not significant, which could be due to the attributes being above the detection threshold, but the panellists were not able to discriminate the ratings (Kemp *et al.*, 2009).

8.4.2.2 Regression Equations and Contour Plots for Aroma

Using regression analysis, it was found that the majority of the aroma attributes were described with a linear regression, with artificial fruity aroma and caramel aroma better described with a quadratic regression (Table 8.18). For artificial fruity aroma, the R^2 value was 0.77 for the linear regression, compared to 0.99 for the quadratic, and for caramel aroma, the R^2 was 0.66 for the linear compared to 0.89 for the quadratic.

Table 8.18: Summary of regression analysis looking at the effect of sucrose on the aroma of vanilla extract.

			Regression Coefficients				
Attribute	**Quadratic or Linear**	**R^2**	**Sugar**	**Water**	**Vanilla Extract**	**Sugar-water**	**Sugar-Vanilla Extract**
Overall Aroma	L	0.93	1.6	1.5	11.5		
Artificial Fruity Aroma	Q	0.99	0.04	1.0	3.2	3.2	3.2
Bourbon Aroma	L	0.94	1.4	1.0	4.7		
Caramel Aroma	Q	0.89	0.4	1.1	4.6	4.8	3.4
Raisin Aroma	L	0.86	1.3	1.1	4.3		
Spicy Aroma	L	0.84	1.3	1.2	4.7		
Vanilla Aroma	L	0.85	1.6	1.3	7.0		

For all the linear regression-based attributes, (overall aroma, bourbon aroma, raisin aroma, spicy aroma and vanilla aroma) the vanilla extract had the highest regression coefficient, and water the lowest, with sugar in the middle. This indicated that vanilla extract was responsible for most of the variation in the rating for each attribute.

For the quadratic regression equations (artificial fruity aroma and caramel aroma) vanilla extract had the highest regression coefficient of the linear terms and sugar the lowest (Table 8.18). This was different to the linear regression for the other aroma attributes, where water was the lowest regression coefficient term. For both artificial fruity aroma and caramel aroma, the regression coefficients were comparable to the value for vanilla extract (~3.2 and ~4.6), indicating that all three terms had similar weighting on the response. This will be looked at in further detail when comparing the contour plots in Figures 8.12 and 8.13.

All contour plots for the linear regression attributes are in Figure 8.12, with Figure 8.13 showing vanilla aroma larger for more detail.

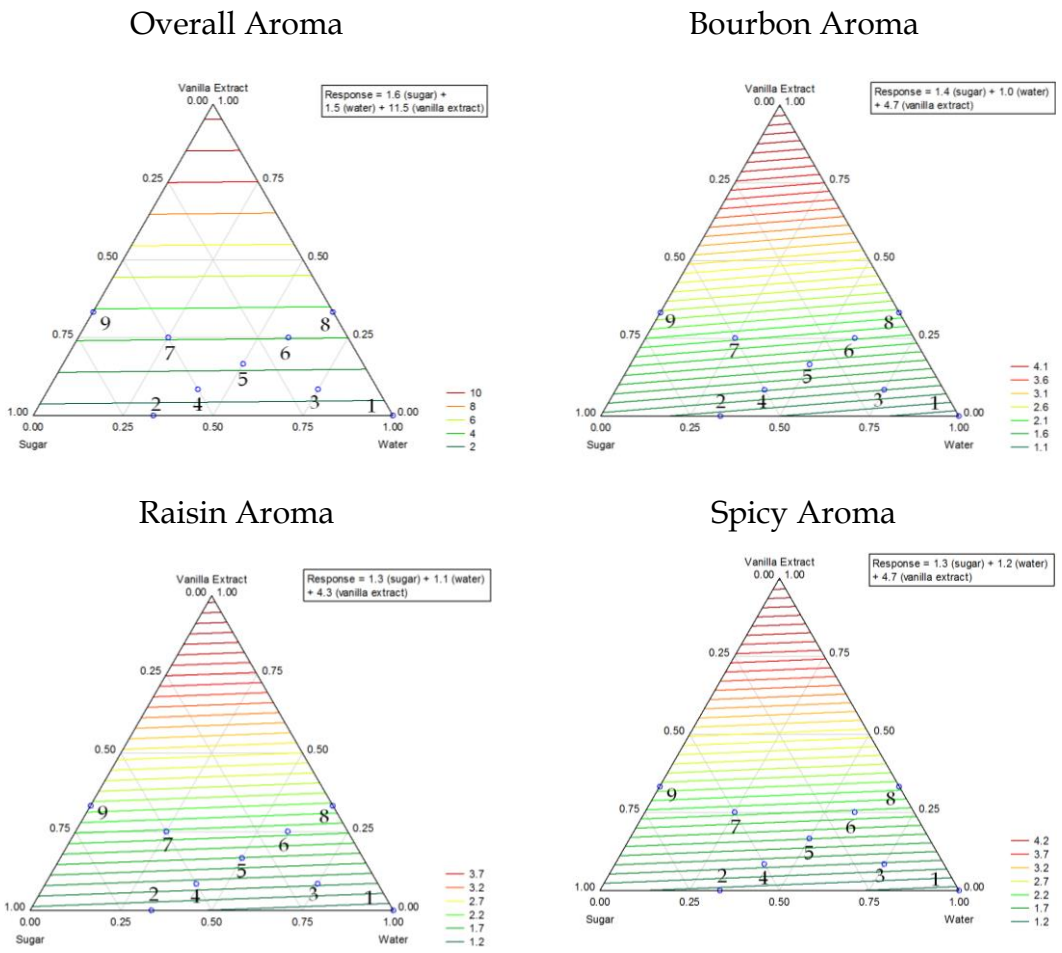


Figure 8.12: Contour plots for the effect of sugar on overall aroma, bourbon aroma, raisin aroma and spicy aroma.

The highest response for most attributes (except artificial fruity aroma and caramel aroma) was when there was the most vanilla extract (Figure 8.13). For overall aroma, bourbon aroma, raisin aroma, spicy aroma and vanilla aroma, the lowest rating was sample 1, the water only sample, with sample 2 rating higher. These results indicated that the sugar increased the response for these attributes. This was possibly due to a 'salting out' effect. When sugar is added to a solution, it is able to cause a 'salting out' effect, an effect by which the increasing concentration of compounds in a solution causes more volatiles to be present in the headspace (Taylor, 1998). Hansson *et al.* (2001) reported that increasing concentrations of sugar were able to increase the flavour compounds detected by GC analysis of the headspace, with the effect being more pronounced with the polar compounds. Baránková and Dohnal (2016) also found that sucrose induced a 'salting out' effect on aqueous solutions containing ethyl butanoate and butyl ethanoate, increasing the headspace concentration detected by GC. This 'salting out' effect has been found to have an effect on flavour when the

sucrose concentration is over 20% (Guichard, 2012), which is above the range of this experiment. There has been little information reported on the effective range of 'salting out' on aroma of different compounds and this experiment found that the 'salting out' effect was apparent at all concentrations of sugar within the experimental range.

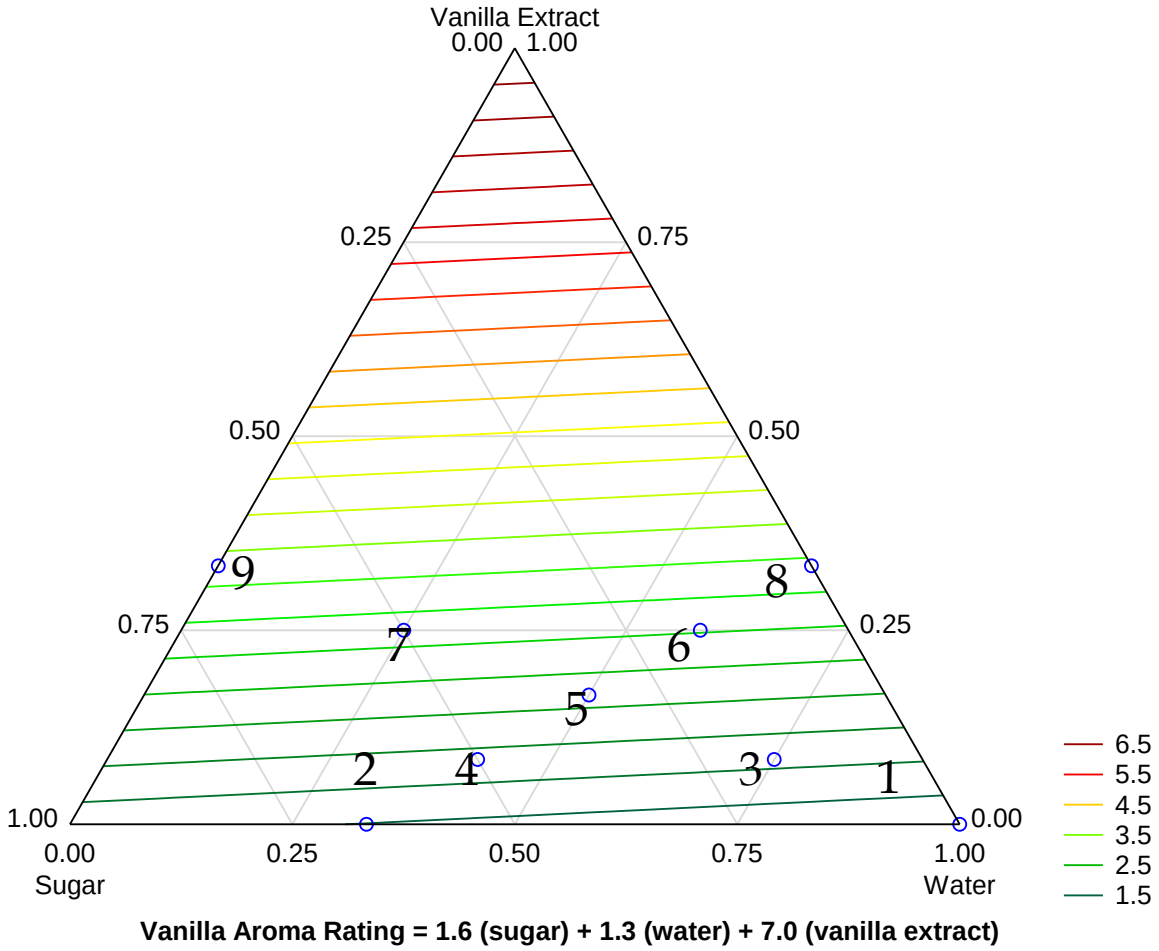
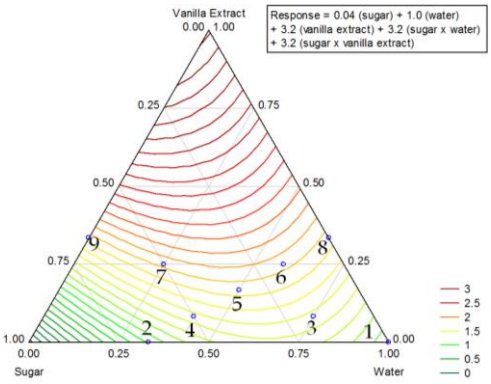


Figure 8.13: Contour plot for vanilla aroma for mixture design investigating effects of sugar on the aroma of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.85, adjusted value 0.80.

Both artificial fruity aroma and caramel aroma were best described with a quadratic regression; the contour plots can be seen in Figure 8.14. For both these attributes, the rating for samples 9, 7, 5, 6 and 8 were almost the same, being in the range of 2-2.5. Sample 3 and 4 were also very similar at about 1.5 and sample 1 and 2 were rated as 1. This was also seen with the mean values in Table 8.17, where there was no significant difference between the samples ($p \geq 0.05$), other than for the two samples that contained no vanilla, both of which were significantly lower. This indicated that the attributes were being detected by the panellists, but were below the discrimination threshold,

and so could not be differentiated. The outcome of this was that these two attributes were present in vanilla extract but could not be discriminated.

Artificial Fruity Aroma



Caramel Aroma

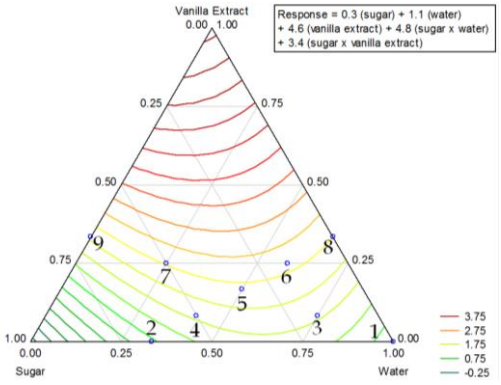


Figure 8.14: Contour plots for effect of sugar on artificial fruity aroma and caramel aroma.

8.4.3 Effect of Sucrose on Flavour Perception of Natural Vanilla Extract

8.4.3.1 Multiple pair comparison results: mean and differences

The mean scores for flavour for the sugar-vanilla extract-water samples were found to have the highest ratings of any attributes, and all of the attributes had significantly different ($p<0.05$) sample ratings. Vanilla flavour had the least separation of samples, with three different groupings in the ratings, butterscotch and bitter flavours separated to a greater degree, with four groups and the other attributes were able to differentiate between the products well, with either five or six different groups in the ratings for the nine samples for these flavour attributes (Table 8.19).

The range of the values was higher than for other experiments, as the concentration of the vanilla extract was up to 10%, compared to around 1.5% used in other experiments, such as in *Chapter 5*.

Table 8.19: Multiple paired comparison results for all attributes tested during investigation into effects of sugar on the flavour profile of natural vanilla extracts. Means within the same column with different letters are significantly different ($p<0.05$) as determined by Tukey's HSD test.

Sample	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
1	1.3^{f}	1.0^{f}	1.0^{c}	1.0^{d}	1.0^{d}	1.6^{cd}	1.0^{ef}	1.0^{e}
2	14.9^{ab}	16.4^{a}	1.2^{c}	1.4^{c}	1.0^{d}	1.1^{d}	1.0^{f}	1.0^{e}
3	7.4^{e}	5.9^{e}	3.2^{ab}	1.6^{c}	1.9^{bc}	1.9^{cd}	1.7^{d}	1.6^{cde}
4	12.8^{c}	13.0^{c}	3.1^{b}	1.8^{bc}	1.6^{c}	1.6^{cd}	1.6^{de}	1.6^{de}
5	10.8^{d}	10.9^{d}	3.5^{ab}	1.7^{c}	2.2^{ab}	2.1^{cd}	2.1^{bcd}	2.1^{bcd}
6	10.6^{d}	6.9^{d}	3.3^{ab}	1.8^{bc}	2.5^{a}	4.4^{b}	2.7^{b}	2.4^{bc}
7	13.6^{bc}	13.9^{bc}	3.9^{a}	2.3^{a}	2.4^{ab}	2.1^{cd}	2.1^{cd}	2.4^{b}
8	15.8^{a}	1.1^{f}	3.3^{ab}	1.4^{cd}	2.0^{abc}	11.0^{a}	4.5^{a}	3.7^{a}
9	15.6^{a}	15.3^{ab}	4.0^{a}	2.2^{ab}	2.4^{ab}	2.7^{c}	2.6^{bc}	2.6^{b}

8.4.3.2 Regression Equations and Contour Plots

For the effect of sucrose on the flavour of vanilla extract, it was found that five of the eight attributes were best described using a quadratic regression compared to a linear regression based on both the R^2 value and the appearance of the contour plot. For overall flavour, vanilla flavour, butterscotch flavour, raisin flavour and bitter flavour the R^2 values were 0.98, 0.93, 0.94, 0.96 and 0.97 for the quadratic regression compared to 0.69, 0.67, 0.64, 0.63 and 0.57 for the linear regression, respectively.

Table 8.20: Summary of regression analysis looking at the effect of sucrose on the flavour of vanilla extract.

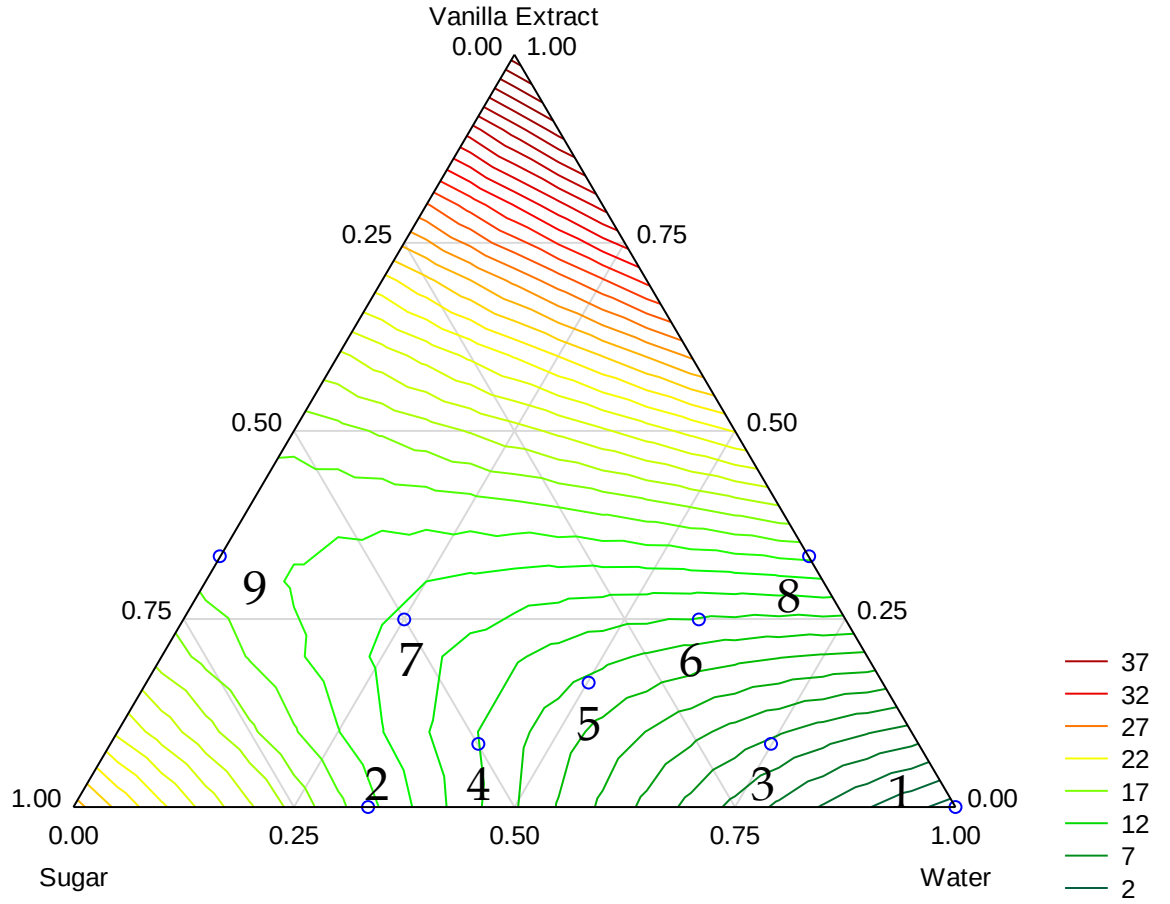
			Regression Coefficients				
Attribute	**Quadratic or Linear**	**R^2**	**Sugar**	**Water**	**Vanilla Extract**	**Sugar-Water**	**Sugar-Vanilla Extract**
Overall Flavour	Q	0.98	25.4	1.5	42.6	-9.6	-70.6
Sweet Flavour	L	0.97	24.1	2.1	1.9		
Vanilla Flavour	Q	0.93	2.4	1.2	6.8	11.4	14.4
Butterscotch Flavour	Q	0.94	0.5	1.0	2.2	3.5	5.3
Raisin Flavour	Q	0.96	1.06	1.05	4.1	5.7	7.6
Bitter Flavour	Q	0.97	7.6	1.7	28.4	-18.5	-53.9
Woody Flavour	L	0.85	0.1	1.5	8.4		
Bourbon Flavour	L	0.93	0.6	1.3	7.3		

From the regression analysis, there were a range of different patterns observed, by which the attributes could be grouped. These groups all had similar contour plots and interactions between the components, so were likely driving the same effects. The groups were:

1. Overall flavour and bitter flavour (Figure 8.15 and Figure 8.16)
2. Vanilla flavour, butterscotch flavour and raisin flavour (Figure 8.17 and Figure 8.18)
3. Sweet flavour (Figure 8.19)
4. Woody flavour and bourbon flavour (Figure 8.20)

Group 1: Overall Flavour and Bitter Flavour

Bitter flavour and overall flavour both had quadratic regression equations (Table 8.20) and followed the same pattern in their responses. Both were driven primarily by vanilla extract, with the highest regression coefficient, and water had the least effect on the response with the lowest regression coefficient. However, as the interaction was quadratic, these factors alone did not explain the variation observed. These are best understood from looking at the contour plots, with overall flavour in Figure 8.15 and bitter flavour in Figure 8.16.



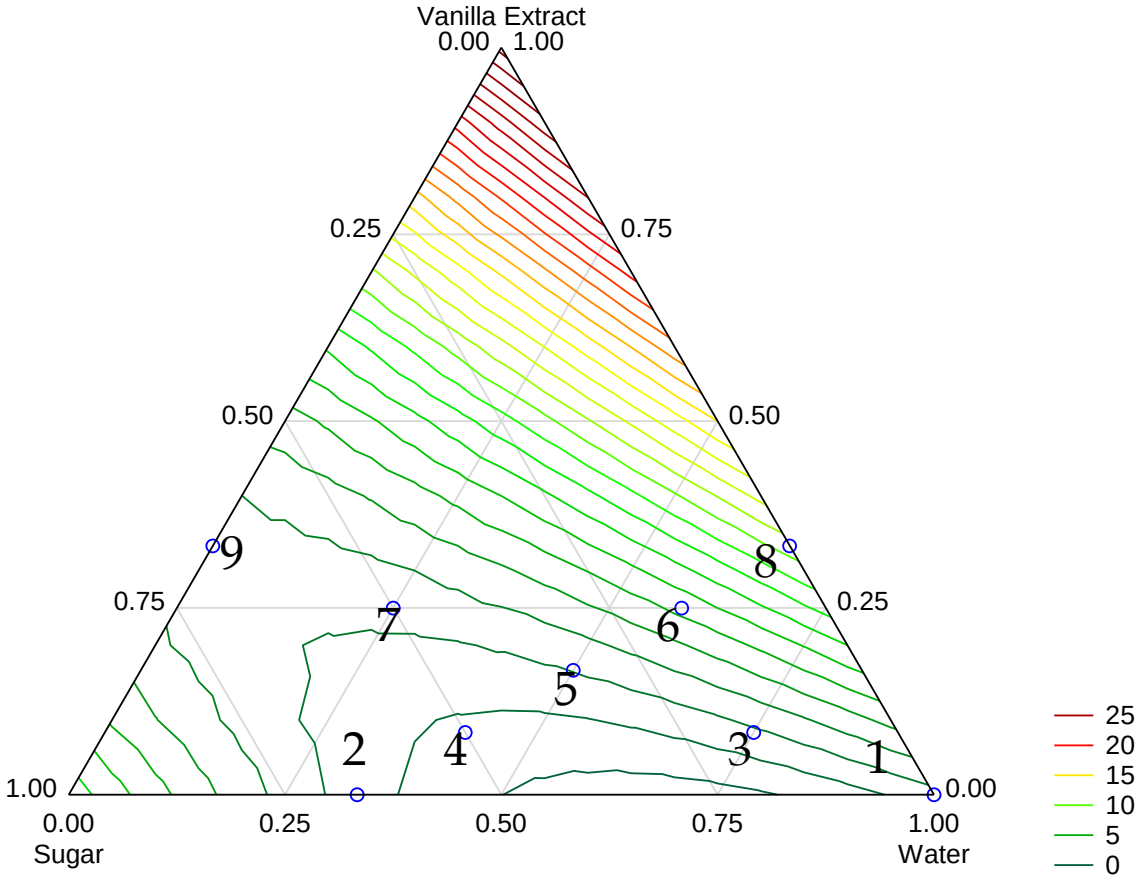
Overall Flavour Rating = 25.4 (sugar) + 1.5 (water) + 42.6 (vanilla extract) - 9.6 (sugar x water) - 70.6 (sugar x vanilla extract)

Figure 8.15: Contour plot for overall flavour for mixture design investigating effects of sugar on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.98, adjusted value 0.96.

For both attributes, the highest response was found with the highest concentration of vanilla extract. As the vanilla extract was a flavouring, it had a much higher flavour intensity than sugar or water, as it was intended to be diluted for use in foods. It also contained ethanol, which has been found to have a bitter taste at 10% concentration

(Scinska *et al.*, 2000). The lowest response for both attributes was obtained with water alone. This would be due to the lack of flavour of water, giving it a low rating for all attributes (Table 8.19, sample 1).

For the samples that contained all three of the components, there was a more complex pattern, due to the quadratic nature of the relationship. For overall flavour, the rating increased from Sample 1, water alone, to Sample 3, to Sample 5. From this central point, at Sample 5, the rating for overall flavour increased regardless of which component was increased (Figure 8.15).



Bitter Flavour Rating = 7.6 (sugar) + 1.7 (water) + 28.4 (vanilla extract) - 18.5 (sugar x water) - 53.9 (sugar x vanilla extract)

Figure 8.16: Contour plot for bitter flavour for mixture design investigating effects of sugar on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.97, adjusted value 0.95.

In contrast, for bitter flavour (Figure 8.16), the response would increase from this central point (Sample 5) with the addition of vanilla extract, if there was no addition of sugar at the same time (Figure 8.16). If sugar was added at the same rate as the vanilla extract, the bitter response stayed the same. At higher concentrations of vanilla extract,

the sugar was able to mask the bitter flavour of the vanilla extract but the proportion of sugar required increased as more vanilla extract was added. It is well known that the addition of sugar to a bitter solution will reduce the bitter flavour (Walters, 1997; Guichard, 2002). This was either caused by the masking of the bitter taste, where the bitter taste was overwhelmed by the sweet taste, or due to the inhibition of the bitter taste by the preferential binding of the sweet compounds to the bitter taste receptors on the tongue (Walters, 1997; Guichard, 2002).

There was also agreement on a suppression effect caused by the presence of both bitter and sweet compounds, whereby the sum of the tastes was less than expected from the concentration of the individual compounds (Walters, 1997; Guichard, 2002). This effect was seen in Samples 2, 4, 5, 7 and 9 where the response was not significantly different from one to the other, also seen in Table 8.19.

Group 2: Vanilla Flavour, Butterscotch Flavour and Raisin Flavour

The second group of attributes with similar regression equations were vanilla flavour, butterscotch flavour and raisin flavour (Table 8.20). These attributes were explained best with a quadratic regression, which resulted in the plots in Figure 8.17 and Figure 8.18.

For these attributes, the largest regression coefficient was found on the interaction terms (Table 8.20), indicating that the relationships in the data were largely determined by a combination of the components rather than one component alone. Of the main effects, the vanilla extract had the highest regression coefficient (Table 8.20); when there was the most vanilla present in the mixture, the response was the highest.

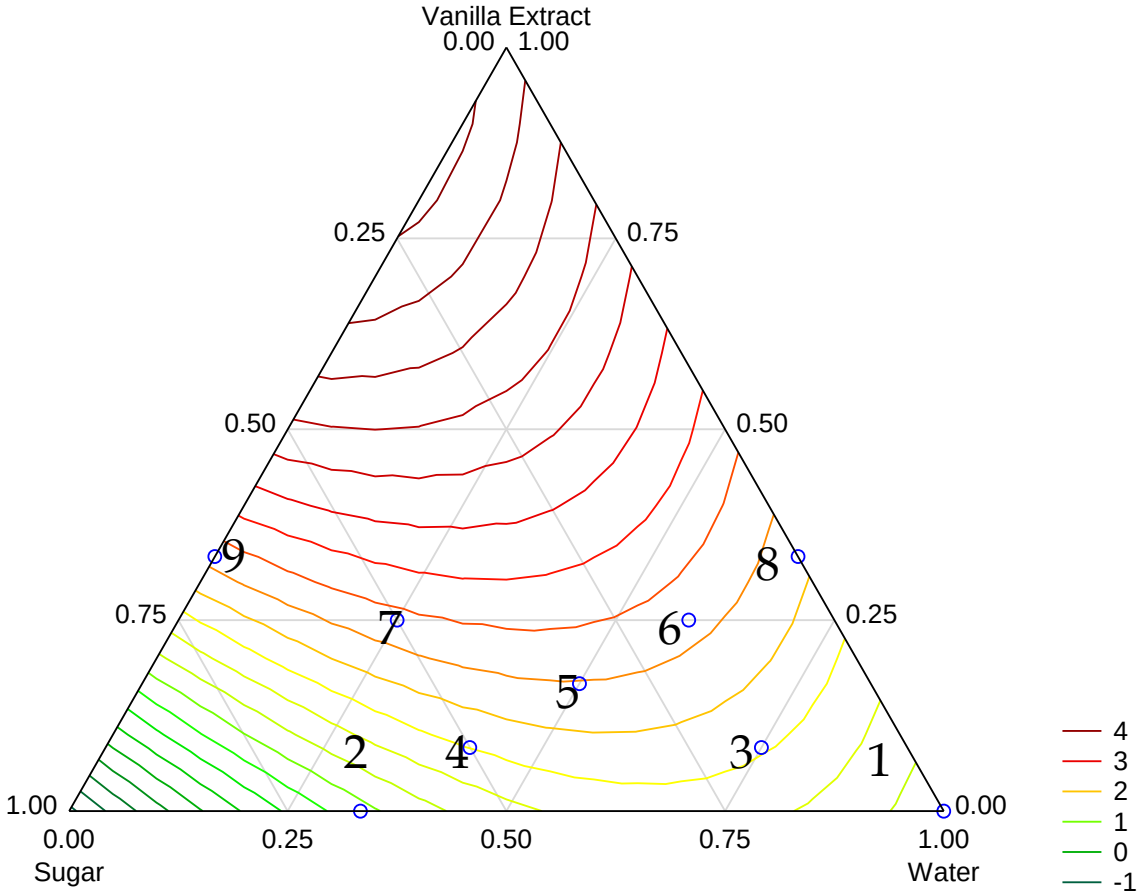
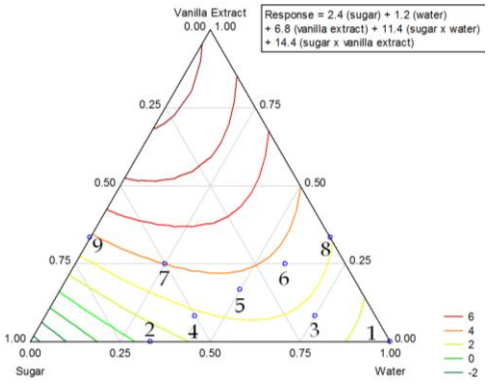


Figure 8.17: Contour plot for raisin flavour for mixture design investigating effects of sugar on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.96, adjusted value 0.93.

For both vanilla flavour and raisin flavour, the response increased from Sample 1 to Sample 3 to Sample 5 (Figure 8.17), as well as increasing from Sample 2 to Sample 4 to Sample 5. Samples with higher sugar or vanilla extract concentration than Sample 5 remained at the same rating on the contour plot. It was possible that these flavours were not being detected at the higher concentrations of vanilla extract or sugar, as other flavours such as woody flavour and bitter flavour would dominate, rating higher than vanilla flavour and raisin flavour (Table 8.19).

Vanilla Flavour



Butterscotch Flavour

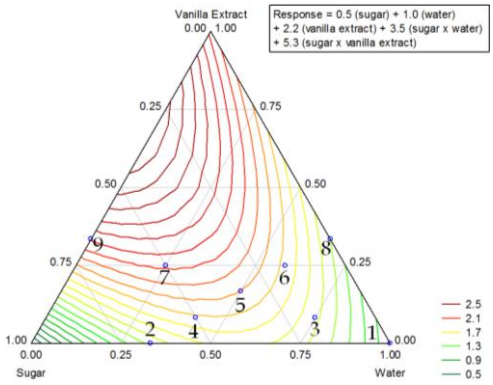


Figure 8.18: Contour plots for effect of sugar on vanilla flavour and butterscotch flavour.

For butterscotch flavour, the plot differed slightly compared to raisin flavour and vanilla flavour (Figure 8.17 and Figure 8.18). The butterscotch flavour response increased from Sample 2 to Sample 4 to Sample 5, as well as from Sample 1 to Sample 3 to Sample 5. From Sample 5, the response increased to Sample 7 and then to Sample 9, the highest value response of the samples. This showed that the butterscotch flavour response was dependant on both the sugar and the vanilla extract concentrations, so both had to increase for the flavour response to increase.

Group 3: Sweet Flavour

The third group of attributes in the regression analysis was that of sweet flavour. Sweet flavour was explained by linear regression (Figure 8.19). With a regression coefficient of 24.1, the sugar component was responsible for the differences seen in the response, with the water and the vanilla extract having minimal effect, with regression coefficients of 2.1 and 1.9, respectively (Table 8.20). Sweet flavour was defined as the sucrose sweetness of the mixture, so it was expected that this attribute would be based largely on the sugar content of the mixtures.

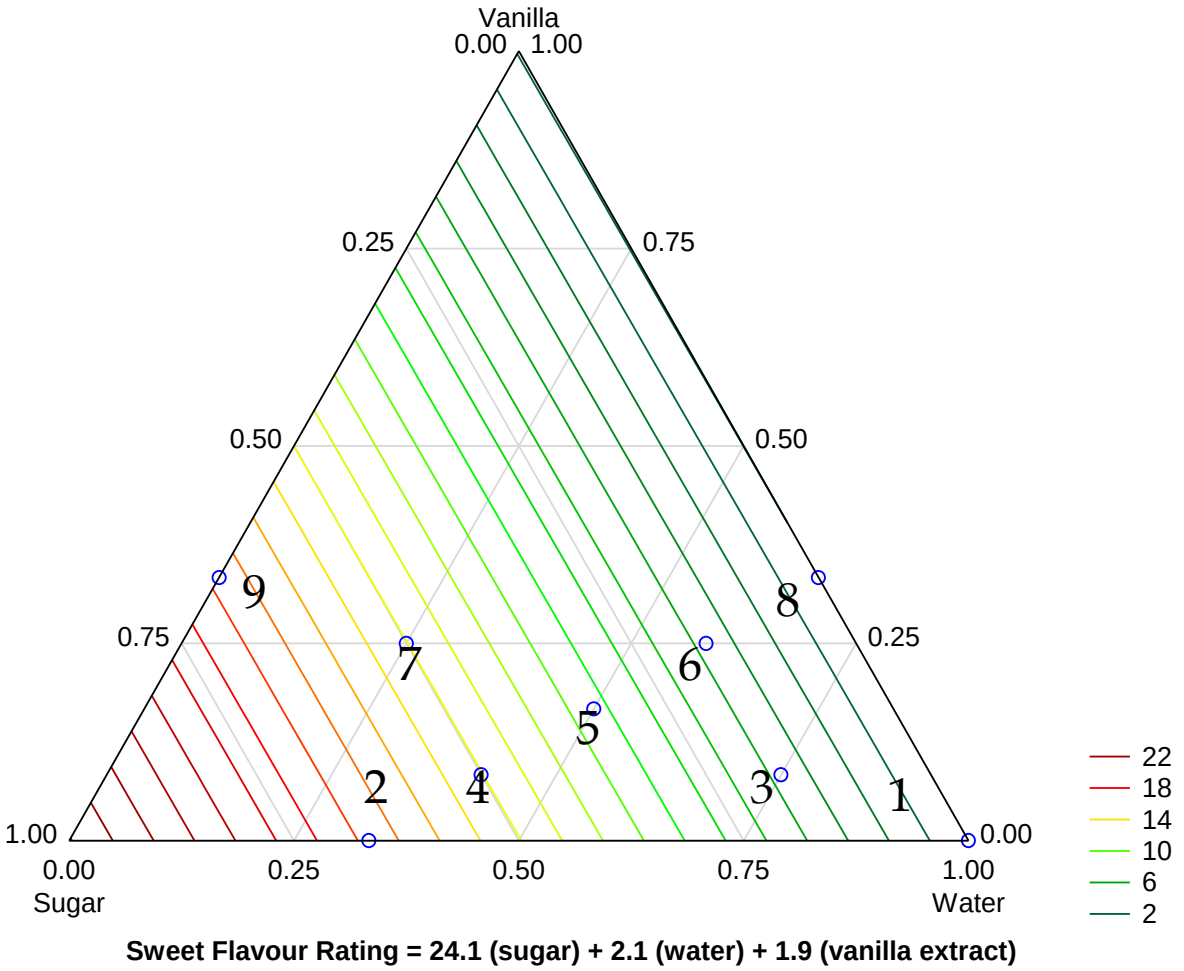


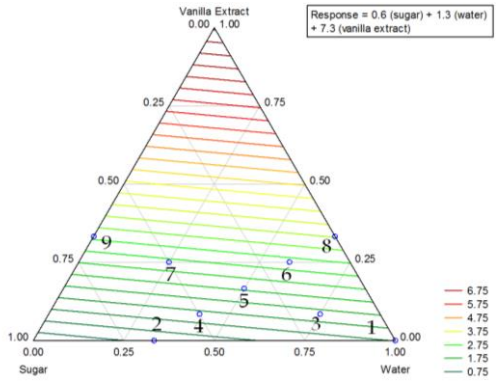
Figure 8.19: Contour plot for sweet flavour for mixture design investigating effects of sugar on the flavour of vanilla extract. Proportions on axes are pseudocomponents. The R-sqr value is 0.97, adjusted value 0.96.

Group 4: Woody Flavour and Bourbon Flavour

The fourth and final grouping seen in the results was that of woody flavour and bourbon flavour (Figure 8.20). For this group, both the regression equations were linear; vanilla extract had the highest regression coefficient and sugar had the lowest (Table 8.20).

The highest response was when the vanilla extract was the highest (Sample 8), and the lowest response was when the sugar content was the highest (Sample 2). The sugar appeared to reduce the rating for bourbon flavour and woody flavour, at any concentration of vanilla extract (Figure 8.20).

Bourbon Flavour



Woody Flavour

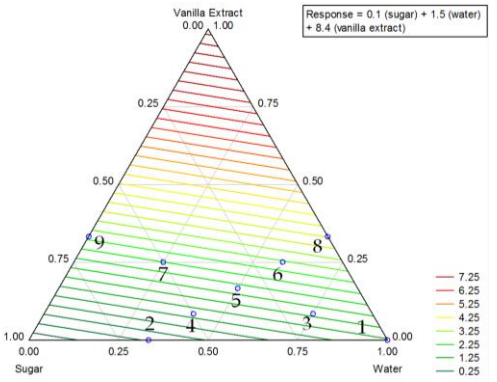


Figure 8.20: Contour plots showing effect of sugar on bourbon flavour and woody flavour.

8.5 Conclusions

For the investigation into the effect of milk fat on the aroma and flavour of vanilla extract, it was found that for artificial fruity aroma, bourbon aroma, caramel aroma, raisin aroma, spicy aroma, vanilla aroma, overall flavour, vanilla flavour, butterscotch flavour, raisin flavour, bitter flavour, woody flavour and bourbon flavour, the rating was reduced at higher levels of milk fat. Overall aroma was rated highest when the vanilla extract was at its highest concentration and lowest when the skim milk was the highest, a direct correlation to the relative aroma intensities of each of the components. Creamy aroma and creamy flavour were both reduced by the presence of vanilla extract. Sweet flavour was rated similarly for all mixtures within the design, indicating that the components in the mixture design, although they do provide a sweet flavour, they were likely below the discrimination threshold and thus were rated the same.

For the investigation into the effect of sugar on the aroma and flavour of vanilla, a range of effects were noted. Overall aroma, bourbon aroma, raisin aroma, spicy aroma and vanilla aroma were all rated higher when the sugar concentration was higher, likely due to a "salting out" effect, where the increased sugar concentration forced more aroma volatiles into the headspace to be detected. Caramel aroma and artificial fruity aroma were not affected by the sugar concentration, likely due to their low ratings making differences hard to detect. Overall flavour and bitter flavour were both highest at high concentration of vanilla and reduced slightly by sugar. This is due to sugar being a less intense food flavouring than vanilla extract, and able to mask bitter flavours in foods. Vanilla flavour, butterscotch flavour and raisin flavour were found

to be dominated by other flavours at high concentrations of vanilla and so could not be easily differentiated. Sweet flavour was rated based purely on the sugar concentration of the mixture, with no effect from the vanilla extract. Woody flavour and bourbon flavour were both masked slightly by the sugar, possibly in a similar mechanism as bitter flavour, with which they were often correlated during sensory analysis.

9. Overall Discussion

The combination of different growing regions of vanilla beans in equatorial countries and different flavour extraction methods used by flavour houses results in many different vanilla extracts with different aroma and flavour characteristics available to the public for consumption. The reasons for the differences in the aroma and flavour characteristics were largely unknown before this research was undertaken, with only a few studies looking at comparing growing regions, and curing methods for sensory characteristics (Heymann 1994; Hariom *et al.* 2006; Van Dyk *et al.* 2010; Takahashi *et al.* 2013a and 2013b). To understand the differences in the aroma and flavour characteristics, the extraction process was first investigated to give a general understanding of the processes, followed by determination of the sensory profiles of vanilla extracts. A limited PLS regression was applied to quantified chemical compounds in the vanilla extracts, and the sensory attributes as determined by the sensory panel to identify which volatile compounds were producing which sensory attributes in the vanilla extracts. Once the key flavour compounds were identified, further processing such as flavour concentration techniques could be investigated for their impact on the aroma and flavour profile in the concentrated extracts. This information is important for producing and marketing vanilla extracts produced in New Zealand from beans sourced around the world. The impact of fat and sugar on the sensory profile of a model food matrix containing vanilla was also investigated. Using this information, it should be possible to predict the effect of changes in the formulation of foods on the sensory profile of vanilla flavoured foods.

Prior to this research, published research focussed on volatile compounds in vanilla products (Toth *et al.* 2010; Perez-Silva *et al.* 2006) and the sensory properties of vanillin rather than vanilla extract (Hansen & Heinis 1991). There was little information available to the public on how the volatiles in vanilla extract related to the flavours that were perceived, how vanilla extract concentrate could be made, how the concentration process would affect the flavour, and how natural vanilla extract was affected by milk fat and sugar in food systems. This lack of information could affect a large number of food manufacturers as vanilla extract is one of the most widely used flavourings in food (Cameron 2011).

An investigation into the effect of different solvents was undertaken (Chapter 4) in order to determine the nature of the volatile compounds that could be recovered in a

vanilla extract using different solvents, thus allowing for better understanding of how the extraction conditions can affect the final volatile profile of vanilla extracts during industrial flavour extractions. Perez-Silva *et al.* (2006) reported on the effect of a limited range of solvents, and presented similar findings to the current research, although not covering the same scope of solvents. The current research found that most of the volatile compounds in vanilla extracts were polar which were easily extracted with hexane or pentane, but as these solvents are not food safe, ethanol is favoured as the food grade solvent. Another aspect investigated was the effect of the vanilla bean size reduction process used prior to flavour extraction.

After determining the impact of some of the extraction conditions on the volatile compounds, the sensory profile of the vanilla extracts were rated by a trained sensory panel (Chapter 5). As a food flavouring ingredient, the aroma and flavour profile is of high importance, affecting the overall acceptability of the product with the consumers. The sensory profiles of 16 vanilla extracts were characterised by a trained sensory panel. To ensure an overall understanding of the aroma and flavour profile of vanilla extracts, the 16 vanilla extracts were selected from a range of types. Most were ethanol flavour extracts, with one glycerol extract. Vanilla beans grown in India, Madagascar, Papua New Guinea, Tonga and Uganda were extracted with ethanol, and 11 vanilla extracts were purchased from supermarkets in New Zealand and Singapore (Chapter 5). This research found that there were differences in the sensory profiles of vanilla extracts from different growing regions with both commercial extracts and specially made extracts from Tonga and Madagascar higher in artificial fruity aroma, vanilla aroma, caramel aroma, raisin aroma, butterscotch flavour, sweet flavour and vanilla flavour. The extracts from India and Papua New Guinea in contrast were higher in bourbon aroma, spicy aroma, overall aroma, raisin flavour, woody flavour, overall flavour, bitter flavour and bourbon flavour. As there were differences in the sensory profiles of the vanilla extracts based on growing region, it was important to determine the regions that were most similar in sensory characteristics, so manufacturers would be able to make better informed decisions about which vanilla beans to source to maintain a consistent sensory profile in their extracts. A comparison of extracts made with glycerol or different concentrations of ethanol showed that the solvent for the extraction did affect the sensory profile, as well as the volatile content, as found in Chapter 4. Extracts produced with low ethanol concentrations or with glycerol (HI3 and HG) were found to have more artificial fruity aroma and be similar in overall

aroma and flavour profile, although much lower in overall aroma and flavour than the higher ethanol extracts. The extract produced with high ethanol concentration (H5) was high in bourbon aroma, bitter flavour, raisin flavour, bourbon flavour and woody flavour and had a higher overall aroma and flavour rating than the medium and low concentration ethanol extracts. Therefore, if manufacturers were to reduce the ethanol concentration used in their extraction process, it is likely that the overall flavour impact of the vanilla extract would reduce. Glycerol extracts, while able to achieve Halal certification, are lower in overall aroma and flavour intensity and would have to be used at higher concentrations in foods to obtain the same flavour intensity as an ethanol extract.

Each of the 16 vanilla extracts characterised for aroma and flavour profile were also analysed by GCMS for volatile chemical composition (Chapter 6). More than 100 volatile compounds were detected in the vanilla extracts, with vanillin at the highest concentration. Fifteen of the higher concentration volatile compounds were quantified using reference standards to allow for a comparison of the concentration of volatiles to the sensory profiles of the vanilla extracts. Thirteen of the compounds used in this study had been identified in vanilla extracts in previous studies, with isovanillin and 3-methyl-2-furoic acid being newly detected compounds. Most of the compounds (11 of 15) had phenolic rings as their base structure; therefore, they could be either breakdown products or precursors of vanillin in the biosynthetic pathway, as vanillin also has phenol as its base structure. As the biosynthetic pathway is still not clear, with a range of different pathways proposed (Knorr *et al.* 1993; Dignum *et al.*, 2001; Yang *et al.*, 2017), any conclusions were not possible at this stage regarding the origin of the compounds. To better understand the role of these volatile compounds in the vanilla extracts, the concentrations were correlated with the 16 aroma and flavour attributes rated by the trained sensory panel. No previous research had attempted this type of analysis, instead other researchers applied GC-O techniques (Pérez-Silva *et al.*, 2006; Brunschwig *et al.*, 2012; Zhang and Mueller, 2012; Takahashi *et al.*, 2013a; Takahashi *et al.*, 2013b) which only provided information about the aroma of the individual volatile compounds instead of how the volatile compounds match up with attributes detected in vanilla extracts.

Using PLS, a number of strong correlations were identified, such as creosol with vanilla aroma and vanilla flavour at low concentrations and woody flavour at higher

concentrations, which had been reported by Shu and Shen (2008) and Ross *et al*. (2010) in other foods. Syringaldehyde was correlated with bourbon aroma and bourbon flavour, 4-hydroxybenzoic acid was correlated with bitter flavour, also reported by Palassarou *et al*. (2017). Guaiacol was correlated with woody flavour and was found at higher concentrations in vanilla extracts that were high in woody flavour, such as PNG, I, L and QO, and previously reported by Pérez-Silva *et al*. (2006) and Zhao *et al*. (2017). One final correlation identified was between vanillyl alcohol and raisin aroma, raisin flavour and butterscotch flavour. Vanillyl alcohol was described by the sensory panel as fudge-like, sweet and toffee, supporting the correlation, and in agreement with Palassarou *et al.* (2017). Although some correlations were identified, the large number of volatile compounds detected in vanilla extract, both in this research and in past research (Toth *et al.* 2006), indicate that the relationship between the volatiles and perceived sensory characteristics is more complex than the scope of this research was able to encompass. A greater number of quantified chemical compounds would allow for more in-depth conclusions to be drawn, but this research provides a foundation for any future work to build on. The use of GC-O would also supplement the correlations noted in this research, allowing for the aroma of all the volatile compounds to be described, even if the reference standard is not available. However, the information gathered in this current research, while not comprehensive, still allows manufacturers to better understand how changes in the volatile composition of their vanilla extracts would affect the sensory profile of the vanilla extract, without requiring the time and cost of using a trained sensory panel.

As vanilla extracts are made into a wide range of products, it was also important to understand how further processing may affect the volatiles and sensory characteristics especially when different concentration techniques were used. This was achieved through the use of a range of different flavour concentration techniques including vacuum concentration, supercritical carbon dioxide extraction, freeze drying and maltodextrin flavour encapsulation of the vanilla extract, combined with monitoring of the volatiles and sensory properties (Chapter 7). Only supercritical carbon dioxide had been investigated in published research (Nyugen *et al*. 1991, Fang *et al*. 2002a, Fu *et al*. 2002, Mukhopadhyay 2007 and Castillo-Ruz *et al*. 2011), with no information available about the sensory profile or volatile content of the product. It was found that all of the processing methods investigated affected the sensory and volatiles in the vanilla extracts, with a reduction in overall aroma and flavour intensity and a reduction in the

total volatile content of the concentrates. The vanillin concentration was standardised for all the sensory and GCMS analysis, so any changes seen were differences in the ratios of volatiles in the extracts, rather than just overall losses of volatiles. As the samples were further processed, more volatiles were lost, leading to a greater change in the sensory profile of the concentrate. The vacuum concentrates were limited by viscosity to approximately 35 mg/ml of vanillin, from an initial concentration of around 10 mg/ml vanillin. Above 35 mg/ml of vanillin the solution could not be removed easily from the flask after vacuum concentration as the viscosity was too high. The vacuum concentrate was able to be made into a powder by dissolving it in maltodextrin, of either DE10 or DE30 to a flavour loading of 30% w/w followed by freeze drying. A higher flavour loading would be recommended in future trials in order to determine the optimum conditions for the strongest flavour, with the ranges for this experiment chosen based on the maltodextrin manufacturers recommendations. This vanilla powder was found to be less similar to the original ethanol extract than the vacuum concentrated extract based on the sensory characteristics.

GCMS analysis revealed that a large number of the volatile compounds were lost during the processing from the vacuum concentrate to the maltodextrin powder. Supercritical carbon dioxide was also trialled as a concentration method and was found to extract primarily vanillin, the main flavour compound in vanilla beans. As CO_2 is non-polar, many of the polar compounds extracted by ethanol in a traditional flavour extraction were not extracted, leading to a sensory profile that differed from the ethanol extract and more closely matched pure vanillin in both aroma and flavour. This was supported by GCMS analysis, with vanillin as the predominant volatile compound, and none of the other previously quantified chemical compounds present. This again supported the findings in Chapter 4, with the range of different extraction solvents trialled showing that more volatiles were extracted with more polar solvents, and vanillin was the primary compound extracted with the non-polar solvents. This information will allow food manufacturers to make more informed decisions about what type of vanilla extract to add to a given food product and how concentration of an extract will alter the flavour profile if this option is provided to the company.

The final aspect of vanilla flavour that was explored was the effect of milk fat and sucrose on the aroma and flavour profile. As vanilla flavouring is used in such a wide

range of foods, such as ice cream, milk, custard, cakes, chocolate and soft drinks, it was important to understand how changing the food composition might affect the flavour provided by the vanilla extract. Stampanoni Koeferli *et al.* (1996) investigated natural vanilla extract, looking at the effect of varying composition of ice cream on the flavour of a natural vanilla ice cream; all other published research had been based around vanillin, a single flavour compound in vanilla extracts (Hansen & Heinis 1991). Using an extreme vertices mixture design (Chapter 8), it was found that when the milk fat was increased, the aroma and flavour of the vanilla extract tended to decrease, with all attributes rated lower in samples higher in milk fat, agreeing with the findings of Stampanoni Koeferli *et al.* (1996). This effect was either due to the increased viscosity of the solution or due to fat soluble aroma and flavour compounds being dissolved and held in fat globules, therefore not released to be detected in the mouth. Therefore, in food applications, if the fat content of a food is decreased to produce a low-fat option, the concentration of vanilla extract would likely have to be decreased to achieve the same vanilla flavour profile, hence food manufacturers need to be aware of this during reformulations.

When the sucrose concentration in an aqueous solution of vanilla extract was increased, the aroma and flavour attributes of the vanilla extract tended to be rated higher by the trained sensory panel. Specifically, overall aroma, bourbon aroma, raisin aroma, spicy aroma and vanilla aroma were all rated higher when more sugar was present in the solution. Artificial fruity aroma and caramel aroma did not seem to be affected much by the sucrose concentration, rated similarly for all samples. For the flavour, bitter flavour was reduced by sucrose, vanilla flavour, butterscotch flavour and raisin flavour were only moderately enhanced, reaching a maximum rating at 5% w/v sucrose concentration and not being rated higher for stronger sucrose solutions. Woody flavour and bourbon flavour were somewhat masked by the sucrose, with lower ratings recorded for higher concentrations of sugar. Therefore, in a food where the sugar concentration is being reduced, it would be recommended for the food manufacturer to increase the vanilla extract concentration to maintain the same overall aroma and flavour impact. However, it should be noted that for sucrose, the attributes in the vanilla extract were affected differently relative to each other, so the final sensory profile desired by the food manufacturer should also be considered when making changes to the concentration of vanilla extract, milk fat and sucrose in a food formulation.

Overall, a number of new aspects about vanilla flavour were identified in the current research which will help improve understanding of the flavouring and its use. The effect of extraction conditions, the sensory profile and how it relates to the volatile compounds in vanilla extract, concentration techniques and the relationship between vanilla extract and food components in a system were all better understood at the end of this research.

10. Conclusions and Recommendations

10.1 Conclusions

The sensory characteristics of a range of commercial vanilla extracts were determined by a trained sensory panel. It was found that there were differences in the vanilla extracts depending on the growing region and extraction method used. Madagascar and Tonga vanilla extracts were most alike in sensory characteristics, with Uganda also similar. This group was characterised as high in butterscotch flavour and raisin aroma/flavour. Indian and Papua New Guinean vanilla extracts were also grouped together, separate to the other regions, characterised as higher in bourbon aroma/flavour and woody flavour. A range of different solvents were used to extract the vanilla extracts from Heilala. It was found that as the ethanol was decreased, the sensory profile became less woody and bourbon and more vanilla aroma and artificial fruity aroma dominated. The glycerol extract was found to have a low overall aroma and flavour impact, being most similar to the low ethanol extract.

When the sensory characteristics of the vanilla extracts were compared to the volatile compounds, as quantified by GCMS, it was found that there were a number of sensory attributes defined by chemical compounds. Bourbon aroma and flavour correlated with syringaldehyde. Vanillyl alcohol was correlated with raisin aroma, raisin flavour and butterscotch flavour. Vanilla aroma and flavour were associated with creosol and vanillin. Sweet flavour was correlated with p-anisic acid, maltol and 4-hydroxybenzoic acid.

This research also confirmed that ethanol was the best solvent to use for vanilla extract. Through the use of GCMS, it was found that a higher concentration of vanillin and more total volatile compounds were extracted with 75% v/v ethanol than 100% ethanol, although both concentrations extracted more vanillin than 50%, 25% and 0% (water) ethanol. Therefore 75% ethanol was the most suitable ethanol concentration for extracting volatiles from cured vanilla beans. Different compounds were able to be extracted from vanilla beans, depending on the polarity and boiling point of the solvent. Hexane and pentane were able to extract the most volatile compounds and water extracted the least number of volatile compounds. Ethanol was found to extract similar compounds to hexane, making it one of the more successful solvents in regards to number of volatiles extracted as well as volume of volatiles, indicating that this

solvent was well suited for use in the food industry to create a vanilla flavour extract. The preparation of the vanilla beans was also compared, looking at hand cut, blended and freeze-dried ground vanilla beans. There was no observable pattern in the volatile compounds extracted from each by ethanol in a one-week extraction.

The recommended method to concentrate vanilla extract without leading to modification of the overall flavour, it was found that vacuum concentration produced a concentrate with the most similar sensory profile to the original ethanol extract. Concentrations of up to 35 mg/ml of vanillin, compared to 1.5 mg/ml of a standard single fold extract, were reached.

It was found that increasing the fat content in a model system caused the aroma and flavour attributes of the vanilla extract to be rated lower by the trained panel, with the exceptions being creamy aroma and flavour, which were rated higher when more cream was present and overall aroma and flavour, which were rated relative to the strength of the ingredients – vanilla extract has a stronger flavour profile than either skim milk or cream, so results in a higher rating. With sugar a 'salting out' effect was observed on the aroma attributes where the ratings increased with increasing sugar concentration. For flavour, the sweet type attributes – sweet, vanilla, butterscotch and raisin flavour were rated higher with higher levels of sugar. The attributes bitter flavour, woody flavour and bourbon flavour were all rated lower when the sugar concentration was higher.

These advances in understanding of vanilla will be able to be applied to a range of foods such as ice cream, flavoured milks, chocolates and powdered products. With understanding of the nature of vanilla extract, it will be possible for flavour manufacturers to better control their processing and maintain quality even with the current global shortages in supply.

10.2 Recommendations to further research

It is recommended to expand the number of compounds quantified beyond the 15 compounds identified by GCMS and use HPLC-MS to be able to detect more compounds, to form a more robust model of the compounds responsible for each sensory characteristic of the vanilla extracts. The use of GC-O to compliment the PLS analysis is also recommended.

Once the concentrations of the main volatiles are determined, it would be interesting to create an ethanol-based solution containing all the volatiles at the correct concentrations to compare based on sensory properties to the original natural vanilla extract. This could confirm the importance of the volatiles, as odour activation values need to be considered along with total concentration within the samples.

Although the importance of volatile compounds in vanilla extract other than vanillin is being better understood, there is still little information about where these compounds originate in the biosynthetic pathways. It would be recommended that the formation of these compounds be investigated further, so that compounds with high impact on the flavour of vanilla extracts are able to be controlled during the processing from the green vanilla beans through to the final vanilla extracts.

The processing of the vanilla extract into a powder could also be optimised, as there were a number of factors, such as vacuum concentration, flavour loading and maltodextrin type that could affect the final product which could be improved upon to create a better powdered vanilla. This research merely proved that the process was possible, it did not aim to optimise the process.

Although there was a lot of investigation into the remaining volatiles in the vanilla extracts after a range of processing conditions, further research could look into where the lost volatiles are going. For example, for the freeze drying, collect the ice from the coil, or for the vacuum concentrate, conduct sensory analysis on the condensate.

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12. Appendices

Appendix 1: Previously Found Compounds in Vanilla

Table A.1 contains a full list of all the compounds that have been identified in vanilla and vanilla products in previous studies. The compounds have been identified using various methods, from retention times in HPLC and GC to tentative identification by comparison to the library records of MS outputs.

Table A1: Complete list of compounds previously found in vanilla and its products. The key for numbers and letters can be found at the end of the table.

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
ACIDS			
(9Z)-Octadec-9-enoic acid	Oleic acid	2,3,5,6,7,10,11,12,13,14	r,u,x
2,2-dimethyl-3-butenoic acid		9	a
2,3-dihydroxypropyl ester 9,12-octadecanoic acid		6	u
2-ethoxycarbonylbenzoic acid	mono ethyl phthalate		t
2-ethylhexanoic acid		14	ah
2-furancarboxylic acid	2-furoic acid	2,14	ah
2-heptenoic acid	hept-2-enoic acid	10	x
2-hydroxy-2-(3-hydroxy-4-methoxyphenyl)acetic acid	isovanillylmandelic acid	2,6	u
2-hydroxybenzoic acid	salicylic acid	6,11	u,t,y
2-hydroxypropanoic acid	lactic acid		t
2-methyl-1(1,1-dimethyl)-2-methylpropanoic acid		2	a
2-methylpropanoic acid	isobutyric acid	2,10,14	e,x,ah
2-methylbutanoic acid		2,14	ah
3-phenyl-2-propenoic acid	cinnamic acid	1,2,5,10,14	a,o,u,x,ah
2-propenoic acid	acrylic acid	2,6	v
3,4-dimethoxybenzoic acid	veratric acid	7	v
3-methylbutanoic acid	isovaleric acid	2,3,6,7,8,9,10,11,13,14	a,e,t,u,x,ah
3-methylpentanoic acid	3-methylvaleric acid	14	ah
3-methyl-2-butenoic acid		2,14	ah
4-(4-hydroxyphenoxy)benzic acid		2,14	ah
4-hydroxy-3-methoxybenzoic acid	vanillic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,b,e,r,u,x,ad,ae,ah
4-hydroxybenzoic acid	p-hydroxybenzoic acid	1,2,4,8,10,11,12,13	a,b,r,u,w,ad
4-methoxybenzoic acid	p-anisic acid	2,5,6,10,11,12	a,m,o,r,u,x,af
4-methyl-2-oxovaleric acid	isopropyl pyruvic acid	7,10,11,12	u
4-oxopentanoic acid	levulinic acid	1,2,3,5,6,7,11,12,13,14	a,e,u
4-phenoxybenzoic acid		11,12	a,u
9,12-octadecadienoic acid	linoleic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,u,x
9-hexadecanoic acid	palmitoleic acid	2,6,7,10,11,12,13	u,x
a,d-dimethyltetonic acid		5	u
ethanoic acid	acetic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,e,r,t,u,x,ah
benzene propanoic acid	hydrocinammic acid	10	x
benzoic acid	dracylic acid	2,10,14	e,q,r,x,ah
butanoic acid	butyric acid	2,10,14	t,x,ah
cyclohexaneacetic acid	cyclohexylacetic acid	2	u
decanoic acid	capric acid	2	f,t,ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
dodecanoic acid	lauric acid	1,2,3,5,6,7,9,10,11,13,14	a,t,u,x
heptadecanoic acid	margaric acid	1,2,3,5,6,7,8,9,10,11,12,13,14	a,r,u,x
heptanoic acid	enanthic acid	2,10,11,14	u,x,y,ah
hexadecanoic acid	palmitic acid	1,2,3,5,6,7,8,9,10,11,12,13,14	a,r,u,x
hexanoic acid	caproic acid	2,10,14	a,t,u,x,ab,ah
hydroxy acetic acid	glycolic acid		t
methanoic acid	formic acid	2,3,4,5,6,7,8,10,11,12,13,14	a,r,t,u
methoxyethanoic acid	methoxyacetic acid	7	t,u
nonanoic acid	pelargonic acid	2,10,14	a,r,y,ah
octadecanoic acid	stearic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,u,x
octanoic acid	caprylic acid	2,9,10,14	a,f,t,x,ab,ah
pentadecanoic acid	pentadecylic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,e,r,u,x
pentanoic acid	valeric acid	2,10,14	x,ah
propanoic acid	ethanecarboxylic acid	2,3,6,10,11,12,13,14	a,u,x,ah
tetradecanoic acid	myristic acid	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,t,u,x
ALCOHOLS			
(2E)-3,7-dimmethylocta-2,6-dien-1-ol	nerol		t
(4-methoxyphenyl)methanol	anisyl alcohol	2,3,6,7,10,11,12,13,14	a,c,h,l,m,o,r,t, u,x,ab,ac,af,ah
(7,7-dimethyl-4-bicyclohept-3-enyl)methanol	myrtenol		t
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	phytol	2,3,6,10,11	u
1-(4-methoxyphenyl)-2-methyl-3-buten-1-ol		6,11,12,14	u
1,2,3,4-butanetetraol	erythritol	9	a
1,2,3-propanetriol	glycerol	2	a
1,2-cyclohexanediol		4	a
1,2-dihydroxybenzene	catechol, pyrocatechol	2,5	r,u
1,2-dimethoxybenzene	veratrol	2,10,14	u,ah
1,2-propanediol	propylene glycol	2,10,14	x,ah
1,3-butanediol	b-butylene glycol	1,2,3,6,8,9,10,11,13,14	a,r,u
1,3-cyclohexanediol, trans-	hexahydroresorcinol	2,12	u
1,4-butanediol	tetramethylene glycol	2,10	a
1-isopropyl-4-methylbenzene	p-cymene	2,14	ah
1-methoxy-2-(4-methylphenyl)methylbenzene		12	a
1-octen-3-ol	octenol, vinyl hexanol	2,10,14	t,x,ab,ah
1-phenyl-1,2-butanediol		1,2	a
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	alpha-terpineol	2	u,ah
2,2,4-trimethyl-3-penten-1-ol		13	a
2,2-dimethylpentan-1-ol	neoheptanol	2,6,11	u
2,3-butanediol	dimethylene glycol, 2,3-butyleneglycol	1,5,10,11	a,t,u,x
2,4-dimethyl-1-heptanol		10	a
2,4-dimethylphenol	2,4-xylenol	2,14	ah
2,6-dimethoxy-4-methylphenol	4-methyl syringol	7,11	u
2,6-dimethoxyphenol	pyrogallol 1,3-dimethyl ether	2	ah
2,6-dimethyl-4-ethyl-4-heptanol		13	a
2-acetoxy-1-propanol		6,12	u
2-butoxyethanol		2,14	ah
2-butyne-1,4-diol		9	a
2-cis-9-octadecenyloxyethanol		2,12	a
2-ethylcyclobutanol		2	u
2-methoxy-4-(2-propenyl)-phenol	eugenol	2,4	a,ab,ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
2-methoxy-4-methylphenol	creosol	2,10,14	t,x,ab,ah
2-methoxy-4-prop-2-enylphenol	engenol, caryophyllic acid	7	u
2-methoxy-4-cresol	2-hydroxy-5-methylanisole	2,5,6,7,10,11,13,14	u
2-methoxy-4-vinylphenol		2,14	ah
2-methoxyphenol	guaiacol, methylcatechol	2,3,6,7,10,11,13,14	f,r,t,u,x,ab,ah
2-methylbutan-1-ol		2,14	t,ah
2-methylbutan-2-ol		2,14	ah
2-methylphenol	o-cresol	2,14	ah
2-methyl-3-buten-3-ol	dimethyl vinyl carbinol	2,14	ah
2-octen-1-ol		2	ah
2-octen-4-ol	butyl propenyl carbinol	2	r
2-phenylethanol	phenylethyl alcohol	2,10,14	t,x,ab,ah
3-(hydroxymethyl)phenol		1,2,6,9,14	a,u
3,4-dimethoxybenzyl alcohol	veratryl alcohol		u
3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	nerolidol	1	a
3,7-dimethyl-2,6-octen-1-ol	geraniol		t
3,7-dimethyl-6-octen-1-ol	citronellol, dihydrogeraniol		t
3,7-dimethylocta-1,6-dien-3-ol	linalyl alcohol, linalool		t
3-hexen-1-ol	pipol	2,14	ah
3-methyl-2-buten-1-ol	prenol	2,14	t,ah
3-methyl-2-propylpentan-1-ol		2,8,12,13	a
3-methylbutan-1-ol	isopentanol	2,14	t,ah
3-methylhexan-2-ol		1,2,11,13	a,u
3-methylpentan-1-ol		8	a,t
3-methylpentan-3-ol		2,14	ah
3-methylphenol	m-cresol	7	u
3-penten-2-ol		2,14	ah
3-phenyl-2-propen-1-ol	cinnamyl alcohol	2,10,14	x,ah
3-phenylpropan-1-ol	benzene propanol	2,10,14	x,ah
4-allylphenol	chavicol	2,14	ah
4-(3-hydroxy-1-propenyl)-2-methoxyphenol	coniferyl alcohol		r
4-(hydroxymethyl)-2-methoxyphenol	vanillic alcohol, 4-hydroxy 3-methoxybenzyl alcohol	2,6,10,11,12,13,14	j,p,u,x,y,ab,ah
4-(hydroxymethyl)phenol	p-hydroxy benzyl alcohol	2,6,10,11,12,13	d,f,j,r,u,x,y,ab
4,5-octanediol		5	u
4-ethenyl-2-methoxyphenol	4-hydroxy-3-methoxystyrene	2,7,10	t,u,x
4-ethenylphenol	4-vinyl phenol, p-vinyl phenol	2,10,14	x,ah
4-ethyl-1,3-benzenediol	4-ethylresorcinol	1,2,4,5,11,14	a,u
4-ethyl-2-methoxyphenol	p-ethyl guaiacol	2	r,t,ah
4-methoxybenzene-propanol		6,11	u
4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	terpinen-4-ol		t
4-methylphenol	p-cresol	2,9,10,14	a,f,t,x,ab,ah
9,12-octadecadien-1-ol	linoleyl alcohol	12	a
9-octadecen-1-ol	oleyl alcohol	12	a
benzene-1,4-diol	hydroquinone, quinol	1,2,11	a,u,ah
butan-1-ol		14	ah
butan-2,3-diol		2,14	ah
but-2-yne-1,4-diol		2	u

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
docosan-1-ol	behenyl alcohol	1,2	a
dodecan-1-ol	lauryl alcohol		t
dodecylcyclohexanol		1	a
heptacosan-1-ol		2,3,5,6,7,10,11,12,13	u
heptadecan-1-ol	heptyl alcohol		t
heptan-1-ol		2,14	ah
heptan-2-ol	amyl methyl carbinol	1,12	a,u
hexacosan-1-ol		2,5,6,10,11,12,14	u
hexadecan-1-ol	cetyl alcohol, palmityl alcohol	2	r
hexan-1-ol	hexyl alcohol	2,14	t,ah
hexan-2-ol		2,6,7,11,13,14	u,ah
hydroxytricosane		2	a
methyl-2-vinylethyl carbinol		10	u
nonan-2-ol			t
nonan-3-ol		2,4,13,14	a,u
o-catechol		1,2,8,9,12	a
octacosan-1-ol	montanyl alcohol	2,3,5,6,10,11,12,13,14	u
octan-1-ol	octyl alcohol, capryl alcohol	2,10,14	t,x,ah
pentan-1-ol		2,14	t,ah
pentan-2-ol	amyl alcohol	2	r,t
pentan-3-ol		2,14	ah
phenol	phenyl alcohol, benzenol	1,2,3,4,6,7,8,9,10,11,12,13,14	a,r,u,x,ah
phenylacetic acid	benzenacetic acid	2	ah
phenylmethanol	benzyl alcohol	2,10,14	t,x,ah
tetradecan-1-ol	myristyl alcohol	2	r
ALDEHYDES			
(2E,4E)-deca-2,4-dienal		2,7,8,9,10,12,14	a,r,u,x
(2E)-3-phenylacrylaldehyde	cinnamaldehyde	2,14	ah
2,2-dimethylpent-4-enal		9	a
2,5-dihydroxybenzaldehyde		2	ah
2,6,6-trimethylcyclohexene-1-carbaldehyde	beta-cyclocitral		t
2,6,6-trimethyl-1,3-cyclohexadiene-1-carbaldehyde	safranal	2,14	ah
2-hydroxy-2-methylpropanal		2	a
2-hydroxybenzaldehyde	salicylic aldehyde	2,14	t,ah
2-hydroxy-3-methoxybenzaldehyde	3-methoxysalicyladehyde, o-vanillin	2	ah
2-methylbutanal		2,14	ah
2-methylbut-2-enal	2-methyl-2-butenal	10	a
2-oxopropanal	pyruvaldehyde	2	a
3,3-dimethylhexanal		4	a,u
3,4-dihydroxybenzaldehyde	protocatechualdehyde	11,12	u
3,5-dimethoxy-4-hydroxybenzaldehyde	syringaldehyde, 5-methoxyvanillin	2,6,14	u,ah
3-ethoxyhexanal		2	ah
3-hydroxy-4-methoxybenzaldehyde	iso-vanillin	2,14	ah
3-methoxybenzaldehyde	m-anisaldehyde	12	a
3-methylbutanal	isovaleradehyde	1,2,4,5,6,7,8,9,10,11,12,13,14	a,r,u,ah
3-methyl-2-butenal	3-methylcrotonaldehyde, senecialdehyde	14	ah
3-methylpentanal	3-methylvaleraldehyde	1,2,4,6,7,8,9,10,11,12,13,14	a,u
4-ethoxy-3-methoxybenzaldehyde	vanillin ethyl ether	2,14	ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
4-hydroxy-3,5-dimethoxybenzaldehyde	syringic aldehyde	1,2,3,5,6,7,11,13,14	a,r,u
4-hydroxy-3-methoxybenzaldehyde	vanillin	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,t,u,x,ah
4-hydroxybenzaldehyde		1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,b,c,h,k,r,s,t,u,ah
4-methoxybenzaldehyde	anisaldehyde	2,11,12,14	e,l,m,t,u,ab,af,ah
5-(hydroxymethyl)-2-furfural		2,14	ah
acetaldehyde	ethanal		e,u
benzaldehyde		2,14	e,t,ae,ah
dec-2-enal	2-decenal	10	x
decanal		2,5,14	u
heptanal		2,14	ah
hept-2-enal	2-heptenal	10	x
hexacosanal		2,3,5,6,7,10,11,12,13,14	u
hexanal	hexanaldehyde	1,2,8,10,13,14	a,ah
nonanal	nonanaldehyde	1,2,11,14	a,r,u,ah
octacosanal		2,6,11	u
octadec-9-enal	octadecenyl	1	a
octanal		2	ah
pentanal	valeraldehyde	2,14	t,ah
phenylacetaldehyde		2	ah
ALKANES			
1,3,5-trimethylcyclohexane		10	u
1,7-Dimethyl-7-(4-methyl-3-penten-1-yl)tricyclo[2.2.1.02,6]heptane	alpha-santalene	2	ah
10-methyleicosane		2	r
11-decylheneicosane		1,2,9,10,12	a
2,2,4,6,6-pentamethylheptane	isododecane	2,12	a
2,2,4-trimethylpentane	icooctane	12	u
2,2,6-trimethylcyclohexane		2,14	ah
2,3,3,4-tetramethylpentane		9	a
2,6,10,15-tetramethylheptadecane		9,12,14	a,u
2,7,10-triethyldodecane		1	a
2-cyclohexyleicosane		10	a
2-methoxy-3-methylbutane		1	a
3-ethyl-2-methylpentane		10	a
3-ethyl-3-methylpentane		10	a
4-ethylbenzaldehyde		14	ah
4-ethylheptane		3,13	a,u
4-ethyltetradecane		2	a
6-ethyl-2-methyldecane		9	a
decane			t
docosane		1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,t,u,ah
dodecane		2,14	t,ah
heneicosane		2,14	r,ah
hentriacontane		3,6,7,11,12,14	u
heptacosane		2,3,5,6,7,10,11,12,13,14	u
heptadecane			t
heptane		2	ah
hexacosane		2,3,5,6,7,10,11,12,13,14	r,u
hexadecane		2,14	t,ab,ah
hexane		2,4,9,14	a,ah
hexatricontane		1,2,4,8,9,10,12	a
icosane	eicosane	2	r,t
nonacosane		2,3,5,6,7,10,11,12,13,14	u
nonadecane		2,14	ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
nonane			t
octacosane		1,11	a,u
octadecane		2,14	ah
octane		2,14	ah
pentacosane		2,3,5,6,7,10,11,12,13,14	u,x,ah
pentadecane		2,14	t,ah
pentatriacontane		1,2,4,9,12,13	a
tetracosane		1,2,3,5,6,7,10,11,12,13,14	r,u,ah
tetradecane		14	t,ah
tricosane		2,3,6,11,14	a,r,u,x,ah
tridecane		2,14	ah
undecane		2,14	t,ah
x-decane			t
ALKENES			
(1aR,4R,4aR,7bS)-1,1,4,7-tetramethyl-1a,2,3,4,4a,5,6,7b-octahydro-1H-cyclopropa[e]azulene	alpha-gurjunene	14	ah
(1E,4E,8E)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene	alpha-caryophyllene, alpha-humulene	14	ah
(1R,4aS,8aS)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	gamma-cadinene	2,14	t,ah
(1R,9S)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	beta-caryophyllene	14	ah
(1S,4aS,8aR)-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	alpha-muurolene		t
(1S,8aR)-1-isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	delta-cadinene	2,14	ah
(1Z,3Z,6Z,10Z)-14-isopropyl-3,7,11-trimethyl-1,3,6,10-cyclotetradecatetraene	cembrene	2,14	ah
(1Z,6Z,8S)-8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	germacrene D	2,14	ah
(3R,4aS,5R)-3-Isopropenyl-4a,5-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	valencene	2,14	ah
1,2,3-trimethoxy-5-methylbenzene	3,4,5-trimethoxytoluene	11	u
1,2,3-trimethylbenzene			t
1,2,4-trimethylbenzene	pseudocumene	2,14	ah
1,2-dimethoxybenzene	veratrole		t
1,2-dimethoxy-4-methylbenzene	methyl creosol	2,14	ah
1,3-dimethylbenzene	xylene		t
1-cyclohexen-1-ylethanone	1-acetylcyclohexene	2,6	u
1-ethyl-4-methylbenzene	p-ethyl toluene		t
1-isopropyl-4,7-dimethyl-1,2-dihydronaphthalene	alpha-calacorene	2,14	ah
1-methoxy-2-vinylbenzene	2-vinylanisole, 2-methoxystyrene	2,14	ah
1-methyl-4-(6-methylhept-5-en-2-yl)benzene	alpha-curcumene	2,14	t,ah
1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohexene	beta-bisabolene		t

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
1-methyl-4-prop-1-en-2-ylcyclohexene	limonene	2,14	t,ah
1-methyl-4-propan-2-ylbenzene	cymene		t
1-methyl-4-propan-2ylcyclohexa-1,3-diene	alpha-terpinene		t
1-methylnaphthalene		2,14	ah
1-tricosene		2,3,5,6,7,11,13,14	u
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	squalene	2,3,5,6,7,11,12,13,14	a,u
2,6-dimethyl-6-(4-methyl-3-penten-1-yl)bicyclo[3.1.1]hept-2-ene	alpha-bergamotene	14	ah
2-ethylnaphthalene		2,14	ah
2-methylnaphthalene		2,14	ah
3-eicosene		1,9	a
3-methylidene-6-propan-2-ylcyclohexene	beta-phellandrene		t
4-butoxybut-1-ene		8	a
4,7,7-trimethylbicyclo[3.1.1]hept-3-ene	alpha-pinene	2,14	t,ah
4-isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	calamenene	2,14	ah
5-eicosane		2,4,8,9,13	a
7,7-dimethyl-4-methylidenebicyclo[3.1.1]heptane	beta-pinene		t
7-methyl-3-methylideneocta-1,6-diene	myrcebe		t
8-isopropyl-1,3-dimethyltricyclo[4.4.0.02,7]dec-3-ene	alpha-copaene	2,14	ah
9-tricosene		1,2,4,6,8,9,10,13,14	a,u,ah
benzene	benzol		t
cyclohexene		2,14	ah
docos-1-ene	docosene	2,3,5,6,10,12,13,14	u
ethenylbenzene	styrene	2,14	t,ah
ethylbenzene	ethylbenzol, phenylethane		t
heptacos-1-ene	heptacosene	2,5,6,7,10,11,12,13,14	u
hexacos-1-ene	hexacosene	2,3,5,6,7,10,11,13	u
hexadec-1-ene	hexadecene	12	a
methylbenzene	toluene	2,14	t,ah
naphthalene	naphthalin, animite	2,14	g,t,ah
non-4-ene		13	a
nonacos-1-ene		2,3,5,6,7,10,11,12,13,14	u
pentacos-1-ene		2,3,5,6,7,10,11,13,14	r,u
propylbenzene	1-phenylpropane, isocumene	2,14	t,ah
tricosene		2	r
x-dodecene			t
x-eicosene			t
x-tetradecene			t
ESTERS			
(1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl)acetate	bornyl acetate	2	ag
(4-formyl-2-methoxyphenyl)acetate	acetovanillin	2	r
(4-methoxyphenyl)methyl(E)-3-phenylprop-2-enoate	anisyl trans-cinnimate	12	m

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
(4-methoxyphenyl)methyl(Z)-3-phenylprop-2-enoate	anisyl cis-cinnamate	12	m
(4-methoxyphenyl)methyl acetate	anisyl acetate	11,12	a,g,u,ab
(4-methoxyphenyl)methyl anisate	anisyl anisate	12	m
(4-methoxyphenyl)methyl formate	anisyl formate	2,10,12	a,g,u
(4-methoxyphenyl)methyl hexadecanoate	anisyl stearate	11,12	u
(4-methoxyphenyl)methyl hexanoate	p-anisyl hexanoate	11	u
(4-methoxyphenyl)methyl hydroxybenzoate	anisyl 4-hydroxybenzoate	12	m
(4-methoxyphenyl)methyl octadec-9-enoate	anisyl oleate	11,12	u
(4-methoxyphenyl)methyl octadecanoate	anisyl petadecanoate		u
(4-methoxyphenyl)methyl petadecanoate	anisyl palmitate	11,12	u
(4-methosyphenyl)methyl tetradecanoare	anisyl tetradecanoate		u
(4-propan-2-ylphenyl)acetate	4-isopropylphenyl acetate	11	u
[(1R,4S,6R)-1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl] acetate	isobornyl acetate	2	ag
[(2S)-2,3-dihydroxypropyl](9Z,12Z)-octadeca-9,12-dienoate	2,3-dihydroxypropyl linoleate	9	a
[(E)-3-phenylprop-2-enyl] (E) -3-phenylprop-2-enoate	cinnamyl cinnamate		g
[(E)-3-phenylprop-2-enyl] benzoate	cinnamyl benzoate		g
2-(2-hydroxybenzoyl)oxybenzoic acid	salsalate	11,12	u
2-(4-methylcyclohex-3-en-1-yl)propan-2-yl acetate	alpha-terpinyl acetate	2	ag
2,3-dihydroxypropyl acetate	glycerolmonoacetate	2,3,5,6,7,8,10,11,12,13,14	a,u
2-acetyloxyethyl acetate	ethylene diacetate	6,11,14	u
2-ethylhexyl(E)-3-(4-methoxyphenyl)prop-2-enoate	2-ethylhexyl-4-methoxycinnamate	6	u
2-ethyl-trans-bicyclohexylmethyl-14-methylpentadecanoate		1,2,9	a
2-hydrocyethyl acetate	ethylene glycol acetate	4,13	a
2-methylpropyl pentanoate	isobutyl valerate		t
2-pentanal propanoate		13	a
2-phenethyl formate		2	ag
2-phenylethylacetate		2,14	t,ah
3,7-dimethyloct-6-enyl 2-methylpropanoate	citronellyl isobutyrate	2	ag
3,7-dimethylacta-1,6-dien-3-yl acetate	linalyl acetate	2	ag
3-Hydroxy-2-butanyl acetate		2,14	ah
3-hydroxypropyl (Z)-octadec-9-enoate	3-hydroxypropyl oleate	12	a
3-hydroxypropyl prop-2-enoate	2-hydroxypropyl acrylate		u
3-methylbutyl 2-hydroxybenzoate	isoamyl salicylate	2	ag
3-methylbutyl acetate	isoamyl acetate	14	ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
4-Formyl-2-methoxyphenyl acetate	vanillin acetate	2,14	ah
4-hexen-1-ol acetate		10	a
4-methyl-3-cyclohexene-1-carboxylic acid, methyl ester		9	a
7-methyl-4-octanol acetate		1,2,8	a
bis(2-ethylhexyl)benzene-1,2-dicarboxylate	dioctyl phthalate	2,6,14	u,ah
bis(2-ethylhexyl) hexanedioate	dioctyl adipate	2,10	a
bis(2-methylpropyl) benzene-1,2-dicarboxylate	diisobutyl phthalate	2,12	a
bis(6-methylheptyl) benzene-1,2-dicarboxylate	isooctyl phthalate	1,2,3,5,6,7,8,9,10,11,12,13,14	a,u
butyl hexanoate	butyl caproate		ag
butyl pentanoate	butyl valerate		t
dibutyl benzene-1,2-dicarboxylate	dibutyl phthalate	2,14	t,ah
diethyl benzene-1,2-dicarboxylate	diethyl phthalate	2,3	t,u,ah
diethyl benzoate			t
dipropyl benzene-1,2-dicarboxylate	dipropyl phthalate		t
ethenyl formate	vinyl formate	1,13	a
ethyl 2-(4-hydroxy-3-methoxyphenyl)acetate	ethyl homovanillate	2,6,7,11	u
ethyl (2E)-3-phenylacrylate	ethyl cinnamate	2,14	ah
ethyl 2-hydroxy-2-methylbutanoate	ethyl-2-methyl butyrate		t
ethyl 2-hydroxyisobutanoate		2,14	ah
ethyl 2-hydroxybenzoate	ethyl salicylate	2	ag
ethyl 2-hydroxypropanoate	ethyl lactate		t
ethyl 2-methoxyacetate	ethyl methoxyacetate		t
ethyl 4-hydroxy-3-methoxybenzoate	ethyl vanillate	6,11,13	u
ethyl 4-oxopentanoate	ethyl levulinate		t
ethyl acetate		2,3,7,14	u,ah
ethyl benzene		2,14	ah
ethyl hexadecanoate	ethyl palmitate	2,6,14	t,u,ah
ethyl hexanoate	ethyl caproate		t
ethyloctadeca-9-12-15-trienoate	ethyl linolenate	10	x
hexadioic acid, bis(2-ethylhexyl) ester		2,14	ah
hexyl 2-hydroxybenzoate	hexyl salicylate	2	ag
hexyl acetate	methamyl acetate	2,3,6,10,11,12,14	t,u
hexyl butanoate		2	ag
m-anisic acid, methyl ester		3,6,11,12	u
methoxymethyl acetate		10	a
methyl (9Z,12Z)-octadeca-9,12-dienoate	methyl linoleate	2	r
methyl 10,13-octadecadienoate		1,2,4,8,9,10,12	a
methyl 11-octadecenoate		9	a
methyl (2E)-3-phenylacrylate	methyl cinnamate	2,14	ah
methyl 2-hydroxyacetate	methyl glycolate		t
methyl 2-hydroxybenzoate	methyl salicylate	2,10,14	t,x,ah
methyl 2-hydroxypropanoate	methyl lactate		t
methyl 2-oxopropanoate	methyl pyruvate	1,2,4,8,10,11,12	a,u
methyl 2-phenylacetate			t
methyl 3-methoxybenzoate	methyl m-anisate	12	a

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
methyl-3-phenylpropanoate		14	ah
methyl 4-(2-hydroxyethoxy)benzoate		11	u
methyl 4-(hydroxymethyl)benzoate		2	r
methyl 4-hydroxy-3-methoxybenzoate	methyl vanillate	2,3,5,6,11,14	t,u,ah
methyl 4-hydroxybenzoate	methyl paraben	1,2,4,8,14	a,ah
methyl 4-methoxybenzoate	methyl anisate	2,12	ab,ah
methyl-9,12-octadecadienoate	methyl linolelaidate	14	ah
methyl acetate	tereton	2,11,14	a,r,u
methyl acrylate	methyl prop-2-enoate	2,5,6,7,10,11,12,13,14	u
methyl benzoate		14	t,ah
methyl butyrate		14	ah
methyl decanoate	methylcaprate	2,14	ah
methyl dodecacoate	methyl laurate	2,14	t,ah
methyl heptadecanoate	methyl margarate		t
methyl heptanoate			t
methyl hexadecanoate	methyl palmitate	2,3,6,7,11,13,14	r,t,u,ah
methyl hexanoate	methyl caproate	2,14	t,ah
methyl icosanoate	methyl arachidate	10	a
methyl nonanoate	methyl pelargonate	2,14	t,ah
methyl octanoate	methyl caprylate	2,14	ah
methyl pentadecanoate			t
methyl pentanoate	methyl valerate		t
methyl propionate		14	ah
methyl tetradecenoate	methyl myristate		t
methyl-2-(4-hydroxyphenoxy)benzoate			u
methyl-3-phenylprop-2-enoate	methyl-trans-cinnamate	2,3,6,10,12,13,14	a,o,t,u,x,ab
methyl-4-hydroxybenzoate		2,5,6,11,14	u
methyl-8-methyldecanoate		2,10,13	a
octyl acetate		14	ah
pentyl 2-hydroxybenzoate	pentyl salicylate	2	ag
pentyl acetate	n-amyl acetate		t
phenylmethyl (E)-3-phenylprop-2-enoate	benzyl cinnamate		g
phenylmethyl acetate	benzyl acetate	2,14	t,ah
phenylmethyl benzoate	benzyl benzoate		g
phenylmethyl butanoate	benzyl butyrate		g
phenylmethyl formate	benzyl formate		t
prop-2-enyl octadecaboate	allyl stearate	1	a
propan-2-yl acetate	isopropyl acetate	1	a,u
propan-2-yl pentanoate	isopropyl valerate		t
propyl 4-hydroxybenzoate	propyl paraben	1,2	a,u
propyl pentanoate	propyl valerate		t
spirohexane-1-carboxylic acid, ethyl ester		12,13	a
ETHERS			
1,1'-dipropylene glycol 2'-methyl ether		14	ah
1,2-dimethoxyethane	ethylene glycol dimethyl ether	2	a
1-methoxyhexane	methyl hexyl ether	2	u
1-methoxypropane	methyl propyl ether	2	u
1-proposypropane	propyl ether	4,12	u
2-ethoxypropane	isopropyl ethyl ether	1,2,4,6,10,11	a,u
2-methosypropane	methyl isopropyl ether	2	a

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
2,5,5,8a-tetramethyl-3,4,4a,5,6,8a-hexahydro-2H-chromene	dihydroedulan II	2,14	ah
3-ethenoxyprop-1-ene	vinyl allyl ether	2,13	a,u
3-methoxypentane	1-ethylpropyl methyl ether	1	a
4-(ethoxymethyl)-2-methoxyphenol	vanillyl ethyl ether	2	n,ab,ah
4-(ethoxymethyl)phenol	p-hydroxybenzyl ethyl ether		n,t
4-hydroxybenzyl methyl ether	alpha-methoxy-p-cresol	2	ah
4-(methoxy)-2-methoxyphenol	vanillyl methyl ether	2,10	n,s,x,ah
anisole	methoxybenzene		t
phenoxybenzene	diphenyl ether		t
anisyl ether ether		12	m
anisyl methyl ether		12	m
dianisyl ether		12	m
isopentyl methyl ether		12	m
methyl cyclobutyl ether		6	u
p-cresyl isopropyl ether			t
p-hydroxybenzylmethyl ether		2	n,r,t
KETONES			
(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	alpha ionone	12	m
(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	beta-ionone	12	m
(E)-oct-3-en-2-one			t
1-(2,4,6-trimethylpehyl)ethanone	2,4,6-trimethylacetophenone	2,9,13	a,r
1-(2,4-dihydroxyphenyl)ethanone	2,4-dihydroxyacetophenone	1,2,6,9	a,u
1-(-furanyl)ethanone		2,14	ah
1-(2-hydroxy-5-methylphenyl)ethanone	o-acetyl-p-cresol	2,3,6,11,12,13,14	r,u
1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone, apocynin	2,3,4,5,6,7,8,9,10,11,13,14	a,r,u,x,ah
1-(4-hydroxy-3-methoxyphenyl)propan-2-one	vanillyl methyl ketone	2,6,10,11,13,14	a,u
13-methyl-oxacyclotetradecane-2-11-dione		2	a
18-heptacosene-2,4-dione		2,14	ah
20-nonacosene-2,4-dione		2,14	ah
1-hydroxyheptan-2-one			t
1-hydroxypentan-2-one			t
1-hydroxypropan-2-one	hydroxy acetone, pyruvic alcohol	1,2,3,4,6,7,9,10,11,13,14	u,ah
1-methoxypropan-2-one	methoxyacetone	1,2,4,8,9,12,13	a,u
1-phenylethanone	acetophenone	2,14	e,ah
2,2,4,4-tetramethylpentan-3-one		2,14	ah
2,3,3,4-tetramethylcyclobutan-1-one		6	u
2,4-dimethylpentan-3-one	diisopropyl ketone	11	u
2-butan-2-ylcyclopentan-1-one		2,10,11,14	u
2-hydroxy-3-methylcyclopent-2-en-1-one		13	a
2-methylpentan-2-one		1,2,4,9,10,12	a
3-buten-2-one	methyl vinyl ketone	2,14	ah
3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one	b-hydroxypropiovanillone	6,11	u
3-hydroxybutan-2-one	2-acetoin	2,3,6,10,11,13,14	t,u,x,ah

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
3-methyl-3-decen-5-one		13	a
3-methylbut-3-en-2-one		4	a
3-methylbutan-2-one	isopropyl methyl ketone	2,7,9,14	a,u,ah
3-methylcyclohex-2-en-1-one		1,2,4,8,9,12,13	a
3-methylcyclohexan-1-one		2	a
3-methylcyclopentan-1-one		1,2,4,6,8,9,10,12,13	a,r,u
3-methylpentan-2-one	sec-butyl-methyl ketone	6,11	u
3-oxabicyclo[3.2.0]heptane-2,4-dione		5,7,10	u
3-penten-2-one	methyl propenyl ketone	2,14	t,ah
4-(4-hydroxy-3-methoxyphenyl)butan-2-one	zingerone, vanillyl acetone	2,6,11,13	r,u
4-(4-hydroxyphenyl)-2-butanone	raspberry ketone	2	r
4,4-dimethyl-2-oxethanone		1	a
4-acetyl-2-hydroxy-5-methylbenzene		4	a
4-butoxy-3-methylbutan-2-one		6,10	a,u
4-cyclopentene-1,3-dione		2,14	ah
4-hexen-3-one		2	ah
4-hydroxy-3-methoxyphenylacetone	methyl vanillyl ketone, guaiacylacetone	2,14	ah
4-hydroxy-4-methylpentan-2-one	diacetone alcohol	2,14	ah
4-methylene-2-oxethanone		10	a
4-phenylbut-3-en-2-one	benzylidene acetone	2	ah
4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one	verbenone, 2-pinen-4-one	2	ah
5-hydroxy-2,3-dimethyl-2-cyclopentene-1-one		6	u
5-hydroxyheptan-2-one			t
6-methylhepta-3,5-dien-2-one		2,14	ah
6,10,14-trimethylpentadecan-2-one	hexahydrofarnesyl acetone	2,6,7,9,11,13,14	a,r,t,u,ah
butan-2-one	methyl ethyl ketone	1,2,14	a,ah
butane-2,3-dione	diacetyl	2,6,7,11,12,13,14	a,e,s,u,ah
cycloheptanone	suberone	2,5,10,14	a,u
cyclohesane-1,3-dione	dihydroresorcinol	2,14	a,u
cyclohexane-1,4-dione	tetrahydroquinone	10	a
cyclohexanone		2,3,5,6,7,10,11,12	u
cyclopent-4-ene-1,3-dione		2,3,5,6,7,11,12,14	u
decan-2-one	octyl methyl ketone		t
di(phenyl)methanone	diphenyl ketone	5	u
hentriacontene-2,4-dione		2,3,5,6,7,10,11,12,14	u
heptacosane-2,4-dione		2	u
heptacosene-2,4-dione		2,3,5,6,10,11,12,13,14	h
heptan-2-one	amyl methyl ketone	2,14	t,ah
heptan-4-one	dipropyl ketone	4	a
heptane-2,4,6-trione	diacetyl ketone	2	r
hex-5-en-2-ol		2	a
hexan-2-one	propyl acetone		t
hexane-2,3-dione		7	u
hexane-2,4-dione		1,2,5,6,8,9,14	a,u
nonacosene-2,4-dione		2,3,5,6,7,10,11,12,13,14	u
nonan-2-one	hexyl methyl ketone		t
octa-3,5-dien-2-one		2,14	ah
octa-4,6-dien-3-one			t
oct-3-en-2-one		2,14	ah
octan-2-one	heptyl methyl ketone	2	t,ah
pentacosane-2,4-dione		2,5,6,7,10,11,13,14	u

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
pentacosene-2,4-dione		14	u
pentan-2-one	ethyl acetone	2,6,7,11	u
pentane-2,3-dione	acetyl propionyl	3,6,7,11,14	u
HETEROCYCLIC			
1-(1H-pyrrol-2yl)ethanone	2-acetyl pyrrole		t
1,3,7-trimethylpurine-2,6dione	caffeine	12	m
1,3-benzodioxole-5-carbaldehyde	heliotropine, piperonal	2,12,14	i,l,s,t,v,z,ah
1,4-dimethylpiperazine	lupetazine		u
1-furan-2-ylethanone	acetyl furan, 2-furl methyl ketone	1,2,3,6,11,12,13	a,u
1-furan-2-ylpropan-1-one	2-propionylfuran	4,13	a
1H-pyrrole-2,5-dione, ethyl-4-methyl		10	x
2-(5H)-furanone		2	ah
2-(hydroxymethyl)-5-hydroxy-4H-pyran-4-one		1,4,10,13	a
2,2,4,5-tetramethyl-1,3-dioxolane		5	u
2,2'-bi 1,3-dioxolane		1	a
2,3-dihydro-1-bnzofuran	coumaran	1,2,3,4,6,7,8,10,11,12,13,14	a,r,u
2,3-dihydro-2,5-dimethylfuran		1,2,3,4,10,12	a,u
2,5-dimethyl furfural			e
2,6,6-trimethyl-10-methylidene-1-oxaspirol[4.5]dec-8-ene	vitispirane	12	a,m,t
2,6-dimethyl-3(2H)-benzo-furanone		4,10,12	a
2,6-dimethyl-4-pyrone		2,12	a
2-butyltetrahydrofuran		1,2,9	a
2-ethyl-1,3-dioxalane		3,5,10	a,u
2-furancarboxylic acid methyl ester		2	r
2-furfural	2-furaldehyde	2,14	ah
2-hydroxy-5-methyl furan			t
2-pentylfuran	2-amyl furan	2,6,8,10	a,t,u,ah
2-propylfuran		2,4,8	a
3,4-dimethyl-5-pentylidene-2(5H)-furanone	bovolide	2	ah
3,4-dimethylfuran-2,5-dione	dimethylmaleic anhydride	2,3,5,6,7,10,11,12,13,14	u,ah
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one		1,2,4,8,9,10,12,13	a
3,5-dimethyl-2,4-(3H,5H)-furandione		1,2,8,9,12	a
3,7-dimethylpurine-2,6-dione	theobromine	12	m
3H-pyran-2,6-dione	glutaconic anhydride	2,3,5,6,11,12,13	u
3-hydroxy-2-methylpyran-4-one	maltol	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,u,ah
3-hydroxy-4,4-dimethyl-dihydro-2(3H)-furanone		9	a
3-hydroxy-4,4-dimethyloxolan-2-one	pantolactone	2,10,14	r,x,ah
3-methyl-5-propylcyclohex-2-en-1-one	celery ketone, livescone	2	ah
3-phenylfuran		2,14	ah
3,5-dihydroxy-2-methylpyran-4-one	hydroxymaltol	2,14	ah
4-(4,5-dimethyl-1,3-dioxolan-2-yl)-2-methoxyphenol	vanillin-2,3-butyleneglycol acetal	2	r,t
4,4,7 a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one	dihydroactinidiolide	12	m,t

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
4-hydroxy-2,5-dimethylfuran-3-one	furaneol, strawberry furanone	2,3,5,6,7,10,11,12,13,14	u
4-methyl-2H-furan-5-one		2,12,14	u
4-methyl-5,6-dihydropyran-2-one	dehydromevalonolactone	2,14	ah
4,5-dihydro-2-methylfuran		2,14	ah
5-butyldihydro-2(3H)-furanone	gamma-octalactone	2,14	ah
5-(hydroxymethyl)furan-2-carbaldehyde	hydroxymethylfurfural	2,3,5,6,7,10,11,12,13,14	r,u
5-(hydroxymethyl)-tetrahydro-2-furanol		6	u
5,6-dihydro-2H-pyran-2-carboxaldehyde		1,6	a,u
5-ethyl-2(5H)-furanone	2-hexen-4-olide	2	ah
5-ethyldihydro-2(3H)-furanone	gamma-hexalactone	2,14	ah
5-ethylfuran-2-carbaldehyde	5-ethylfurfural	3,6,11,14	u
5-hydroxy-2-(hydroxymethyl)pyran-4-one	kojic acid	2,3,5,6,10,11,12,13,14	u
5-isopropyldihydro-3(2H)furandione		9	a
5-methyl-2-furfural		2,14	ah
5-methyl-3H-furan-2-one		1,2,4,8,9,10,12,13	a
5-methyldihydro-2(3H)-furanone	gamma-valerolactone	2,14	ah
5-methylfuran-2-carbaldehyde	5-methyl-2-furfural	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,e,r,t,u
5-methyl-tetrahydro-2-furan-methanol		1,2	a
5-pentyloxolan-2-one	coconut aldehyde		t
5-pentyldihydro-2(3H)-furanone	gamma-nonalactone, apricolin	2	ah
6-undecyloxan-2-one	hexadecalactone	9	a
dihydro-3-methyl-2(3H)furanone	alpha-methyl-gamma-butyrolactone	2,14	ah
dihydro-5-isopropyl-3(2H)-furanone		2	u
dihydroxydihydromaltol		2	r
ethyl furan		2	ah
furan-2,5-dione	maleic anhydride	2	a,u
furan-2-carbaldehyde	furfural	1,2,3,4,5,6,8,9,10,11,12,13,14	a,e,r,t,u,x,ab
furan-2-ylmethanol	furfuryl alcohol, 2-furancarbinol	1,2,3,4,5,6,7,8,9,10,11,12,13,14	a,r,t,u
furan-2-ylmethyl acetate	furfuryl acetate		g
furan-2-ylmethyl benzoate	furfuryl benzoate		g
furan-3-ylmethanol	3-furanmethanol	6	u
furfuryl hydroxy methyl ketone			t
hydroxydihydromaltol		2,3,5,6,7,10,11,12,13,14	u
hydroxymaltol		2	r
methyl 5-oxotetrahydro-2-furancarboxylate		2,3,10	u
methyl furan-2-carboxylate	2-furan carboxylic acid methyl ester	2,3,5,6,10,12,14	a,u
methyl pyridine-3-carboxylate	methyl nicotinate		t
oxolan-2-one	gamma-butyrolactone	2,3,6,7,10,11,14	t,u,x,ah
oxolan-2-ylmethyl acetate	tetrahydro-2-furfuryl acetate	1	a
pyran-4-one		2,12	a,u
tetrahydro-4,4,6,6-tetramethyl-2H-pyran-2-one		9,10	a,u
tetrahydroxymethylfurfuryl alcohol		2	r
thiophene	thiofuran		t

IUPAC Name	Common Name	Country(s) of origin	Reference(s)
trans-2,3-dimethyl-tetrahydro-2-furanol		1,2	a

Country of Origin		References					
1	Bali	a	Adedeji et al. (1993)	o	Gnadinger (1925). Sourced from Toth et al. (2010)	ac	Simony (1953). Sourced from Toth et al. (2010)
2	Bourbon (Madagascar)	b	Anwar (1963). Sourced from Toth et al. (2010)	p	Goris (1924). Sourced from Toth et al. (2010)	ad	Stoll and Pray (1960). Sourced from Toth et al. (2010)
3	Comoros	c	Bohnsack (1965). Sourced from Toth et al. (2010)	q	Goris (1947). Sourced from Toth et al. (2010)	ae	Tiermann and Haarmann (1976). Sourced from Toth et al. (2010)
4	Costa Rica	d	Bohnsack and Seibert (1965). Sourced from Toth et al. (2010)	r	Hartman et al. (1992). Sourced from Toth et al. (2010)	af	Walbaum (1909). Sourced from Toth et al. (2010)
5	Hawaii	e	Bohnsack (1967). Sourced from Toth et al. (2010)	s	Kleinert (1963). Sourced from Toth et al. (2010)	ag	Werkhoff and Guntert (1996). Sourced from Toth et al. (2010)
6	India	f	Bohnsack (1971a). Sourced from Toth et al. (2010)	t	Klimes and Lamparsky (1976). Sourced from Toth et al. (2010)	ah	Zhang and Mueller (2012)
7	Indonesia	g	Bohnsack (1971b). Sourced from Toth et al. (2010)	u	Lee (2006). Sourced from Toth et al. (2010)		
8	Jamaica	h	Bonnet (1968). Sourced from Toth et al. (2010)	v	Lhugenot et al. (1971). Sourced from Toth et al. (2010)		
9	Java	i	Busse (1900). Sourced from Toth et al. (2010)	w	Morison-Smith (1964). Sourced from Toth et al. (2010)		
10	Mexico	J	Chevalier et al. (1972). Sourced from Toth et al. (2010)	x	Pérez-Silva et al. (2006)		
11	Papua New Guinea	k	Chovin et al. (1954). Sourced from Toth et al. (2010)	y	Prat and Subitt (1969). Sourced from Toth et al. (2010)		
12	Tahiti	l	Cowley (1973). Sourced from Toth et al. (2010)	z	Pritzer and Jungjunz (1928). Sourced from Toth et al. (2010)		
13	Tonga	m	da Costa and Pantini (2006). Sourced from Toth et al. (2010)	aa	Schulte-Elte et al. (1978). Sourced from Toth et al. (2010)		
14	Uganda	n	Galetto and Hoffman (1978). Sourced from Toth et al. (2010)	ab	Shiota and Itoga (1975). Sourced from Toth et al. (2010)		

Table A2: List of the hydrocarbons identified by Ramaroson-Raonizafinimana et al (1997). The relative compositions are for each class of compound.

	Relative Composition (%)		
Compound	***V. fragrans***	***V. tahitensis***	***V. madascariensis***
Alkane			
n-decane		0.6	
n-dodecane	0.1	0.4	1.0
n-tetradecane	0.1	0.2	0.2
n-pentadecane	0.8	2.4	6.0

Compound	Relative Composition (%)		
	V. fragrans	*V. tahitensis*	*V. madascariensis*
n-hexadecane	0.4	0.4	0.3
n-heptadecane	1.0	2.9	4.1
n-octadecane	0.8	7.9	0.2
n-nnadecane	0.6	2.2	1.8
n-eicosane	14.0	1.8	1.7
n-deneicosane	2.9	4.6	3.4
n-docosane	15.3	7.8	15.2
n-tricosane	8.6	4.0	8.4
n-tetracosane	21.9	9.0	14.9
n-pentacosane	5.5	2.3	7.1
n-hexacosane	10.8	7.5	9.0
n-heptacosane	4.5	2.7	3.5
n-nonacosane	10.3	12.8	6.9
n-triacontane	3.5	10.8	2.8
n-hentriacontane	8.1	6.0	3.9
n-dotriacontane	1.0	1.7	1.0
n-tritriacontane	1.0	0.7	1.0
n-tetratriacontane	0.7	3.1	0.3
n-pentatriacontane	0.5	1.9	0.2
n-hexatriacontane	0.2	4.9	0.1
3-methylalkane			
3-methylpentadecane	0.4	0.3	0.2
3-methylheptadecane	0.8	0.4	0.1
3-methylnonadecane	1.6	0.5	0.1
3-methyleicosane	2.0	0.6	0.2
3-methyldocosane	34.7	11.4	64.3
3-methyltetracosane	40.9	26.4	26.4
3-methylhexacosane	14.5	54.2	6.4
3-methylhentriacontane	3.1	5.0	2.1
3-methyltritriacontane	2.0	1.2	0.2
5-ethylalkane			
5-ethyltetradecane	0.5	0.4	2.6
5-ethylhexadecane	1.0	0.8	2.8
5-ethyloctadecane	1.5	1.0	6.3
5-ethylpentacosane	9.5	10.0	504.0
5-ethylheptacosane	22.3	184.0	20.4
5-ethylnonacosane	26.2	41.5	10.1
5-ethylhentriacontane	36.4	25.9	5.2
5-ethyltritricontane	2.5	2.0	2.2
Alkene			
1-tetradecene			0.2
1-hexadecene	0.8	0.2	1.6
1-octadecene	1.4	0.1	1.7

	Relative Composition (%)		
Compound	***V. fragrans***	***V. tahitensis***	***V. madascariensis***
1-eicosene	0.6	0.9	0.9
1-docosene	0.6	0.8	0.5
1-tricosene	2.0	1.0	0.9
1-pentacosene	0.6	2.0	14.2
1-heptacosene	1.4	21.1	1.3
1-nonacosene	25.2	23.2	19.4
1-hentriacontene	55.3	38.5	50.5
1-dotriacontene	0.7	0.4	1.2
1-tritriacontene	11.4	11.8	7.6

Appendix 2: Standard Curves

A 2.1 Standard Curves for GCMS

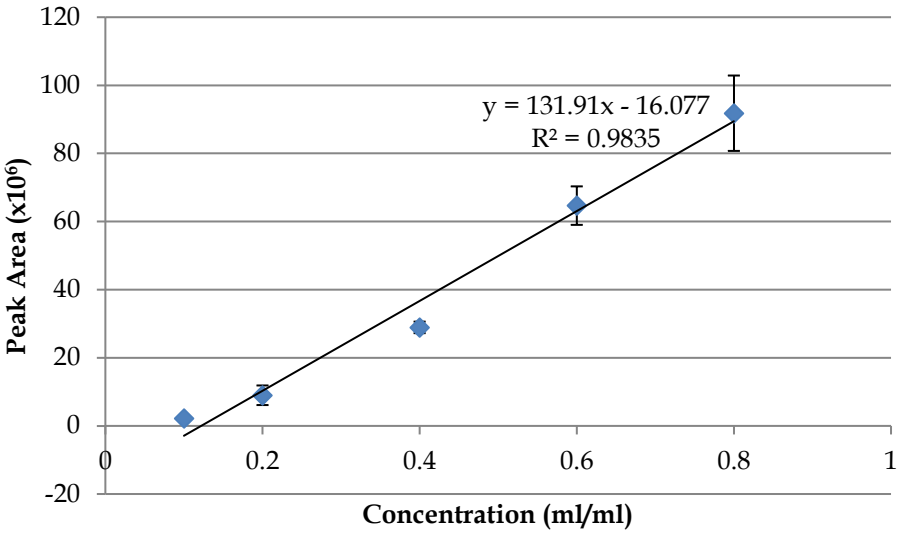

Figure A1: Standard curve on GCMS for hexanoic acid. Retention time 16.95 mins.

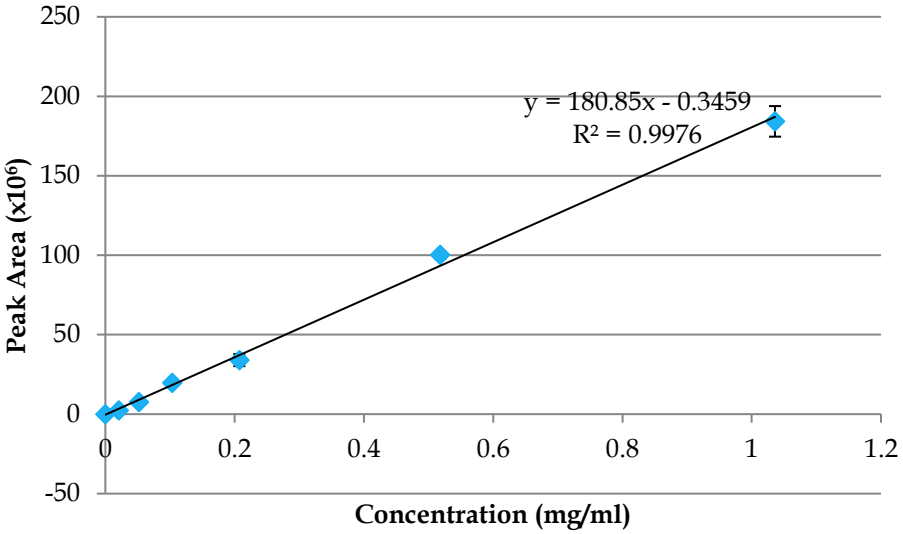

Figure A2: Standard curve on GCMS for p-cresol. Retention time 20.10 mins.

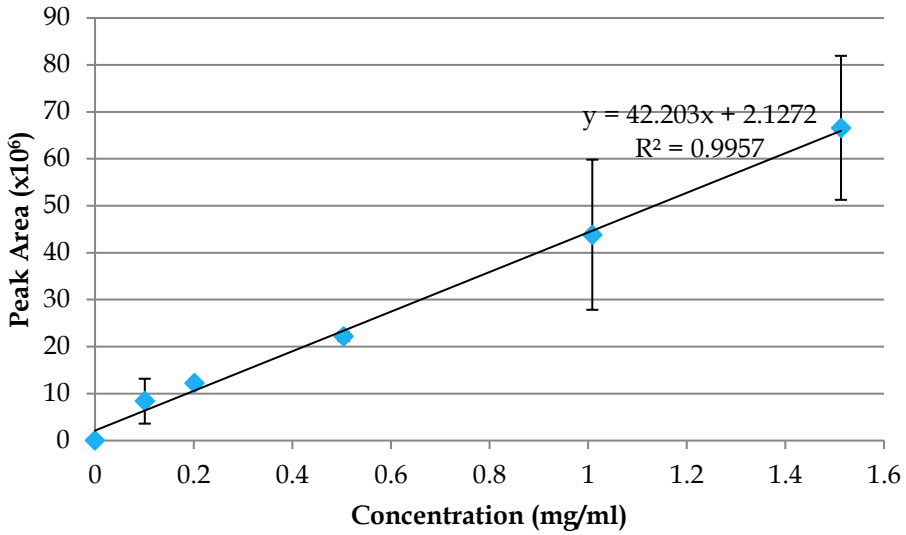

Figure A3: Standard curve on GCMS for vanillic acid. Retention time 20.20 mins.

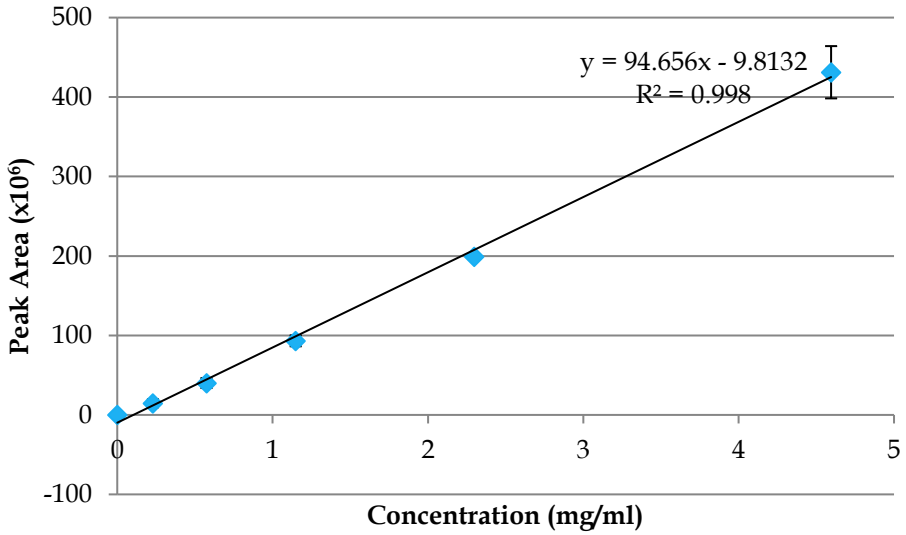

Figure A4: Standard curve on GCMS for guaiacol. Retention time 20.25 mins.

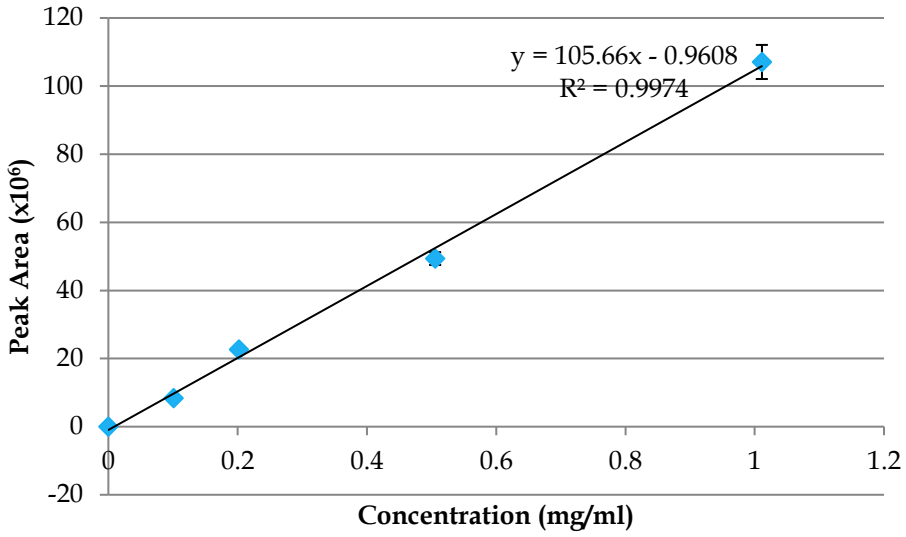

Figure A5: Standard curve on GCMS for maltol. Retention time 21.05 mins.

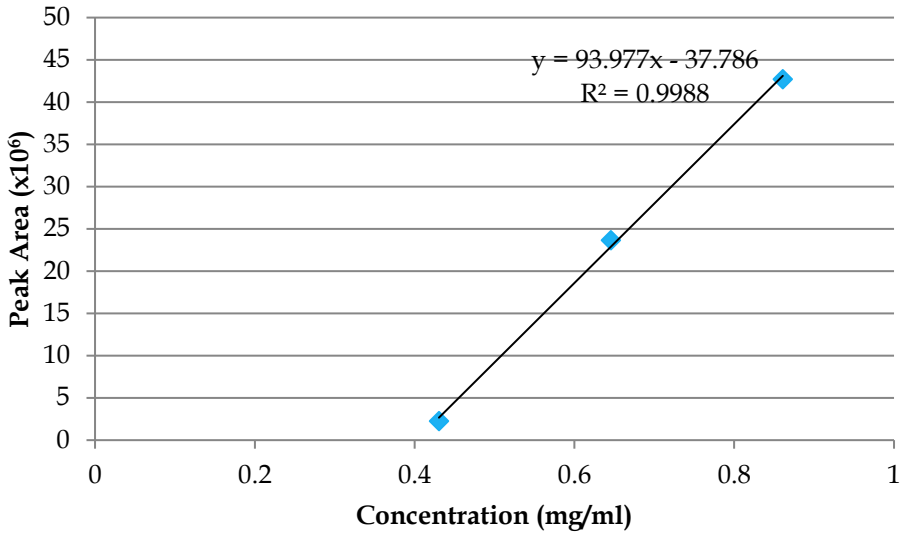

Figure A6: Standard curve on GCMS for 3-methyl-2-furoic acid. Retention time 22.15 mins.

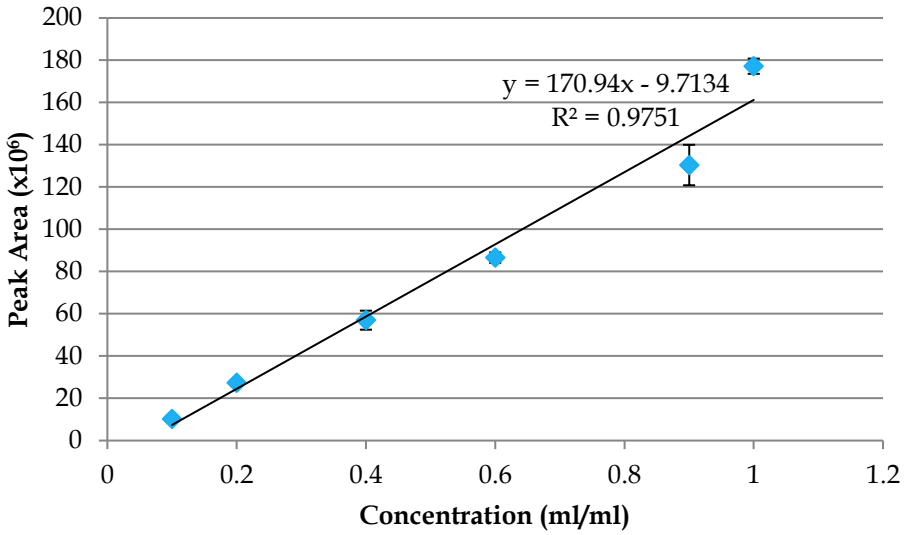

Figure A7: Standard curve on GCMS for creosol. Retention time 23.35 mins.

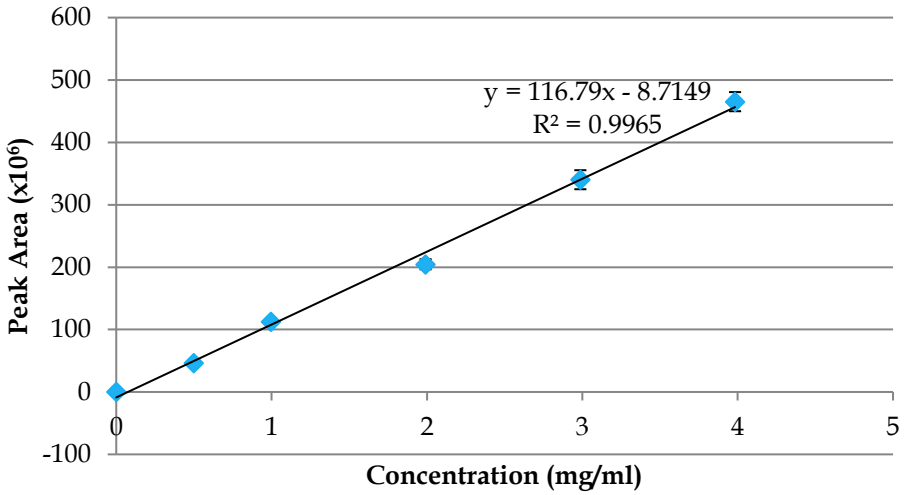

Figure A8: Standard curve on GCMS for 4-hydroxybenzaldehyde. Retention time 28.40 mins.

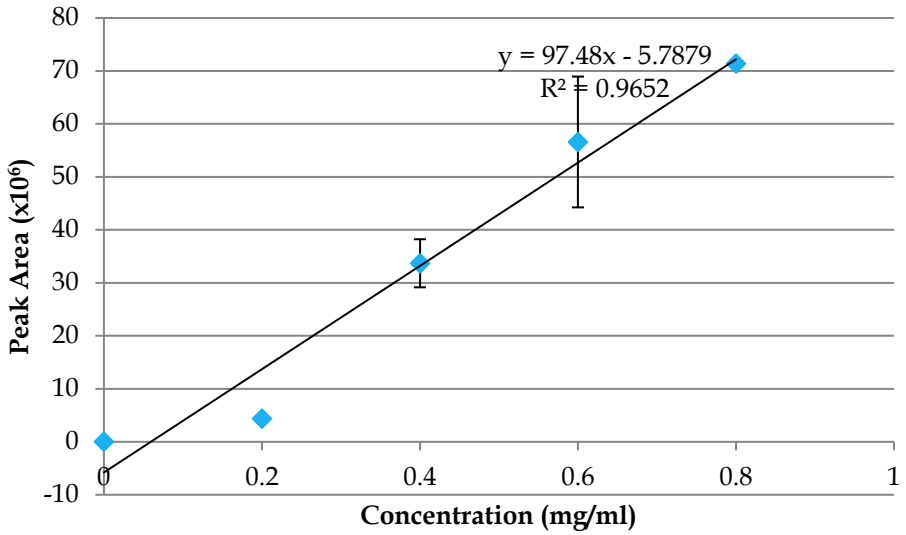

Figure A9: Standard curve on GCMS for p-anisic acid. Retention time 29.95 mins.

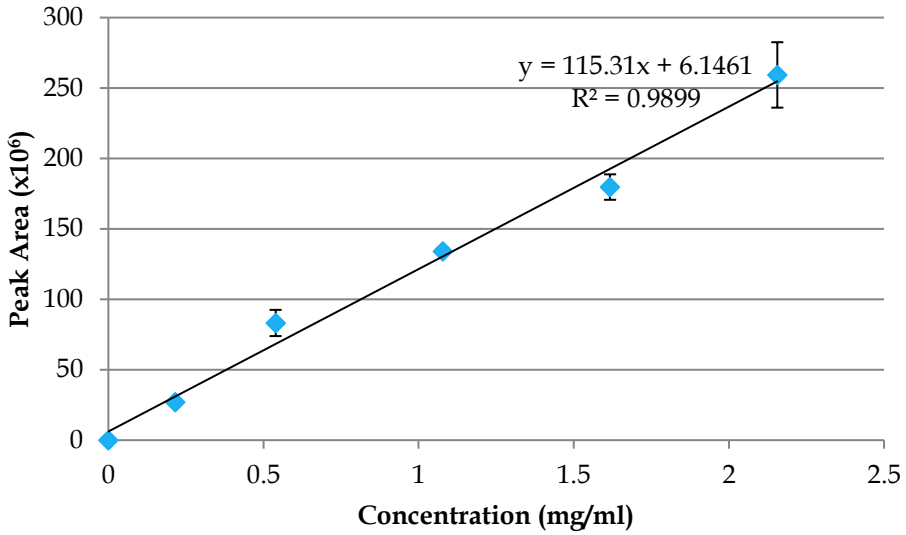

Figure A10: Standard curve on GCMS for vanillyl alcohol. Retention time 30.80 mins.

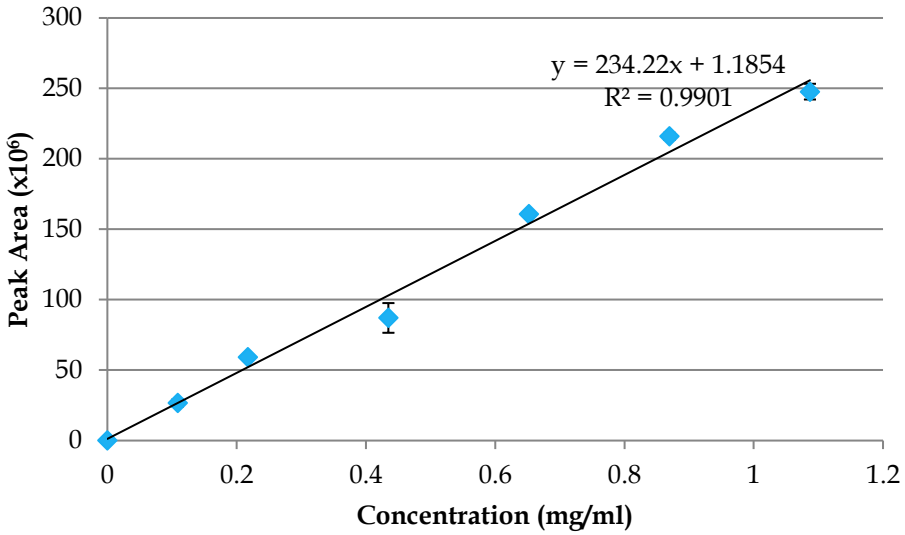

Figure A11: Standard curve on GCMS for 3,4-dimethoxybenzaldehyde. Retention time 31.4 min.

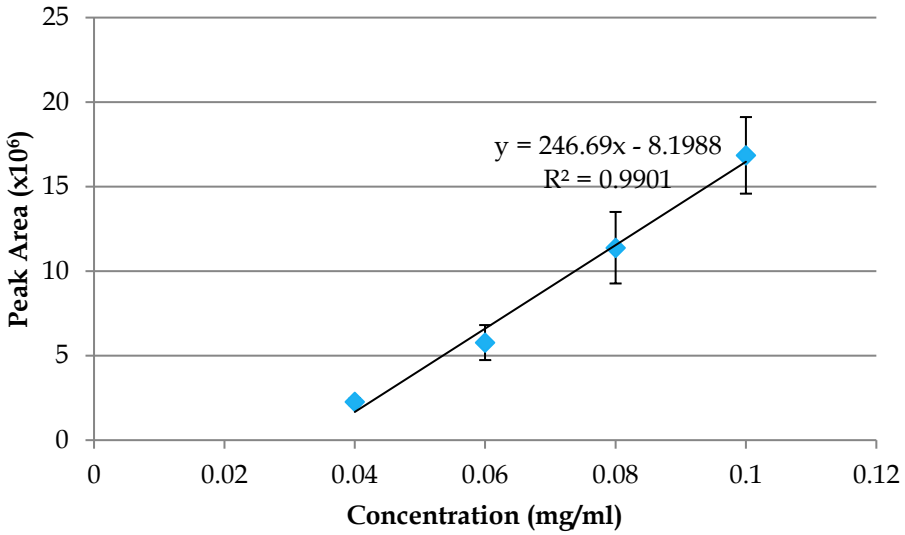

Figure A12: Standard curve on GCMS for isovanillin. Retention time 31.60 mins.

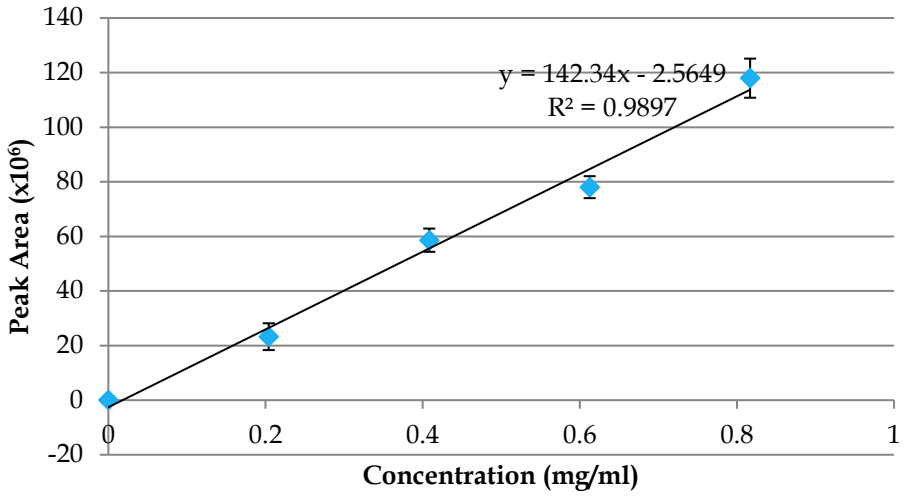

Figure A13: Standard curve on GCMS for acetovanillone. Retention time 31.75 mins.

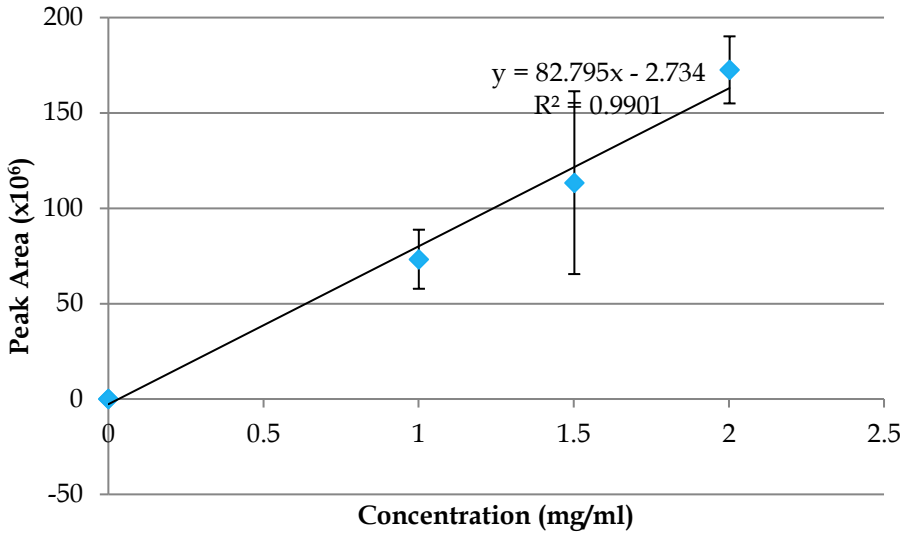

Figure A14: Standard curve on GCMS for 4-hydroxybenzoic acid. Retention time 32.15 mins.

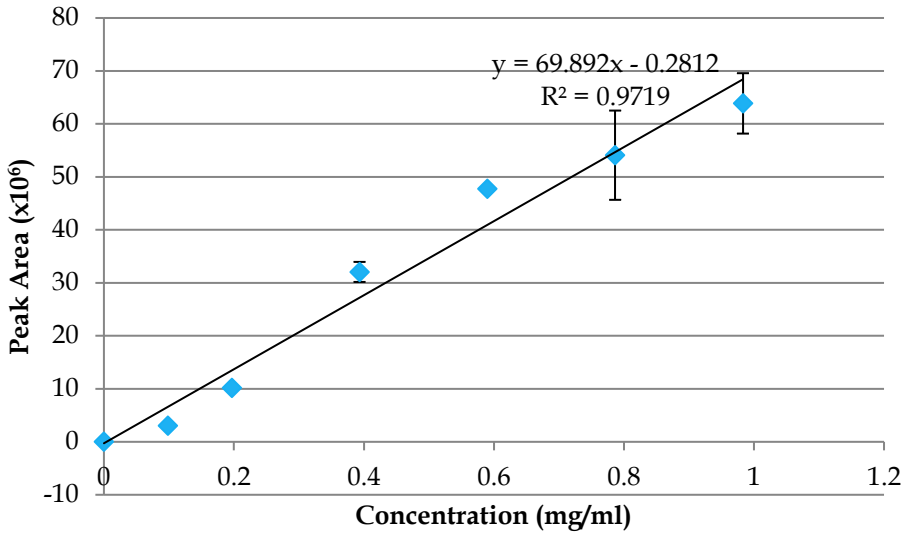

Figure A15: Standard curve on GCMS for syringaldehyde. Retention time 35.75 mins.

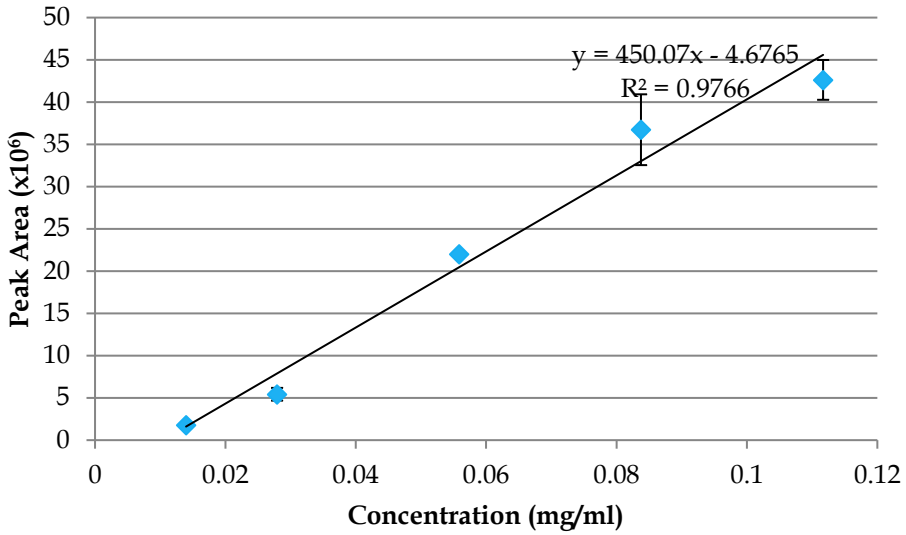

Figure A16: Standard curve on GCMS for ethyl homovanillate. Retention time 35.80 mins.

A 2.2 Standard Curves for HPLC

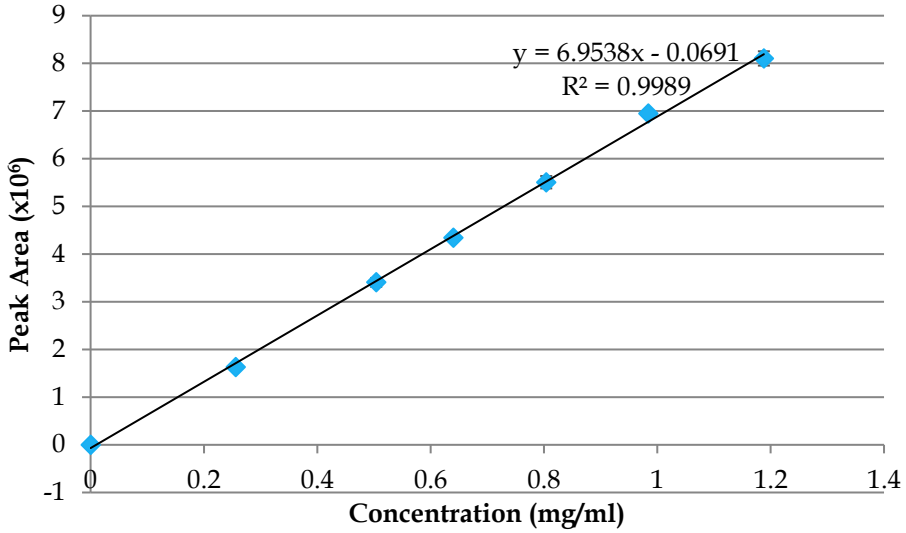

Figure A17: Standard curve for HPLC for 4-hydroxybenzoic acid. Retention time 11.7 mins.

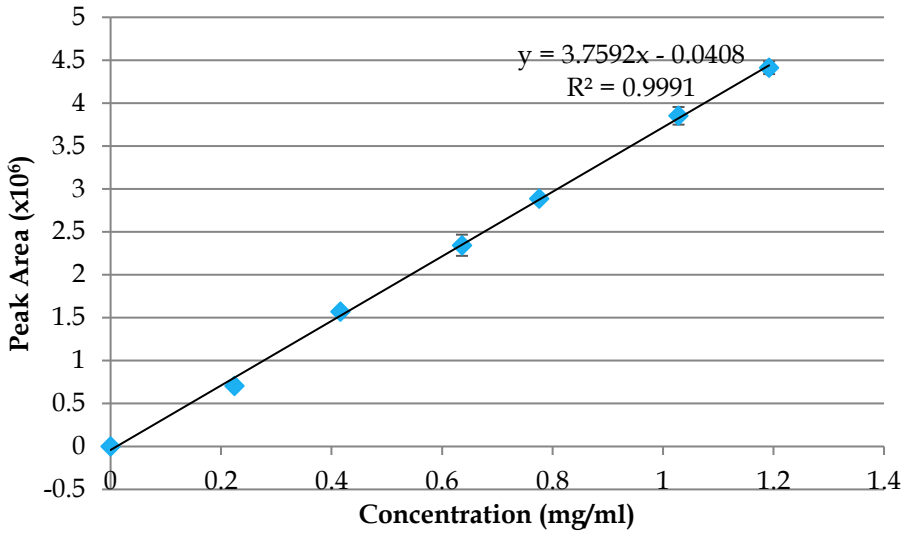

Figure A18: Standard curve for HPLC for vanillic acid. Retention time 13.1 mins.

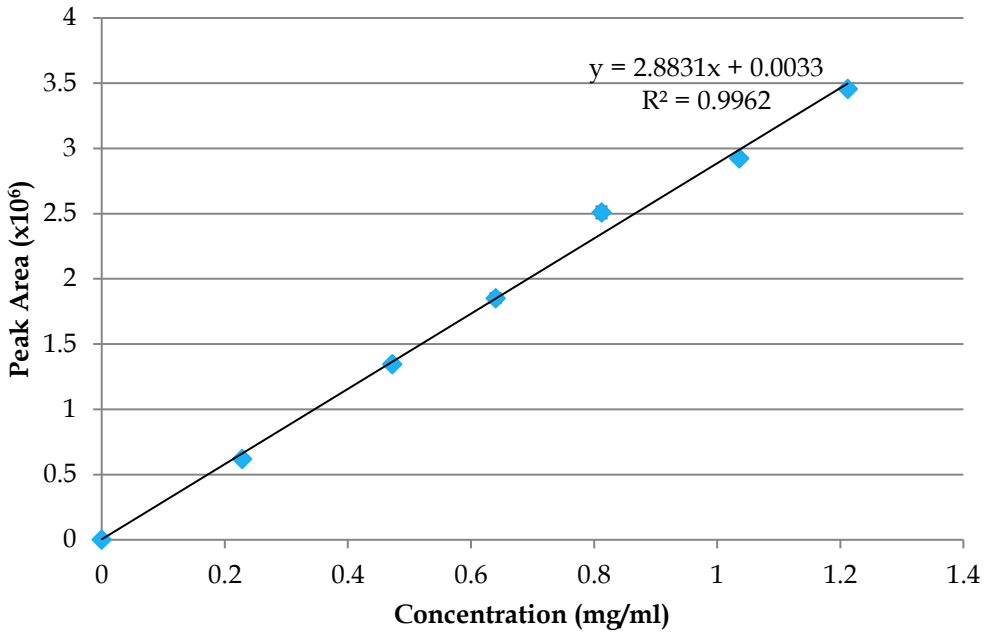

Figure A19: Standard curve for HPLC for 4-hydroxybenzaldhyde. Retention time 14.4 mins.

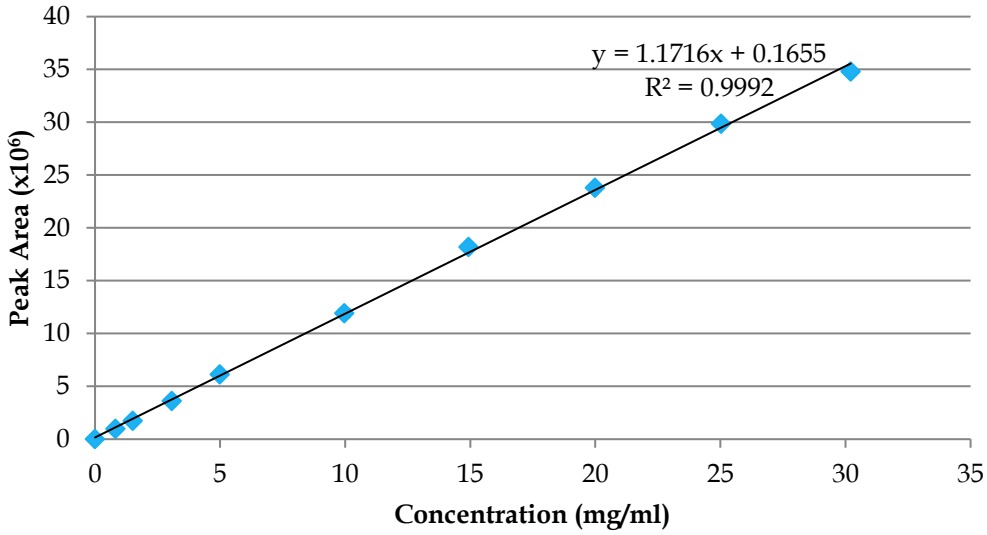

Figure A20: Standard curve for HPLC for vanillin. Retention time 15.8 mins.

A2.3 Standard Curves for GC

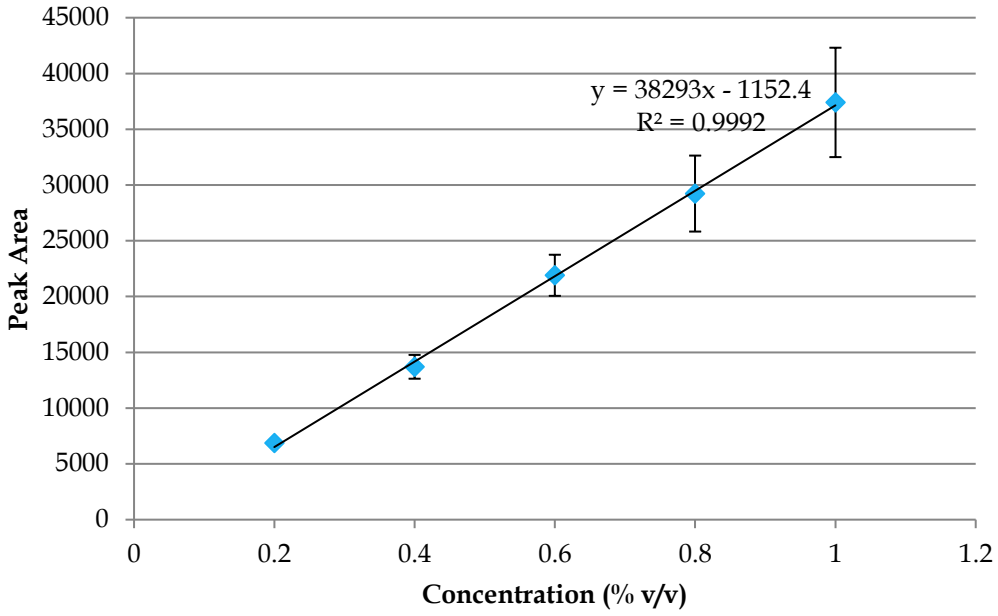

Figure A21: Standard curve for GC for ethanol, under 1% concentration (v/v). Retention time 0.75 mins.

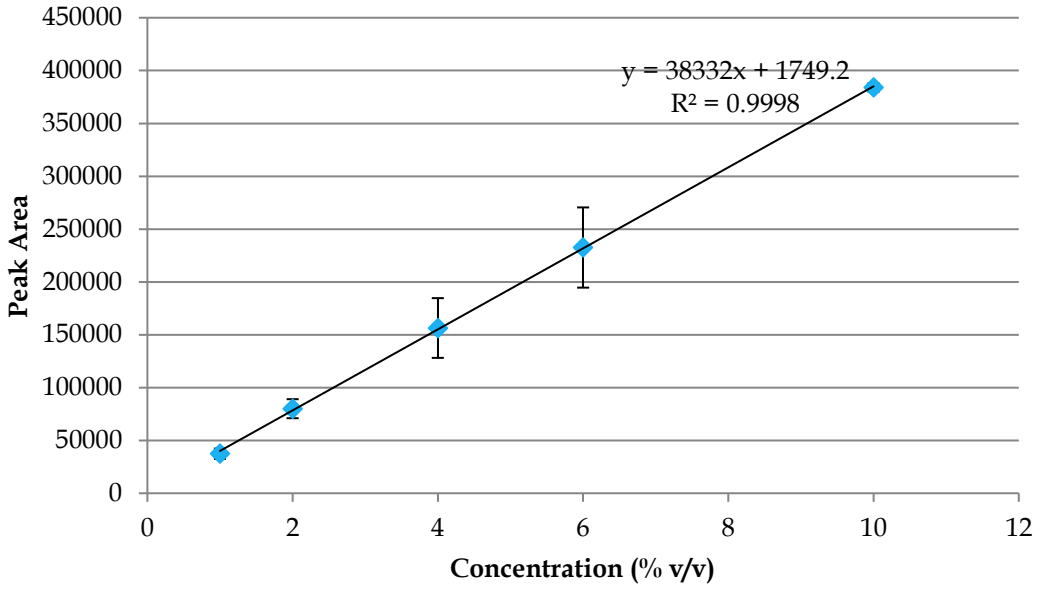

Figure A22: Standard curve for GC for ethanol, under 1-10% concentration (v/v). Retention time 0.75 mins.

Appendix 3: Mass Balance for Vanilla Extracts Produced From Different Growing Regions

Table A3: Details of mass balances for extraction of vanilla extract from vanilla beans of different growing region. Values are means ± standard error where n=2.

	Total Mass (g)	Moisture content (% w/w g water/ g vanilla bean)	Solids mass (g)	Total Liquid mass (g)	Water mass (g)	Ethanol Mass (g)	Ethanol concentration (% w/w g ethanol/g extract)
India							
Beans	200.05 ± 1.21	17.90 ± 0.04	164.25 ± 0.89	35.81 ± 0.16	35.81 ± 0.34	0	0
Extraction solution	415.24 ± 0.53	-	0	415.24 ± 1.51	187.06 ± 0.68	228.18 ± 0.64	54.95 ± 0.12
Spent Beans	269.80 ± 1.03	45.80 ± 1.32	146.23 ± 4.56	123.57 ± 3.59	109.11 ± 1.02	14.46 ± 0.94	13.70 ± 2.89
Extract	345.49 ± 1.26	-	0	345.49 ± 2.06	131.77 ± 8.56	213.72 ± 2.16	61.86 ± 0.57
Madagascar							
Beans	199.65 ± 0.71	19.28 ± 0.00	161.15 ± 0.57	38.49 ± 0.13	38.49 ± 0.31	0	0
Extraction solution	416.21 ± 1.35	-	0	416.21 ± 1.35	187.03 ± 0.63	229.18 ± 0.72	55.06 ± 0.00
Spent Beans	288.13 ± 0.57	48.55 ± 2.12	148.23 ± 5.79	139.90 ± 6.36	93.66 ± 0.95	46.09 ± 1.00	34.68 ± 2.40
Extract	327.73 ± 1.49	-	0	327.73 ± 1.49	144.47 ± 10.76	183.26 ± 1.89	55.92 ± 0.32
Papua New Guinea							
Beans	200.94 ± 0.64	14.42 ± 0.02	171.95 ± 0.62	28.98 ± 0.15	28.98 ± 0.49	0	0
Extraction solution	417.8 ± 1.23	-	0	417.80 ± 1.57	188.38 ± 1.04	229.42 ± 0.67	54.91 ± 0.07
Spent Beans	284.12 ± 0.87	45.25 ± 0.21	155.55 ± 6.21	128.57 ± 7.54	89.93 ± 0.84	38.64 ± 1.09	34.45 ± 2.78
Extract	334.62 ± 1.84	-	0	334.62 ± 1.89	143.84 ± 11.26	190.78 ± 2.11	57.01 ± 0.47
Tonga							
Beans	200.17 ± 0.30	46.30 ± 0.00	107.49 ± 0.16	92.68 ± 0.14	92.68 ± 0.14	0	0
Extraction solution	416.90 ± 1.55		0	416.90 ± 1.55	187.54 ± 0.34	229.36 ± 1.21	55.02 ± 0.09
Spent Beans	273.12 ± 2.50	62.79 ± 0.79	101.64 ± 3.17	171.48 ± 0.67	136.86 ± 14.44	34.60 ± 4.61	21.33 ± 2.93
Extract	343.95 ± 4.35		0	343.95 ± 4.35	147.92 ± 14.32	196.03 ± 4.56	56.99 ± 0.61
Uganda							
Beans	199.82 ± 0.49	27.89 ± 0.00	144.09 ± 0.34	55.72 ± 0.23	55.72 ± 0.51	0	0
Extraction solution	417.32 ± 1.51	-	0	417.32 ± 1.68	188.44 ± 0.49	228.88 ± 0.84	54.85 ± 0.16
Spent Beans	254.94 ± 1.46	49.03 ± 0.03	129.95 ± 4.37	124.99 ± 0.60	95.27 ± 12.23	29.72 ± 3.54	26.81 ± 1.98
Extract	362.2 ± 2.45	-	0	362.20 ± 3.25	163.04 ± 11.54	199.16 ± 4.21	54.99 ± 0.79

Appendix 4: Summary Data from PCA of Sensory Analysis of Vanilla Extracts

A4.1 Vanilla Extracts from Different Growing Regions

A4.1.1 Aroma

Table A4: Eigenvalues and percent of variation explained for PCA of aroma attributes, comparing vanilla extracts from different growing regions.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	4.117	1.729	0.926	0.228
Variability (%)	58.814	24.703	13.229	3.254
Cumulative %	58.814	83.517	96.746	100.000

Table A5: Factor loadings for aroma attributes in the PCA of the vanilla extracts for different growing regions.

	PC 1	PC 2	PC 3	PC 4
Overall Aroma	-0.552	0.772	-0.288	0.128
Artificial Fruity Aroma	0.718	0.631	-0.040	-0.290
Bourbon Aroma	-0.638	0.234	0.708	-0.192
Caramel Aroma	0.715	0.322	0.546	0.294
Raisin Aroma	0.994	-0.064	0.089	0.001
Spicy Aroma	-0.819	0.570	0.004	0.063
Vanilla Aroma	0.848	0.498	-0.182	-0.003

Table A6: Correlation matrix for aroma attributes in the PCA of the different growing regions vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	0.065	0.304	-0.266	-0.624	**0.899**	-0.032
Artificial Fruity Aroma	0.065	**1**	-0.283	0.610	0.670	-0.248	**0.931**
Bourbon Aroma	0.304	-0.283	**1**	-0.050	-0.586	0.646	-0.553
Caramel Aroma	-0.266	0.610	-0.050	**1**	0.739	-0.382	0.666
Raisin Aroma	-0.624	0.670	-0.586	0.739	**1**	-0.851	0.795
Spicy Aroma	**0.899**	-0.248	0.646	-0.382	-0.851	**1**	-0.412
Vanilla Aroma	-0.032	**0.931**	-0.553	0.666	0.795	-0.412	**1**

A4.1.2 Flavour

Table A7: Eigenvalues and percent of variation explained for PCA of flavour attributes, comparing vanilla extracts from different growing regions.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	5.254	2.146	1.033	0.566
Variability (%)	58.381	23.847	11.479	6.294
Cumulative %	58.381	82.227	93.706	100.000

Table A8: Factor loadings for flavour attributes in the PCA of the vanilla extracts for different growing regions.

	PC 1	PC 2	PC 3	PC 4
Overall Flavour	0.889	-0.344	-0.149	-0.263
Sweet Flavour	-0.728	-0.411	0.504	0.215
Vanilla Flavour	-0.639	-0.722	-0.265	0.006
Butterscotch Flavour	-0.890	-0.266	0.369	-0.020
Raisin Flavour	0.690	0.012	0.708	-0.148
Bitter Flavour	0.879	-0.472	0.067	-0.012
Straw Flavour	0.540	0.726	0.080	0.418
Woody Flavour	0.929	-0.337	0.150	-0.027
Bourbon Flavour	0.572	-0.636	-0.124	0.503

Table A9: Correlation matrix for aroma attributes in the PCA of the different growing regions vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	-0.638	-0.282	-0.750	0.543	**0.937**	0.109	**0.927**	0.614
Sweet Flavour	-0.638	**1**	0.630	**0.940**	-0.182	-0.416	-0.562	-0.468	-0.110
Vanilla Flavour	-0.282	0.630	**1**	0.663	-0.638	-0.239	**-0.888**	-0.391	0.129
Butterscotch Flavour	-0.750	**0.940**	0.663	**1**	-0.353	-0.633	-0.653	-0.682	-0.397
Raisin Flavour	0.543	-0.182	-0.638	-0.353	**1**	0.650	0.376	0.747	0.226
Bitter Flavour	**0.937**	-0.416	-0.239	-0.633	0.650	**1**	0.133	**0.986**	0.789
Straw Flavour	0.109	-0.562	**-0.888**	-0.653	0.376	0.133	**1**	0.258	0.048
Woody Flavour	**0.927**	-0.468	-0.391	-0.682	0.747	**0.986**	0.258	**1**	0.714
Bourbon Flavour	0.614	-0.110	0.129	-0.397	0.226	0.789	0.048	0.714	**1**

A4.2 Vanilla Extracts from Heilala Vanilla

A4.2.1 Aroma

Table A10: Eigenvalues and percent of variation explained for PCA of aroma attributes, comparing vanilla extracts from Heilala vanilla.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	4.915	1.218	0.683	0.184
Variability (%)	70.208	17.398	9.761	2.634
Cumulative %	70.208	87.606	97.366	100.000

Table A11: Factor loadings for aroma attributes in the PCA of the range of vanilla extracts from Heilala.

	PC 1	PC 2	PC 3	PC 4
Overall Aroma	0.928	-0.184	0.320	0.052
Artificial Fruity Aroma	-0.656	0.299	0.686	-0.102
Bourbon Aroma	0.938	-0.027	0.200	0.281
Caramel Aroma	0.730	0.631	-0.236	-0.111
Raisin Aroma	0.955	0.169	0.121	-0.209
Spicy Aroma	0.970	0.241	0.010	0.017
Vanilla Aroma	-0.596	0.780	-0.019	0.190

Table A12: Correlation matrix for aroma attributes in the PCA of the Heilala vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	-0.450	**0.954**	0.481	**0.884**	0.860	-0.692
Artificial Fruity Aroma	-0.450	**1**	-0.515	-0.441	-0.471	-0.559	0.591
Bourbon Aroma	**0.954**	-0.515	**1**	0.590	0.858	**0.911**	-0.530
Caramel Aroma	0.481	-0.441	0.590	**1**	0.799	0.857	0.041
Raisin Aroma	**0.884**	-0.471	0.858	0.799	**1**	**0.965**	-0.480
Spicy Aroma	0.860	-0.559	**0.911**	0.857	**0.965**	**1**	-0.387
Vanilla Aroma	-0.692	0.591	-0.530	0.041	-0.480	-0.387	**1**

A4.2.2 Flavour

Table A13: Eigenvalues and percent of variation explained for PCA of flavour attributes, comparing vanilla extracts from Heilala vanilla.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	7.486	1.149	0.320	0.045
Variability (%)	83.181	12.764	3.557	0.497
Cumulative %	83.181	95.946	99.503	100.000

Table A14: Factor loadings for flavour attributes in the PCA of the range of vanilla extracts from Heilala.

	PC 1	PC 2	PC 3	PC 4
Overall Flavour	0.960	0.243	0.133	-0.040
Sweet Flavour	0.794	0.582	0.150	-0.086
Vanilla Flavour	0.991	0.052	-0.113	0.042
Butterscotch Flavour	0.766	0.591	-0.242	0.077
Raisin Flavour	0.988	-0.111	-0.099	-0.036
Bitter Flavour	0.929	-0.139	0.330	0.088
Straw Flavour	0.889	-0.366	-0.261	-0.083
Woody Flavour	0.922	-0.357	0.136	-0.059
Bourbon Flavour	0.940	-0.325	-0.053	0.094

Table A15: Correlation matrix for flavour attributes in the PCA of the Heilala vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	**0.927**	**0.948**	0.844	**0.910**	**0.899**	0.733	0.819	0.812
Sweet Flavour	**0.927**	**1**	0.797	**0.910**	0.708	0.699	0.461	0.550	0.541
Vanilla Flavour	**0.948**	0.797	**1**	0.820	**0.984**	**0.880**	**0.889**	0.878	**0.925**
Butterscotch Flavour	0.844	**0.910**	0.820	**1**	0.712	0.556	0.522	0.458	0.548
Raisin Flavour	**0.910**	0.708	**0.984**	0.712	**1**	**0.898**	**0.949**	**0.940**	**0.966**
Bitter Flavour	**0.899**	0.699	**0.880**	0.556	**0.898**	**1**	0.784	**0.947**	**0.909**
Straw Flavour	0.733	0.461	**0.889**	0.522	**0.949**	0.784	**1**	**0.920**	**0.961**
Woody Flavour	0.819	0.550	0.878	0.458	**0.940**	**0.947**	**0.920**	**1**	**0.970**
Bourbon Flavour	0.812	0.541	**0.925**	0.548	**0.966**	**0.909**	**0.961**	**0.970**	**1**

A4.3 Commercial Vanilla Extracts

A4.3.1 Aroma

Table A16: Eigenvalues and percent of variation explained for PCA of aroma attributes, comparing commercial vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	3.742	1.682	0.829	0.643	0.087	0.018
Variability (%)	53.461	24.022	11.841	9.180	1.239	0.257
Cumulative %	53.461	77.483	89.324	98.504	99.743	100.000

Table A17: Factor loadings for aroma attributes in the PCA of the commercial vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Overall Aroma	0.901	-0.267	-0.298	0.103	-0.089	0.093
Artificial Fruity Aroma	-0.642	-0.503	0.569	-0.084	-0.001	0.067
Bourbon Aroma	0.713	-0.607	0.066	-0.287	0.188	-0.013
Caramel Aroma	0.711	0.349	0.593	-0.091	-0.113	-0.018
Raisin Aroma	0.376	0.906	0.085	-0.087	0.145	0.055
Spicy Aroma	0.697	-0.179	0.230	0.651	0.062	-0.020
Vanilla Aroma	0.936	-0.119	-0.029	-0.320	-0.077	-0.027

Table A18: Correlation matrix for aroma attributes in the PCA of the commercial vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	-0.616	0.738	0.370	0.055	0.668	**0.856**
Artificial Fruity Aroma	-0.616	**1**	-0.092	-0.288	-0.637	-0.283	-0.532
Bourbon Aroma	0.738	-0.092	**1**	0.339	-0.225	0.447	**0.816**
Caramel Aroma	0.370	-0.288	0.339	**1**	0.624	0.503	0.645
Raisin Aroma	0.055	-0.637	-0.225	0.624	**1**	0.070	0.256
Spicy Aroma	0.668	-0.283	0.447	0.503	0.070	**1**	0.455
Vanilla Aroma	**0.856**	-0.532	**0.816**	0.645	0.256	0.455	**1**

A4.3.2 Flavour

Table A19: Eigenvalues and percent of variation explained for PCA of flavour attributes, comparing commercial vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	4.872	2.011	1.285	0.510	0.242	0.080
Variability (%)	54.130	22.350	14.280	5.662	2.687	0.892
Cumulative %	54.130	76.480	90.759	96.421	99.108	100.000

Table A20: Factor loadings for flavour attributes in the PCA of the commercial vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Overall Flavour	0.799	-0.350	0.311	-0.110	0.361	0.005
Sweet Flavour	0.363	-0.653	0.590	0.287	-0.084	-0.068
Vanilla Flavour	0.868	0.357	-0.321	0.065	-0.056	0.089
Butterscotch Flavour	-0.687	-0.207	-0.447	0.513	0.150	-0.006
Raisin Flavour	0.426	0.827	0.219	0.216	0.194	0.044
Bitter Flavour	0.806	-0.273	-0.506	0.113	0.011	-0.085
Straw Flavour	0.730	0.561	0.266	0.212	-0.158	-0.107
Woody Flavour	0.853	-0.456	-0.016	0.114	-0.124	0.188
Bourbon Flavour	0.886	-0.100	-0.403	-0.175	0.027	-0.107

Table A21: Correlation matrix for flavour attributes in the PCA of the commercial vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	0.640	0.442	-0.618	0.166	0.573	0.388	**0.780**	0.645
Sweet Flavour	0.640	**1**	-0.091	-0.243	-0.214	0.210	0.136	0.628	0.104
Vanilla Flavour	0.442	-0.091	**1**	-0.503	0.602	**0.764**	**0.761**	0.614	**0.841**
Butterscotch Flavour	-0.618	-0.243	-0.503	**1**	-0.422	-0.211	-0.651	-0.446	-0.493
Raisin Flavour	0.166	-0.214	0.602	-0.422	**1**	0.030	**0.844**	-0.008	0.169
Bitter Flavour	0.573	0.210	**0.764**	-0.211	0.030	**1**	0.331	**0.816**	**0.935**
Straw Flavour	0.388	0.136	**0.761**	-0.651	**0.844**	0.331	**1**	0.386	0.453
Woody Flavour	**0.780**	0.628	0.614	-0.446	-0.008	**0.816**	0.386	**1**	**0.764**
Bourbon Flavour	0.645	0.104	**0.841**	-0.493	0.169	**0.935**	0.453	**0.764**	**1**

A4.4 All Vanilla Extracts Combined

A4.4.1 Aroma

Table A22: Eigenvalues and percent of variation explained for PCA of aroma attributes, comparing all vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigenvalue	2.734	1.747	1.537	0.474	0.282	0.204	0.022
Variability (%)	39.050	24.960	21.952	6.770	4.028	2.921	0.320
Cumulative %	39.050	64.010	85.961	92.731	96.760	99.680	100.000

Table A23: Factor loadings for aroma attributes in the PCA of all vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Overall Aroma	0.948	-0.137	-0.065	0.032	0.151	-0.216	0.088
Artificial Fruity Aroma	-0.170	-0.246	0.823	0.472	0.094	-0.036	-0.012
Bourbon Aroma	0.911	-0.123	0.099	-0.007	0.153	0.349	-0.008
Caramel Aroma	0.171	0.889	0.300	0.110	-0.258	0.089	0.063
Raisin Aroma	0.017	0.911	-0.231	0.114	0.310	-0.065	-0.047
Spicy Aroma	0.883	-0.044	-0.292	0.232	-0.253	-0.098	-0.078
Vanilla Aroma	0.409	0.175	0.785	-0.414	-0.009	-0.113	-0.046

Table A24: Correlation matrix for aroma attributes in the PCA of all the vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	-0.144	**0.821**	-0.028	-0.033	**0.845**	0.319
Artificial Fruity Aroma	-0.144	**1**	-0.044	0.023	-0.332	-0.289	0.342
Bourbon Aroma	**0.821**	-0.044	**1**	0.067	-0.095	**0.707**	0.391
Caramel Aroma	-0.028	0.023	0.067	**1**	**0.667**	0.101	0.405
Raisin Aroma	-0.033	-0.332	-0.095	**0.667**	**1**	0.000	-0.056
Spicy Aroma	**0.845**	-0.289	**0.707**	0.101	0.000	**1**	0.045
Vanilla Aroma	0.319	0.342	0.391	0.405	-0.056	0.045	**1**

A4.4.2 Flavour

Table A25: Eigenvalues and percent of variation explained for PCA of flavour attributes, comparing all vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
Eigenvalue	4.498	2.323	0.996	0.514	0.404	0.170	0.061	0.023	0.011
Variability (%)	49.981	25.807	11.066	5.707	4.491	1.894	0.673	0.253	0.128
Cumulative %	49.981	75.788	86.854	92.561	97.052	98.946	99.619	99.872	100.000

Table A26: Factor loadings for flavour attributes in the PCA of all vanilla extracts.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
Overall flavour	0.850	0.345	-0.204	-0.129	0.122	0.263	0.122	0.023	-0.003
Sweet Flavour	-0.371	0.775	0.113	-0.053	0.491	-0.043	-0.054	-0.013	-0.019
Vanilla Flavour	-0.147	0.882	0.266	0.146	-0.273	0.147	-0.097	0.040	0.010
Butterscotch Flavour	-0.741	0.195	0.532	-0.319	-0.065	-0.078	0.125	0.031	0.019
Raisin Flavour	0.647	-0.337	0.664	-0.065	0.009	0.120	-0.031	-0.079	-0.019
Bitter Flavour	0.888	0.263	-0.046	-0.294	-0.159	-0.149	-0.030	0.032	-0.066
Straw Flavour	0.777	-0.266	0.378	0.376	0.164	-0.082	0.028	0.082	0.000
Woody Flavour	0.949	0.026	-0.018	-0.277	0.069	-0.068	-0.080	0.008	0.076
Bourbon Flavour	0.595	0.729	-0.027	0.249	-0.113	-0.155	0.093	-0.073	0.016

Table A27: Correlation matrix for flavour attributes in the PCA of all the vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Straw Flavour	Woody Flavour	Bourbon Flavour
Overall flavour	**1**	-0.022	0.101	**-0.643**	0.334	**0.832**	0.448	**0.836**	**0.686**
Sweet Flavour	-0.022	**1**	**0.625**	0.467	-0.420	-0.184	-0.390	-0.280	0.275
Vanilla Flavour	0.101	**0.625**	**1**	0.372	-0.210	0.072	-0.250	-0.181	**0.581**
Butterscotch Flavour	**-0.643**	0.467	0.372	**1**	-0.188	**-0.520**	**-0.545**	**-0.627**	-0.364
Raisin Flavour	0.334	-0.420	-0.210	-0.188	**1**	0.455	**0.803**	**0.604**	0.089
Bitter Flavour	**0.832**	-0.184	0.072	**-0.520**	0.455	**1**	0.480	**0.929**	**0.684**
Straw Flavour	0.448	-0.390	-0.250	**-0.545**	**0.803**	0.480	**1**	**0.635**	0.344
Woody Flavour	**0.836**	-0.280	-0.181	**-0.627**	**0.604**	**0.929**	**0.635**	**1**	**0.512**
Bourbon Flavour	**0.686**	0.275	**0.581**	-0.364	0.089	**0.684**	0.344	**0.512**	**1**

Appendix 5: PLS Regression Analysis of Sensory Attributes and Chemical Compounds in Vanilla Extracts

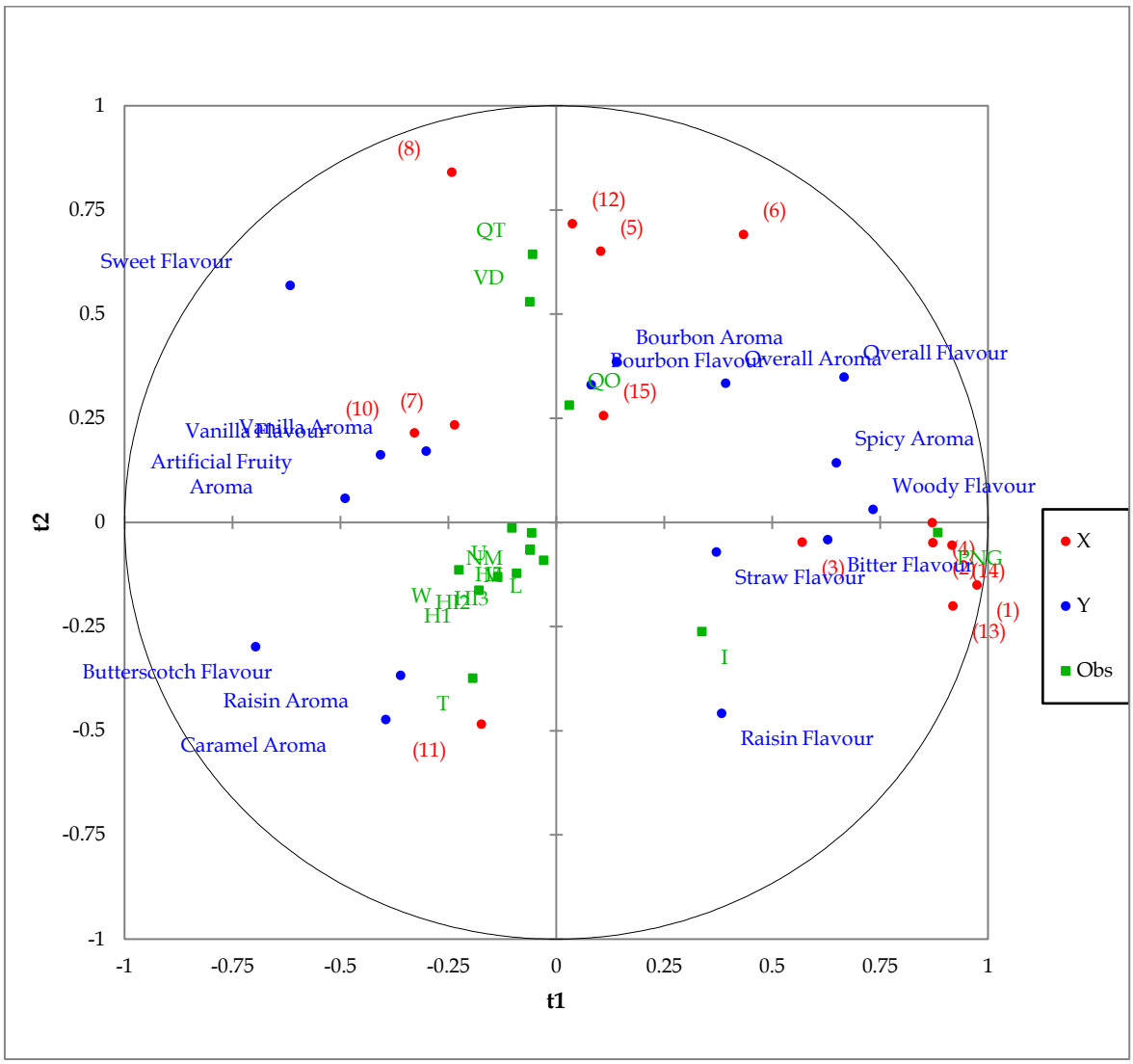

Figure A23: Biplot of t1 against t2 for a PLS regression comparing 16 vanilla extracts with 15 quantified chemical compounds using 16 sensory attributes.

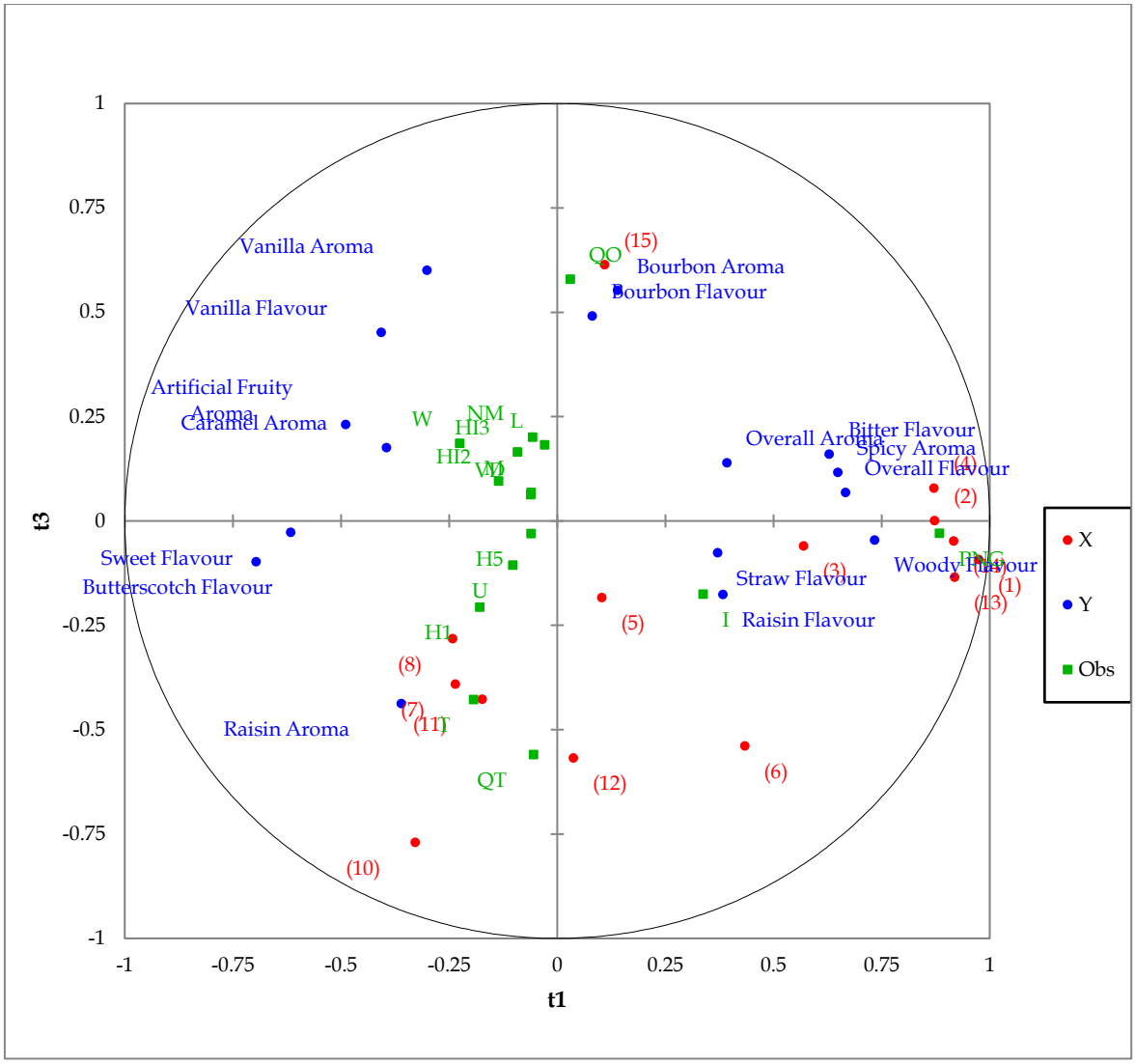

Figure A24: Biplot of t1 against t3 for a PLS regression comparing 16 vanilla extracts with 15 quantified chemical compounds using 16 sensory attributes.

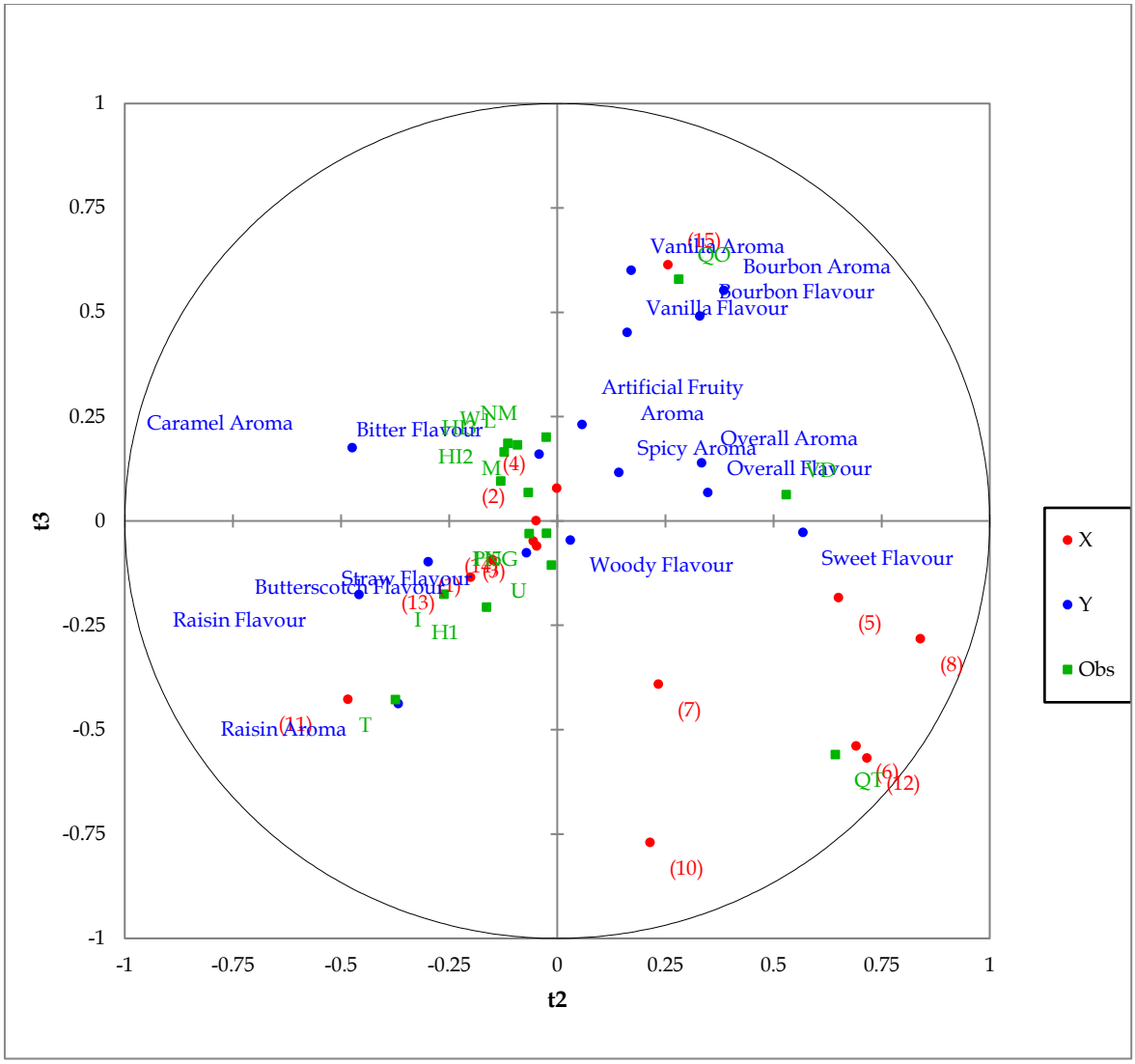

Figure A25: Biplot of t2 against t3 for a PLS regression comparing 16 vanilla extracts with 15 quantified chemical compounds using 16 sensory attributes.

Table A28: Regression coefficients determined by PLS. Numbers in parentheses correspond to chemical name coding in Table 6.2. Continued in Table 8b.

	R^2	Intercept	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Overall Aroma	0.352	4.17	1.05	-0.076	1.84	0.79	9.21	0.13	0.041
Artificial Fruity Aroma	0.542	3.29	-1.65	3.26	-3.09	-0.37	-0.19	-0.058	-2.49
Bourbon Aroma	0.500	2.83	-0.48	-0.29	0.076	0.67	10.98	0.010	-2.34
Caramel Aroma	0.436	2.85	-0.46	-3.45	0.28	-0.24	-3.32	0.12	0.27
Raisin Aroma	0.538	2.81	-0.14	-12.25	2.56	-0.90	-10.38	-0.97	4.92
Spicy Aroma	0.460	2.39	1.30	4.41	0.74	0.63	3.41	0.075	-0.62
Vanilla Aroma	0.548	3.27	-0.98	-4.06	0.095	0.068	4.11	0.076	-0.92
Overall Flavour	0.601	4.15	2.03	4.99	1.76	1.08	9.19	0.20	-0.31
Sweet Flavour	0.716	4.11	-2.05	-3.93	-1.61	-0.44	6.08	0.078	-0.22
Vanilla Flavour	0.447	3.67	-1.04	0.30	-1.33	-0.088	2.00	-0.46	-1.56
Butterscotch Flavour	0.667	2.99	-1.48	-2.02	-1.72	-0.76	-6.01	-0.11	-0.11
Raisin Flavour	0.634	1.94	2.03	-5.22	3.98	0.24	-5.93	-0.67	3.19
Bitter Flavour	0.423	2.12	1.18	4.64	0.45	0.52	1.16	0.027	-0.82
Straw Flavour	0.322	2.33	0.70	-1.70	1.43	0.19	0.24	0.012	0.98
Woody Flavour	0.352	1.92	2.01	3.39	1.89	0.70	2.05	0.09	0.52
Bourbon Flavour	0.358	2.67	-0.62	2.68	-1.07	0.47	8.22	0.008	-2.81

Table A28b: Continuation of Table A31.

	(8)	(10)	(11)	(12)	(13)	(14)	(15)
Overall Aroma	1.39	-1.39	-1.72	0.14	0.66	0.10	2.83
Artificial Fruity Aroma	-0.46	-1.09	-2.25	0.47	-4.57	0.036	-0.31
Bourbon Aroma	1.08	-3.54	3.74	-0.006	-4.34	-0.016	4.23
Caramel Aroma	-0.56	-0.020	1.28	-0.61	0.67	-0.21	-0.96
Raisin Aroma	-0.33	4.08	6.14	-0.87	2.95	-0.63	-2.84
Spicy Aroma	0.18	-0.93	-1.02	0.064	2.32	0.32	1.03
Vanilla Aroma	0.41	-1.81	-1.21	-0.35	-3.84	-0.26	2.27
Overall Flavour	1.21	-1.29	-2.10	0.40	3.16	0.42	2.33
Sweet Flavour	1.48	0.031	-2.25	0.97	-5.95	-0.32	0.70
Vanilla Flavour	0.002	-1.29	-1.65	0.11	-3.45	-0.056	0.85
Butterscotch Flavour	-0.81	0.89	0.97	-0.054	-2.42	-0.22	-1.81
Raisin Flavour	-0.60	1.53	4.63	-1.33	6.57	-0.12	-0.47
Bitter Flavour	-0.27	-0.91	-0.61	-0.12	2.35	0.31	0.60
Straw Flavour	0.17	0.26	0.95	-0.30	1.83	-0.031	0.36
Woody Flavour	0.17	-0.17	0.27	-0.14	4.63	0.32	0.66
Bourbon Flavour	0.58	-3.06	-3.77	0.24	-4.08	0.11	2.94

Appendix 6: Tables of Compounds Found in Supercritical Carbon Dioxide Flavour Extractions

Compounds identified in supercritical carbon dioxide extracts using GCMS for trials 1,2 and 4.

Table A29: Twenty most concentrated compounds found in the supercritical carbon dioxide extract (Trial 1), as identified by mass spectrum (NIST 2008 Library).

Retention Time (mins)	Peak Area (% total)	Identification of compound
29.05	0.89	Unidentified
29.35	40.13	Vanillin
31.25	1.7	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
36.65	1.81	Unidentified
45.35	1.53	n-Propyl 9,12-octadecadienoate
47.20	1.85	1-Heptacosanol
47.55	1.86	Dotriacontane
49.20	0.9	Dotriacontane
50.45	1.16	1-Eicosene
50.75	1.61	Tetrapentacontane
53.75	0.86	Tetracosane
53.80	2.06	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
54.60	1.74	E,E-3,13-Octadecadien-1-ol
54.70	1.26	Bruceantin
55.40	2.77	Unidentified
56.60	1.04	Heptacosyl heptafluorobutyrate
56.80	1.93	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
56.90	2.29	Oxirane, hexadecyl-
57.75	1.53	1(2H)-Naphthalenone, octahydro-5-hydroxy-8a-methyl-
57.95	6.19	E,E,Z-1,3,12-Nonadecatriene-5,14-diol

Table A30: Twenty most concentrated compounds found in the supercritical carbon dioxide extract (Trial 2), as identified by mass spectrum (NIST 2008 Library).

Retention Time (mins)	Peak Area (% total)	Identification of compound
28.80	1.07	Vanillin lactoside
28.95	1.31	Vanillin
31.25	2	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
45.35	1.49	n-Propyl 9,12-octadecadienoate
47.20	1.83	1-Heptacosanol
47.55	1.81	Dotriacontane
50.45	1.14	1-Eicosene

50.75	1.72	Tetrapentacontane
53.80	3.58	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
54.60	1.96	E,E-3,13-Octadecadien-1-ol
54.70	1.66	Bruceantin
55.40	3.76	Unidentified
55.50	1.55	Unidentified
55.70	1.26	Tricosane-2,4-dione
56.60	1.54	Heptacosyl heptafluorobutyrate
56.80	2.76	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
56.90	2.41	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
57.75	1.92	1(2H)-Naphthalenone, octahydro-5-hydroxy-8a-methyl-
57.95	6.91	E,E,Z-1,3,12-Nonadecatriene-5,14-diol

Table A31: Twenty most concentrated compounds found in the supercritical carbon dioxide extract (Trial 4), as identified by mass spectrum (NIST 2008 Library).

Retention Time (mins)	Peak Area (% total)	Identification of compound
25.20	3.14	2-Undecenal
28.45	7.28	Benzaldehyde, 4-hydroxy-
28.60	0.74	Benzaldehyde, 3-(chloroacetoxy)-4-methoxy-
28.780	0.9	Heneicosane
29.20	2.22	Phenol, 2-methoxy-4-(methoxymethyl)-
29.65	41.13	Vanillin
42.30	1.03	Tricosane-2,4-dione
47.20	1.99	1-Heptacosanol
47.55	2.2	Dotriacontane
50.75	1.8	Dotriacontane
53.80	1.91	Oxirane, hexadecyl-
54.60	1.62	E,E-3,13-Octadecadien-1-ol
54.70	0.95	Bruceantin
55.40	2.71	Unidentified
55.50	0.49	Unidentified
56.60	1.33	Heptacosyl heptafluorobutyrate
56.80	1.37	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
56.90	1.37	Oxirane, hexadecyl-
57.75	0.84	1(2H)-Naphthalenone, octahydro-5-hydroxy-8a-methyl-
57.95	5.37	E,E,Z-1,3,12-Nonadecatriene-5,14-diol

Appendix 7: Summary Data from PCA of Sensory Characteristics of Various Concentrated and Powdered Vanilla Extracts

A7.1 PCA of Aroma Attributes

Table A32: Eigenvalues and percent of variation explained for PCA of aroma attributes, comparing various concentrated and powdered vanilla extracts.

	PC 1	PC 2	PC 3
Eigenvalue	5.624	0.837	0.318
Variability (%)	80.344	11.961	4.542
Cumulative %	80.344	92.306	96.847

Table A33: Factor loadings for PCA of aroma attributes, comparing various concentrated and powdered vanilla extracts.

	PC 1	PC 2	PC 3
Overall Aroma	0.958	0.152	-0.130
Artificial Fruity Aroma	0.637	0.757	-0.109
Bourbon Aroma	0.961	-0.134	-0.159
Caramel Aroma	0.892	-0.019	0.441
Raisin Aroma	0.837	-0.456	-0.229
Spicy Aroma	0.984	0.016	0.021
Vanilla Aroma	0.955	-0.121	0.130

Table A34: Correlation matrix for PCA of aroma attributes, comparing various concentrated and powdered vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Aroma	Artificial Fruity Aroma	Bourbon Aroma	Caramel Aroma	Raisin Aroma	Spicy Aroma	Vanilla Aroma
Overall Aroma	**1**	**0.720**	**0.933**	**0.793**	**0.739**	**0.918**	**0.888**
Artificial Fruity Aroma	**0.720**	**1**	0.516	0.508	0.229	0.650	0.494
Bourbon Aroma	**0.933**	0.516	**1**	**0.781**	**0.870**	**0.920**	**0.935**
Caramel Aroma	**0.793**	0.508	**0.781**	**1**	**0.668**	**0.899**	**0.888**
Raisin Aroma	**0.739**	0.229	**0.870**	**0.668**	**1**	**0.841**	**0.794**
Spicy Aroma	**0.918**	0.650	**0.920**	**0.899**	**0.841**	**1**	**0.908**
Vanilla Aroma	**0.888**	0.494	**0.935**	**0.888**	**0.794**	**0.908**	**1**

A7.2 PCA of Flavour Attributes

Table A35: Eigenvalues and percent of variation explained for PCA of flavour attributes, comparing various concentrated and powdered vanilla extracts.

	PC 1	PC 2	PC 3
Eigenvalue	4.239	2.302	0.695
Variability (%)	52.991	28.780	8.683
Cumulative %	52.991	81.770	90.453

Table A36: Factor loadings for PCA of flavour attributes, comparing various concentrated and powdered vanilla extracts.

	PC 1	PC 2	PC 3
Overall Flavour	0.950	0.059	-0.018
Sweet Flavour	-0.664	0.562	0.372
Vanilla Flavour	0.603	0.477	0.583
Butterscotch Flavour	-0.159	0.893	-0.405
Raisin Flavour	0.772	-0.362	-0.129
Bitter Flavour	0.681	0.703	-0.131
Woody Flavour	0.860	0.387	-0.073
Bourbon Flavour	0.841	-0.428	0.118

Table A37: Correlation matrix for PCA of flavour attributes, comparing various concentrated and powdered vanilla extracts. Values in bold are different from 0 with a significance level alpha=0.05.

Variables	Overall Flavour	Sweet Flavour	Vanilla Flavour	Butterscotch Flavour	Raisin Flavour	Bitter Flavour	Woody Flavour	Bourbon Flavour
Overall Flavour	**1**	-0.525	0.538	-0.076	**0.714**	**0.681**	**0.804**	**0.814**
Sweet Flavour	-0.525	**1**	0.024	0.468	**-0.725**	-0.131	-0.386	**-0.728**
Vanilla Flavour	0.538	0.024	**1**	0.105	0.288	**0.672**	0.629	0.303
Butterscotch Flavour	-0.076	0.468	0.105	**1**	-0.351	0.567	0.208	-0.582
Raisin Flavour	**0.714**	**-0.725**	0.288	-0.351	**1**	0.247	0.458	0.655
Bitter Flavour	**0.681**	-0.131	**0.672**	0.567	0.247	**1**	**0.853**	0.276
Woody Flavour	**0.804**	-0.386	0.629	0.208	0.458	**0.853**	**1**	0.578
Bourbon Flavour	**0.814**	**-0.728**	0.303	-0.582	0.655	0.276	0.578	**1**