

**Development of a grapefruit-flavoured spirit
with the opalescence properties of pastis**

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of the degree of
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Attestation of authorship

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

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Confidentiality

None

Abstract

In the form of a potable spirit, an extract of grapefruit skin has been found to develop an attractive opalescence when diluted to below about 38 % ethanol (v/v). This phenomenon is analogous to the pastis effect, called louching, common in many drinks popular in some countries bordering the Mediterranean. The main objective of this research was to develop spirit liquor with commercial potential from grapefruit skin, largely a waste commercial product, as the dominant if not exclusive ingredient other than alcohol and water. This would require making extracts by distillation of undried skins, which would develop a dense opalescence significantly below 40 % v/v ethanol, the common alcoholic strength of spirits as sold in New Zealand.

The product concept was thus a clear liquid which when poured over ice for example, would yield a cool opalescent drink with a characteristic grapefruit flavour. In the case of citrus, the chemical basis of louching is the greater solubility of citrus skin terpenes, principally limonene, in ethanol than in water. The louch point is synonymous with the chemical expression critical micelle concentration, detected here by light scattering at the arbitrary wavelength of 450 nm. Early results with an obvious opalescence showed that the alcohol concentration at which the terpenes ceased to be soluble in the grapefruit distillate was about 38 % (v/v). This point was similar to that for pastis (Pernod brand), where the principle louchable ingredient is anethole. However, the light scattering was much greater for pastis. Thus, a grapefruit spirit sold at 40 % ethanol with the louching intensity of pastis should require increasing the solubility of limonene in solutions with a lower ethanol concentration. This in turn should allow higher concentrations of limonene in true solution in 40 % ethanol, theoretically resulting in a more intense louch in the final drink.

Thus, a broad range of hydrocolloids and surfactants was tested in an attempt to increase the solubility of limonene in lower ethanol concentrations. None of hydrocolloids or surfactants lowered the louch point of a standard limonene concentration in ethanol/water.

The informal flavour assessment showed that the spirit flavour from grapefruit zest alone lacked intensity. It was then thought that better flavour might be obtained by using the whole grapefruit (zest, pith, and juice) rather than zest alone. The additional of pith or pith plus juice caused no significant change in the louch point or light

scattering. However, the final flavour was informally judged to be more intense than that derived from zest alone.

The problem still remained that the light scattering of grapefruit distillate was never as high as that achieved by pastis, even though it was found (by gas chromatography) that the concentration of limonene (w/w) in the grapefruit spirit was at least as high as that of anethole in pastis. The cause was due to the fact that as a louching compound, anethole was a solid with a flat crystal structure because the melting point (21.4°C) is well above that of a cool drink (Handbook of Chemistry and Physics, 1947). By contrast, limonene remains a liquid even in an iced drink because its melting point is far below 0°C. Flat crystals would obviously scatter light far more than would a micelle containing a liquid, in this case limonene.

Although such a grapefruit distillate did not louch as well as pastis, it could still have market potential on the basis that it would be made from the distinctive New Zealand cultivar of grapefruit. Thus a formal sensory assessment was conducted, using a focus group. The grapefruit distillates at 40 % ethanol were perceived by most panelists as refreshing, clean, fruity, and citrusy in aroma, but somewhat deficient in grapefruit flavor, and there was a common perception of strong chemical finish.

At this stage of development a commercial proposition cannot be sensibly made.

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Chapter 1

Introduction

Purpose of this research

The purpose is to develop a potable spirit from grapefruit skins with the behavioural properties of pastis, a drink popular in France and Mediterranean countries. As sold, pastis and related drinks are coloured but clear. On dilution with water or ice, a compound(s) in the drink that was soluble in 40 % ethanol or higher ceases to be so, forming micelles or crystals that scatter light. Pastis thus becomes cloudy. The phenomenon is called ‘louching’ from the French verb *loucher* meaning ‘to look askance’ (French Dictionary, retrieved on 14 May 2008), which is consistent with the marked change in appearance on dilution.

In this chapter, there are short reviews of alcoholic spirit available and the international spirit market. This is followed by a discussion of the importance of geographical distinctiveness in drinks, then leading to the chemistry of louching phenomenon in pastis and grapefruit extracts and the planned experiments. These involve distillation trials, dilution with water and possible ways of modifying the ‘louche point’, the concentration of ethanol below which louching occurs. The method chosen for sensory assessment in this research is also outlined.

Spirit drinks available internationally

A distilled beverage is a consumable liquid containing ethyl alcohol (ethanol) recovered and concentrated by distillation of fermented substances such as fruit, vegetables, or grains (Lea & Piggott, 2003). The word spirit generally refers to distilled beverages low in sugars and containing at least 35 % alcohol by volume. Vodka, gin, whisky, brandy, rum, tequila and pastis are examples of well-known spirits.

Vodka is essentially ethanol diluted to between 35 and 50 % (v/v) ethanol, and as traditionally prepared is multiply distilled from fermented carbohydrate bases, which can include potato, and cereals among other fermentable raw materials (Lea & Piggott, 2003). Vodka is required triple distilled and normally filtered through charcoal because vodka simply requires high-purity alcohol. Vodka spirit is normally

subjected to further processing with activated carbon in order to reduce the concentrations of trace congeneric materials, which may impart sensory character. This may be achieved by either dispersing or agitating powdered charcoal in a large volume of spirit followed by its removal by filtration (Lea & Piggott, 2003). Vodka originated in Poland and Russia and although now sold internationally its ethos remains integral to Eastern European countries. Vodka may also be flavoured using a variety of materials such as orange and lemon peels, ginger, cloves, peppers, blackcurrant and sugar, and in these expressions begins to resemble gin.

Gin is made by flavouring alcohol with botanical substances as for flavoured vodka. However there is one defining flavour ingredient that sets gin apart. Gin is always flavoured in part with juniper berry, the fruit of *Juniperus communis* in the family Cupressaceae. The berry is also widely used as a flavourant in traditional European cuisine, which probably explains its use in spirits. The name gin itself is derived from either the French 'genièvre' or the Dutch 'jenever', which both mean 'juniper'. The key ingredients are multiply-distilled neutral alcohol (flavourless, colourless), water to dilute the spirit to marketable strength, juniper berries and a range of other plant substances such as coriander, angelica, and orange peel (Lea & Piggott, 2003). The exact composition of botanicals defines the qualities of one gin from another, and are confidential recipes (Williams, 1949).

Whisky (or whiskey in Eire and the U.S.A) is distilled from fermented grain mash and aged in oak casks. The flavour of whisky mostly derives from extraction of flavour compounds and colour from toasted oak barrels previously used to hold port wine and sherry. The 'age' of a whisky is the time between distillation and bottling when the spirit is in contact with the wood. This reflects how much the oak has interacted with the whisky, changing its chemical makeup and taste. Exposure to low concentrations of oxygen diffusing through the wood from the atmosphere also contributes to the complexity of whisky flavour (Belitz & Grosch, 2004).

Brandy (derived from the Dutch word *brandewijn*, meaning 'burnt wine') (Hornby, 2005) is a general term for distilled wine, and can be made from grape wine, fermented pomace, or fermented fruit juice. All that is required is that the liquid be allowed to ferment and that the resulting mildly-alcoholic product not be heated past the boiling point of water (Cyril, 1978). But unless specified otherwise, brandy is made from grape wine.

Rum is a spirit drink produced exclusively by alcoholic fermentation and distillation of sugar industry products. The fermentable raw material is either sugar cane itself, molasses, or syrup produced in the manufacture of cane sugar (Dave, 2003). Rums are then aged in oak barrels for months and years to remove harsh flavours through multiple reactions that are variously understood, and to add distinct characteristics from compounds leaching from the oak interior. The interior condition of the barrel determines the colour of rum produced after storage (Williams, 1949).

Rums are characteristically dry tasting, with a slight molasses flavour. Light rum is sometimes referred to as white or silver rum and is a very subtle liquor, much like vodka with a sweet note. These rums are generally aged for up to a year and filtered before bottling. This process gives light rums their clean, light flavour and makes this variety the most common rum for cocktails. Dark rums are the richest rums, which develop their flavour from charred oak barrels.

Tequila is a spirit made primarily in the area surrounding Tequila, a town in the western Mexican state of Jalisco. Tequila is made from blue agave (*Agave tequilana azul*), a desert-adapted lily which is native to Mexico (Lemon, 2000). Agave juice – which is extracted from the core of the stem after a heating procedure to hydrolyse storage inulin – is fermented for 30 to 48 hours. Double distillation yields a colourless liquid that retains the characteristic flavour compounds of the source plant. After blending and dilution to commercial spirit strength it can be sold as conventional (clear, colourless) tequila. Other variations depend on oak barrel aging to produce a background whisky-like colour and oak flavours. Such oak aged tequila is more expensive (Lemon, 2000). Thus all types of tequila start with the colourless distilled spirit, with added complexity as required. The consumption and exportation of this beverage has a high relevance in Mexico, not only economically but also culturally. Tequila is certainly one of the most recognized Mexican icons not only nationally but also internationally (Lea & Piggott, 2003). In this respect tequila is a geographically-distinct drink as is discussed in more detail later.

Pastis is an anise-flavored liqueur and apéritif from France which has its origin in absinthe, a drink created in the late eighteenth century in France. Absinthe comprised distilled alcohol (Lea & Piggott, 2003), flavoured with anise and other herbs including the flower and leaves of the medicinal plant *Angelica absinthium*, also called wormwood. Absinthe was banned in the 1910s because it contained a toxic terpene

thujone (Figure 1), which although used for medicinal purposes, was totally unsuited to a mainstream alcoholic drink. After absinthe was banned, the southern French company Pernod Ricard reformulated the drink without the banned wormwood component (Lea & Piggott, 2003), with a focus on the aniseed flavour from star anise, sugar and a lower alcohol content. The drink was called pastis, which remains particularly popular in France and other Mediterranean-bordering countries.

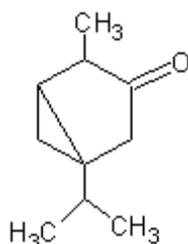


Figure 1 Structure of thujone

In the global market vodka, gin, whisky, brandy, rum, tequila and pastis are the major products in the flavoured spirits. However, when compared to other alcoholic drinks beer is the major sector of alcohol beverages, with total beer sales globally estimated 1578 billion litre in 2005 (Figure 2).

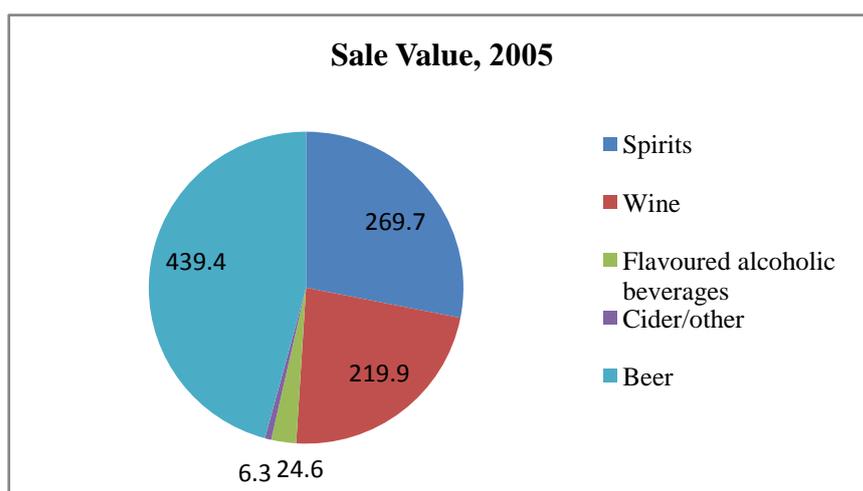


Figure 2 Alcohol beverages as a global industry in 2005

In 2005, the top five spirits brands were entirely nationally explicit. The major spirits brands to be attributed in the top 10 in amount, were Bacardi rum and Diageo's

Smirnoff vodka. Local commercial spirits producers govern the market, and tend to be geographically fragmented due to the persistent popularity of traditional national specialties. There are many and diverse regional brands of flavoured spirits. For example, Cachaca is continuously distilled from sugar cane in Brazil (Lea & Piggott, 2003). Some countries, particularly India, have local brands based on local extra neutral alcohol plus flavourings. These spirits are given the local designations of whisky, brandy, and rum and are known as 'Indian-made foreign liquors'.

Geographical distinctiveness in spirit drinks

If a person in a Western culture were asked to identify the country(s) most associated with whisky, the most common answer would undoubtedly be Scotland. Likewise, champagne would be identified with France and tequila with Mexico. Thus these and other alcoholic drinks have acquired a geographical distinctiveness arising from historical origin. This distinctiveness has major commercial value, in the sense that country of origin or the region is important in creating premium market positioning.

For example, the European Union legally enforces 'cognac' as the exclusive name for brandy produced and distilled in the Cognac area of France, and 'armagnac' from the Gascony area of France (Encyclopedia-Britannica Online Encyclopedia, retrieved on 1 May, 2008). Since these are considered Protected Designation of Origin (POD) status, they refer not just to style of brandy but brandies from a specific region, e.g. a brandy made in California in a manner identical to the method used to make cognac, and which tastes similar to cognac, cannot be called cognac as it is not from the Cognac region of France.

Champagne, the most famous of sparkling wines, is probably the most recognised wine in the world. It is also one of the most-copied wine region names. The term 'champagne' should be used only in one of two ways: to describe a sparkling wine from the Champagne region of France (*appellation d'origine contrôlée*), a legally defined geographical area of production (Section 5388(c) of Title 26 of the United States Code), or to describe a method of making sparkling wine. The French object to others naming their wine 'champagne', but not to the copying of the production process. Thus non-champagne sparkling wines are often labelled 'methode champenoise', which indicates similarity of drinking experience.

The Mexican 'denomination of origin' law has defined the area in which the blue agave is grown as the source of authentic tequila. It includes Jalisco state and some regions of other states all of which have similar reddish volcanic soil and climate (Lemon, 2000).

Sauvignon blanc is a green-skinned grape variety originating from the Bordeaux region of France. Sauvignon blanc was first introduced to New Zealand in the 1970s (Clarke, 2001), and was heavily planted in the geographical area known as Marlborough. As grown and vinified in Marlborough, the grapes produce wines with highly distinctive sensory characteristics. Marlborough sauvignon blanc is acclaimed throughout the world as a definitive benchmark style. Marlborough wines do not have an *appellation d'origine contrôlée* status, but under the Fair Trading Act 2006 (Ministry of Economic Development, 2007) a claim that a wine was vinified from Marlborough grapes when it was not, is illegal. One group of New Zealand wine makers has established 'The Gimblett Gravels Winegrowing District' as a registered trademark of the Gimblett Gravels Winegrowers Association. The Association and registered brand were developed to define and then name a winegrowing district using principles that are not catered for within New Zealand's proposed Geographic Indications Act legislation (Park, 2005).

With the exception of Marlborough sauvignon blanc, certain pinot noir styles from Central Otago, and the Gimblett trademark, New Zealand alcoholic drinks have no iconic status internationally except perhaps for the name 'New Zealand' itself. In seeking to develop iconic drinks it seems sensible to focus on plants strongly identified with New Zealand as a source of flavour. New Zealand's 42 Below vodka (now a trademark of the multinational company Bacardi Limited) produces vodka that is flavoured with kiwifruit and manuka honey among other flavours. The international success of 42 Below vodka may be due in part to the relative uniqueness and geographical distinctiveness of the two flavours. Kiwifruit, although not a native of New Zealand is strongly identified with the country. Manuka honey, from the flower of the native shrub *Leptospermum scoparium*, has a distinctive flavour that is (purportedly) imparted to the vodka (according to promotional claims on the bottle).

In AUT University research is in progress to support the development of geographically-distinct alcoholic drinks, with wine and spirit flavours derived from wood species strongly identified with New Zealand (Kang, 2008), a gin-like spirit

flavoured with miro berries (*Prumnopitys ferruginea*), and a tequila-like spirit from ti kouka or cabbage tree (*Cordyline australis*) (Patel, 2008).

A particular variety of grapefruit thrives in the warmer parts of New Zealand, and is common in domestic gardens, as well as being a commercial crop (Wright, 2007). It is a variety of *Citrus paradisi*, which has gold skin and an orange flesh, and was considered distinctive enough to be named as New Zealand 'Goldfruit' to give the fruit an exotic new image in export markets (Grapefruit (Gold fruit), 2007). The fruit is well suited to New Zealand's temperate climate. On the face of it, the fruit may be distinctive enough to confer geographical distinctiveness when used to flavour alcoholic drinks.

In a chance discovery in 2005, an AUT undergraduate student found that distillate of grapefruit peel was able to develop an attractive opalescence when diluted with water in the manner of louching of pastis, the distinctive French spirit discussed above. This phenomenon is the basis of this thesis.

The student (Lui, 2005) found that a distillate of grapefruit peel and ethanol produced a spirit that was clear when undiluted at about 38% (v/v), but which developed an attractive opalescence when diluted with water. The product concept is a clear, perhaps faintly yellow spirit, which when poured over ice for example, would yield a cool opalescent drink with a characteristic grapefruit flavour. The chemical basis of louching is the greater solubility of citrus peel terpenes, principally limonene, in ethanol than in water (Hengde & Kazuhiro, 2005). The undergraduate's research showed that the alcohol concentration at which the terpenes ceased to be soluble in the spirit was about 38% (v/v). In physical chemistry terms this is called the critical micelle concentration, the point at which the terpenes form their own opalescent phase in the surrounding water-alcohol mixture.

The proposed spirit is not to be confused with a geographically distinctive drink from Italy and sold worldwide, limoncello. It is a lemon-based liqueur which is made by soaking lemon peels in neutral grain alcohol. The final drink is a thick, sweet dessert cordial with an intense lemon flavour. As sold it is already cloudy probably because the alcoholic content (between 17 and 25 % (v/v)) is below the likely louch point of 38% (v/v).

The proposed drink is a spirit with an alcoholic content of between 40 and 45% (v/v) that would louch at about 38 % (v/v) or below.

Pastis and the louching phenomenon

Pastis (Figure 3) is the Mediterranean-sourced drink in which the distinctive flavour is created through a combination of star anise and several aromatic herbs and plants. Star anise is the star-shaped pericarp of the fruit of *Illicium verum*, a small native evergreen tree of southwest China (McGee, 2003). The distinctive star-shaped spice is characterized by a hot and sugary taste and pervasive aniseed scent. The active flavour compound is anethole, which is also the characterising flavour of anise (aniseed), the botanically unrelated herb *Pimpinella anisum*. An alcoholic extract of star anise forms a dense opalescent haze when it is diluted with water to a defined alcoholic strength (Lea & Piggott, 2003). This cloudiness is called ‘louching’ the name origins of which were discussed earlier.

The traditional way to enjoy pastis is with water, or in a contemporary way with juice, cola or other carbonated soft drinks. Due to its aniseed taste and other botanical flavours, pastis is also used in cooking of meat, vegetable, green salad and fruit dishes (McGee, 2003).



Figure 3 Undiluted and diluted pastis

Anethole (or more accurately *trans*-anethole) is the compound that accounts for the distinctive licorice flavour of star anise (aniseed), fennel, star anise and anise myrtle. It may also be referred to as *p*-propenylanisole, anise camphor, isoestragole, or oil of

aniseed. It is unrelated to glycyrrhizic acid, the compound which makes licorice taste sweet. The full chemical name of this structurally planar compound is *trans*-1-methoxy-4-(prop-1-enyl) benzene (Belitz & Grosch, 2004) (Figure 4).

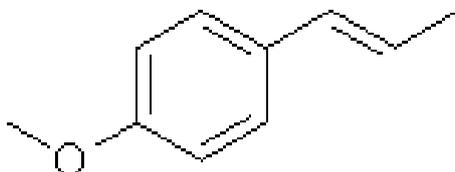


Figure 4 Structure of anethole

Anethole appears as white crystals at room temperature. Its melting point is 21°C, and its boiling point is 234°C. Anethole is distinctly sweet as well as having its flavouring properties. It is perceived as being pleasant, sweet, but not powerful to the taste even at higher concentrations. It is very different from citrus flavour.

Citrus family and terpenes

Citrus is a common name for the genus of flowering plants in the family Rutaceae, and the name generally applies to the fruits as well. The familiar citrus fruits – orange, lemon, grapefruit, mandarin, lime fruit, bergamot, bitter orange, etc. – each has its own characteristic aroma. Originating in tropical Southeast Asia (Reineccius, 2006), the plants are notable for their fragrance, partly due to terpenes and other essential oils contained in the peel and juice. The juice also contains a high concentration of citric acid giving the fruits their characteristic sharp flavour. The yellow and orange colours of citrus fruits develop only in climates with a cool or cold winter. In tropical regions citrus fruits remain substantially green until maturity (Fisher & Scott, 1997), hence the tropical ‘green orange’. The characteristic colour of the fruits is from carotenoids and flavonoids. The flavour of the juice is determined by the ratio of sugars to organic acids, mainly citric acid, overlaid by the presence of low levels of aromatics (Fisher & Scott, 1997).

In all citrus fruits, the essential oil is contained in the numerous, balloon-shaped oil sacs or glands situated irregularly just below the surface of the coloured portion of the peel (the flavedo). The white inner mesocarp (the albedo) of the peel does not contain

any oil sacs but does carry the bitter glycoside such as hesperidin in lemon, orange, and tangerine, or naringin in grapefruit (Reineccius, 2006).

Citrus oils are characterized by the presence of a large proportion of monoterpenes ($C_{10}H_{16}$) and lesser proportions of sesquiterpenes ($C_{15}H_{24}$). These terpenes are the solvent for a range of the oxygenated compounds comprising alcohols, aldehydes, ketones, acids, and esters, which are responsible for the characteristic odour and flavour profiles (Belitz & Grosch, 2004). The terpenoid composition of the various citrus oils is similar, the principal component being D-limonene. The terpenes possess little intrinsic odour or flavour value, but it would be incorrect to say they have no flavouring effect. Citrus oil from which the terpenes have been removed is significantly flatter and lacks the characteristic freshness associated with complete peel oil. The principal components of citrus oil are listed in Table 1.

Table 1 Principal components of citrus oils

<u>Monoterpenes</u>	<u>Acids</u>	<u>Aldehydes</u>
α -Pinene	Acetic	Acetaldehyde
β -Pinene	Capric	Citral
γ -Terpinene	Caprylic	n-Decanal
α -Terpinene	Formic	Octanal
D-limonene		
Myrcene	<u>Alcohols</u>	<u>Esters</u>
p-Cymene	Citronellol	Geranyl acetate
Terpinolene	Geraniol	Linalyl acetate
Sabinene	Linalool	Methyl-N-methyl- anthranilate
Camphene	1-Nonanol	
	Octanol	
<u>Sesquiterpenes</u>	α -Terpineol	
Bisabolene	Terpinen-4-ol	
Cadinene		
Caryophyllene		

From Reineccius, 2006

Orange (*Citrus sinensis*) and lemon (*Citrus limon*) originated in the Himalayas but were introduced to the Mediterranean area early in recorded history (Wikipedia, 2008). The juices are rich in citric acid and ascorbic acid (vitamin C) and are used in cooking for their freshness and sourness. However, the characteristics are due to the essential oil of the peel and are dominated by the aldehyde citral (geranial plus its

optical isomer neral, about 5 %) (Figure 5) combined with smaller amounts of linear aliphatic aldehydes (C₇ to C₁₃).

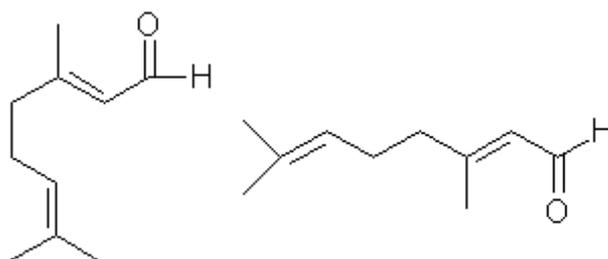


Figure 5 Structure of geranial (left) and neral (right)

As with most citrus oils, d-limonene (Figure 6) is by far the major component (about 65 %) (Fisher & Scott, 1997).

Grapefruit (*Citrus paradisi*) is said to have developed by natural hybridisation between pummelo and sweet orange (*Citrus sinensis*) and was first reported growing in Barbados in the West India around 1750 (Grapefruit, 2007). Commercial grapefruit varieties were mainly developed in Florida, and several types are currently cultivated in many tropical and subtropical regions of warm and humid climates. The peel oil has a strong and desirable aroma useful in industrial flavouring of foods, beverages, pharmaceutical, products, perfumes, and cosmetics, as is common with other *Citrus* oils. There are two natural groups of grapefruits depending upon colour of the flesh; the nominally white (common) types, including Duncan, Marsh seedless, Triumph, and New Zealand grapefruit, and the pigmented types, including Redblush, Thompson, and Foster pink. Limonene comprises between 85 and 96 % of grapefruit essential oil while myrcene, sabinene, α -pinene, and γ -terpinene have been reported at less than 2.5 % in total (Belitz & Grosch, 2004). Nootkatone, octanal, nonanal, decanal dodecanal, octyl acetate, citronellyl acetate, citral, and cavone have been reported in the oil at low levels (< 1.0 %) but have been identified as the major contributors to the aroma (Fisher & Scott, 1997). Some examples of grapefruit oil odour threshold concentrations are shown in Table 2.

Table 2 Odour threshold in water of some aroma compounds in grapefruit peel oils

Compound	Threshold concn. (mg L ⁻¹)
Ethanol	100
Limonene	0.01
(+)-Nootkatone	0.001
(-)-Nootkatone	1.0
1-p-Menthene-8-thiol	0.00000002

From (Belitz & Grosch, 2004)

Limonene (methyl-1,4-isopropenyl cyclohexene) is the major component by weight of grapefruit peel oils, but has a weak aroma (Belitz & Grosch, 2004). As noted above, it is the oxygenated terpenes present in low concentrations in these oils that have the major flavour impact. Although hydrocarbons like limonene may not have much aroma, they do act as a solvent for the powerful odorants.

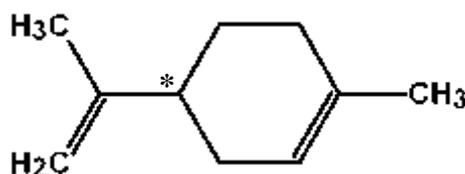


Figure 6 Structure of limonene. The chiral carbon atom is marked *. Only the D-enantiomer occurs in citrus

As a pure solvent, d-limonene can replace a wide variety of products, including mineral spirits, methyl ethyl ketone, acetone, toluene, glycol ethers, and of course fluorinated and chlorinated organic solvents. Thus it can be used as a wipe cleaner, in a dip bath, or in spray systems as a direct substitute for most other organic solvents. As with most organic solvents, d-limonene is not water soluble.

Nootkatone is an essential aroma compound of grapefruit peel oils. It is a sesquiterpene ketone (Figure 7) and its concentration increases with increasing of ripening of grapefruit. The two enantiomers differ significantly in their aroma intensity as shown in Table 2 (Belitz & Grosch, 2004).

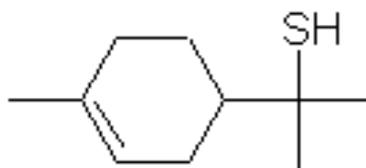


Figure 7 Structure of (-)-nootkatone.

1-p-Menthene-8-thiol from grapefruit peel oils (Figure 8) has a strong flavour impact because sulphur groups bind strongly to the olfactory receptors (Fisher & Scott, 1997). 1-p-Menthene-8-thiol has a fruity odour even at a very low concentration, $0.00000002 \text{ mg L}^{-1}$ in water (Belitz & Grosch, 2004) and it makes an important contribution to the aroma of grapefruit. This compound and other tertiary thiols are possibly formed by the addition of hydrogen sulfide to metabolites of isoprene metabolism (Coultate, 2001). These tertiary thiols are some of the most intense aroma substances.

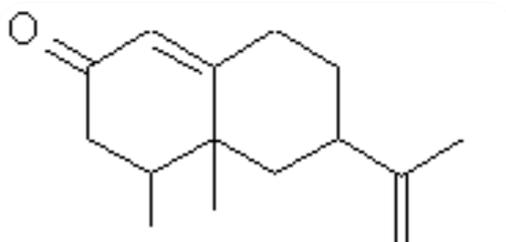


Figure 8 Structure of 1-p-menthene-8-thiol

Phenolic substances in the form of flavonoids are important source of bitterness in fruit juice, particularly citrus juice. The best known is naringin (naringenin-7-neohesperidoside) (Figure 9) a glycoside of the flavonone naringenin with the disaccharide neohesperidose, which occur in grapefruit. Its bitterness can be detected at a concentration of 200 mg L^{-1} (Coultate, 2001).

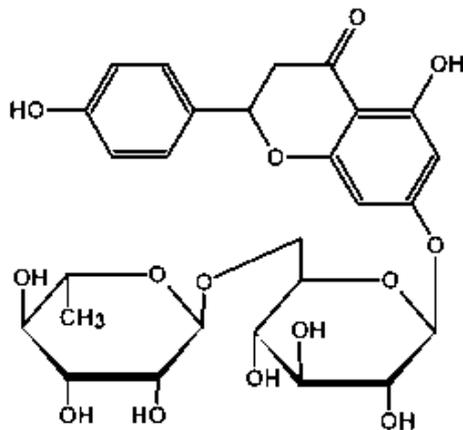


Figure 9 Structure of naringin

Returning again to limonene, this was the compound mostly responsible for the louch phenomenon described in this thesis. As the project developed it became clear that a high concentration of citrus oils in a spirit would be required to produce a ‘quality louch’. But as will be shown, it was found that high concentrations of citrus oils meant that the louch point could be well above 40 % (v/v).

To overcome this problem, a range of compounds were added to the distillates in an attempt to lower the louch point. In simple terms, the proposed role of these additions was to make the limonene more soluble in increasingly aqueous water:ethanol mixtures. The compounds chosen for study were hydrocolloids, which would also contribute to viscosity, and some low molecular weight surfactants. The latter were not necessarily food-grade but were tested to see how the high-louch point problem might be overcome.

Hydrocolloids and surfactants

Hydrocolloids are polymers that can be dispersed in water to produce thickening, gelling, or cloudiness. Most hydrocolloids are polysaccharides. Outlined below is a selection of hydrocolloids to be used in this research.

Gum arabic (gum acacia) is the dried exudate from the stem and branches of tree of the genus *Acacia*, which belongs to family Leguminosae (McClements, 2004). Gum arabic consists of a mixture of arabinogalactan oligosaccharide, polysaccharide and glycoproteins. As a food additive, it is a useful if rather expensive hydrocolloid emulsifier, texturiser and film-former, widely used in the drinks industry to stabilize flavours and essential oils, for example in soft drink concentrates. Gum arabic is also

applied as a flavour fixative in the production of encapsulated, powdered aroma concentrates.

Xanthan gum is the extracellular polysaccharide from *Xanthomonas campestris* and some related microorganisms. It is produced on a nutritive medium containing glucose, NH_4Cl , a mixture of amino acids, and minerals (Belitz & Grosch, 2004). Xanthan gum can be regarded as a cellulose derivative. The main chain consists of 1,4-linked β -glucopyranose residues. On average, every second glucose residue bears in the 3 carbon atom position a trisaccharide of the structure β -D-manp-(1,4)- β -D-glcpA(1,2)- α -Dmanp as the side chain (Figure 10). The practical importance of xanthan gum is based on its emulsion-stabilising and particle-suspending abilities (turbidity problems, essential oil emulsions in beverage). Due to its high thermal stability, it is useful as a thickening agent in food canning.

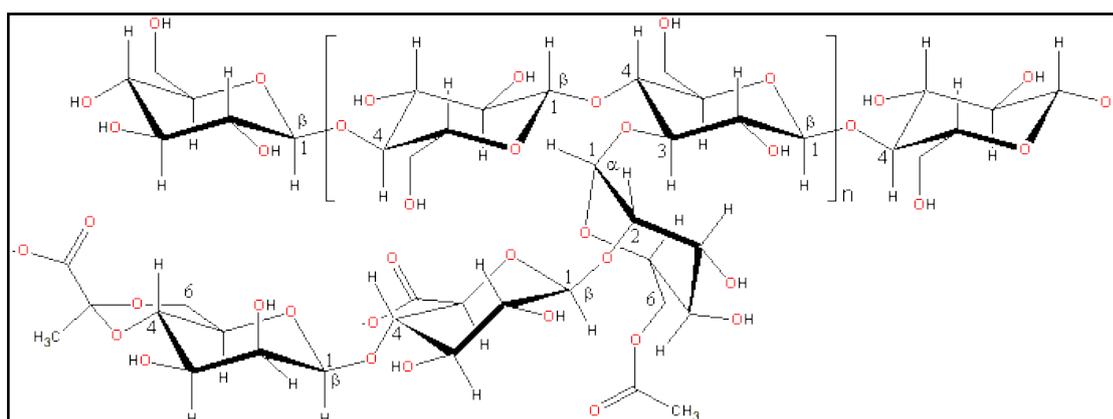


Figure 10 Structure of xanthan gum, where the upper sugar residues represent the cellulosic chain

Alginates are hydrocolloids extracted from certain brown seaweeds (the Pheophyceae) (Lea & Piggott, 2003). Alginates are linear copolymers of β -D-mannuronic acid (designated M in alginate models) and α -L-guluronic acid (G) (Figure 11) which can be distributed as sequences of M and sequences of G or sequences of variously alternating M and G residues. The different sequences and overall different molecular weights lead to appreciable differences in their functional characteristics. As supplied commercially, alginates are in the sodium or potassium salt forms, and are used as thickening, gelling, and stabilizing agents in a variety of food emulsions such as dressings and fruit beverages (Moe, Draget, Gudmund, & Smidsrod, 1995). Gel

formation depends on a high proportion of adjacent G residues and the presence of added calcium ions that lead to a heat-stable gel structure.

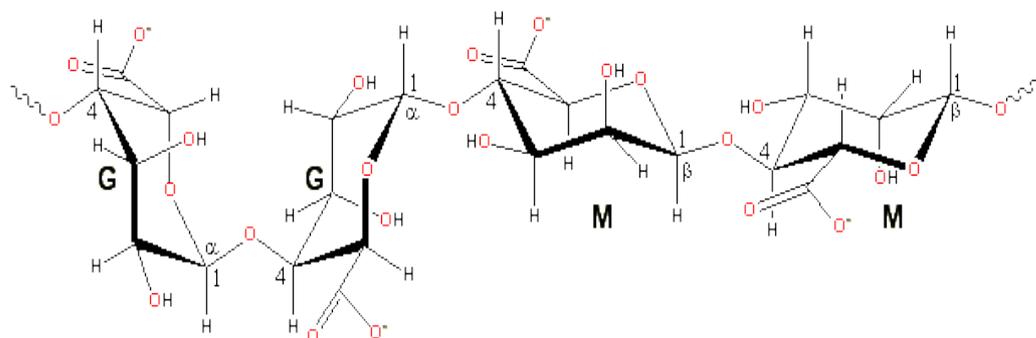


Figure 11 Structure of an alginate showing β -D-mannuronic acid (designated M in alginate models) and α -L-guluronic acid (G)

Cellulose is the most abundant natural polysaccharide, being the major structural component of land plants (Coffey, Bell, & Henderson, 1995). Cellulose is a high molecular weight linear polymer consisting of D-glucose units joined together by β -(1-4) O-glycosidic bonds (Figure 12). In its native state, cellulose is not usually suitable for use as a texture modifier in processed foods because it forms strong intermolecular hydrogen bonds that make it insoluble in water. Nevertheless, it can be isolated and chemically modified in a number of ways to produce products that are useful as food ingredients. The most common cellulose derivatives used in food are methylcellulose (MC) and hydroxymethylcellulose (HPMC). These ingredients consist of cellulose molecules that have been chemically modified by adding substituents to the cellulose back bone on free hydroxyl groups.

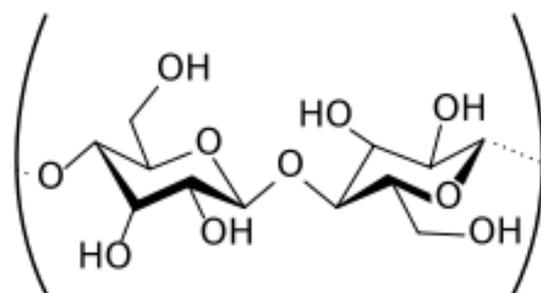


Figure 12 The repeating structure of cellulose

MC and HPMC are soluble in cold water, but for explainable chemical reasons tend to become insoluble when solutions are heated above a critical temperature between 50

and 90° C (Hoefler, 2004). MC and HPMC both form reversible gels or highly viscous solutions on heating (Williams & Phillips, 2003). MC and HPMC are nonionic polymers and therefore have good solubility over a range of pH values and salt concentrations, as well as good compatibility with other ingredients. These products have been used as texture modifiers in a variety of food products, including dressings, sauces, creams, and desserts.

Cyclodextrins (α , β , and γ) are non-reducing cyclic glucose oligosaccharides resulting from cyclomaltodextrin gluconotransferase-catalysed degradation of starch (Yu, Dandekar, Toledo, Singh, & Patil, 2007). Cyclodextrins are not strictly hydrocolloids, but among other functionalities they are stabilisers for oil in water emulsions (Yu et al., 2007). As models they resemble a bottomless bowl-shaped (truncated cone) molecule stiffened by hydrogen bonding between the 3-OH and 2-OH groups around the outer rim (Figure 13). The defining utility of cyclodextrins is their ability to sequester hydrophobic compounds in their hydrophobic cavity.

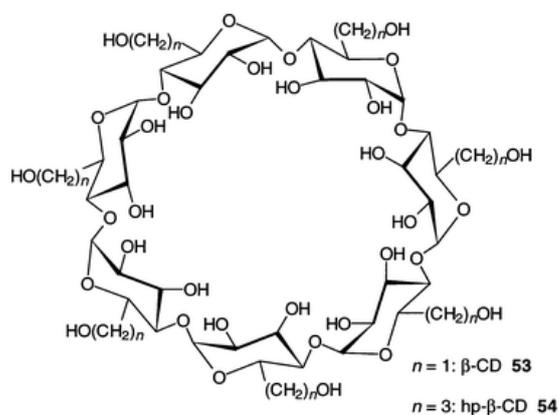


Figure 13 Structure model of a cyclodextrin

Sucrose is a disaccharide of glucose and fructose. The glucose and fructose units are joined by an O-glycosidic bond in the α orientation (Figure 14). Like cyclodextrins, sucrose is not a hydrocolloid, but is not used just to sweeten food and beverage products. It has wide range of other functionalities, including adding colour and texture to baked goods. Sucrose acts as a bulking agent (ice cream, baked goods) and preservative (jams, fruits), and it imparts a satisfying body 'mouthfeel', to beverages. In nominally non-sweet foods like salad dressings and sauces, sugar enhances flavour, and it balances acid content in tomato and vinegar-based products.

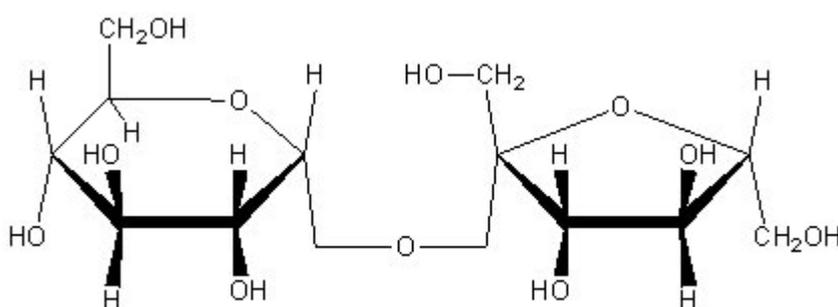


Figure 14 Structure of sucrose, with the glucose moiety on left

A surfactant is capable of absorbing to an oil:water interface and acts to protect emulsion droplets from coalescence (Belitz & Grosch, 2004).

Span 20 (sorbitan monolaurate) and Span 80 (sorbitan monooleate) are nonionic surfactants derived from sorbitol, the sugar alcohol of sorbose aldose sugar in the same family as glucose. Spans are available in food grade and are used as solubilisers or emulsifying agent. Triton X-100 is a nonionic surfactant which has a hydrophilic polyethylene oxide group (on average it has 9.5 ethylene oxide units) and a hydrocarbon lipophilic or hydrophobic group. It is commonly used detergent in biochemistry laboratories. Triton X-100 is available in non food grade.

As will become clear in this thesis, none of the above hydrocolloids and surfactants improved the louching properties of the grapefruit distillate. Nonetheless a louchable spirit was developed that could still have market potential on the basis that it could be made from a distinctive New Zealand cultivar of grapefruit. It had to be consumer-assessed in some way. The focus group interview method was chosen.

Focus group interview

A focus group interview is a structured process used to obtain detailed information about a particular topic. It is particularly useful for exploring attitudes and feelings and to draw out issues that may be unknown to the researcher. For example, in the world of marketing, focus groups are as seen as an important tool for acquiring feedback regarding new products, as well as various social issues. In particular, focus groups allow companies or researchers wishing to develop, package, name, or test market a new product, to discuss, view, and or test the new product before it is made available to the public (Casey & Krueger, 1994).

A focus group typically comprises six to nine participants who are brought together for no more than 1.5 hours to discuss a clearly defined topic (Thomson, 1994). Typically, focus groups are composed of persons representing a particular segment of the population. A group facilitator keeps the discussion on track by asking a series of open-ended questions meant to stimulate discussion. The session is voice- and sometimes vision- recorded and transcripts are prepared.

The experimental plan

The outline of the experimental approach is shown in Table 3. There are five main areas of work leading to an overall discussion and conclusion. The method of spirit preparation will be distillation from undried grapefruit peel. The resulting distillate will be stored at several temperatures to monitor changes with time for reasons discussed later. The sensory assessments will be assessed by focus group interviewing for whatever grapefruit spirits and existing commercial spirits can reasonably be tested. Attributes will be described aroma, colour, and flavour.

Table 3 Outline the experimental approach

Events	Analysis	Reported in Chapter
Measurement of ethanol concentration	Enzymatic method	2
Louching in Pernod and grapefruit distillate under various temperatures	Distillation, louching determination, enzymatic method	3
Compare louching in grapefruit distillate of different weights of grapefruit zest	Distillation, louching determination, enzymatic method	3
Louching of limonene and addition of assorted hydrocolloids	Distillation, louching determination, enzymatic method	4
Louching in whole grapefruit distillate and comparison between California and New Zealand grapefruit	Distillation, louching determination, enzymatic method	5
Compare louching of anethole compared with limonene	Distillation, louching determination, enzymatic method, and gas chromatography determination	6
Focus group assessment of grapefruit-flavoured spirit	Focus group interview	7
Final remarks		7

Chapter 2

Materials and Methods

Sources of grapefruit

Whereas the objective was to create a spirit based on the common New Zealand grapefruit, most experiments were done with a yellow-skinned and yellow/pink-fleshed grapefruit imported from California, U.S.A. (Figure 15). These were chosen because the work began in February and local fruit was not available until June. These fruits were typically 5 cm in diameter at the equator and were bought from Foodtown, Quay St. Auckland.

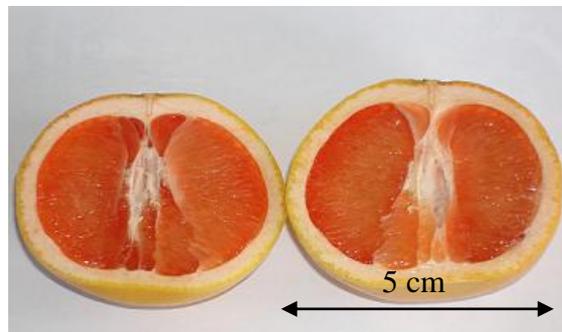


Figure 15 Typical appearance and size of the California fruit

Approximately half way through the project the local oblate and yellow/gold-skinned and -fleshed fruit (Figure 16) became available from domestic Auckland sources. Vary many homes in Auckland and the upper North Island have a grapefruit tree, much of the fruit from which goes to waste. These fruit were more variable in size and in proportions of zest, pith and flesh (defined below), as might be expected from uncontrolled domestic sources. The maximum diameters ranged from about 8 to 12 cm.

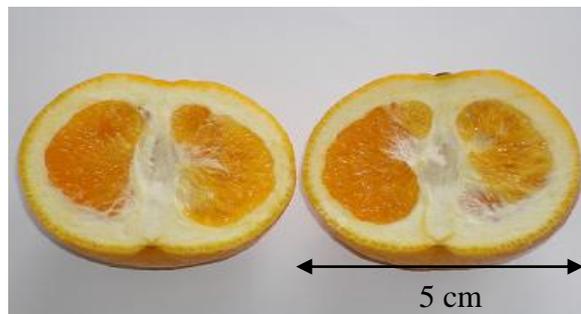


Figure 16 Typical appearance and size of the domestic New Zealand grapefruit

The flesh of the grapefruit is obviously that fraction of the grapefruit from which aqueous juice can be extracted. The pith, also known as albedo, is the white fraction immediately surrounding the flesh. The zest (skin) is the thin layer of segments, membranous, somewhat bitter walls; very juicy, acid to sweet-acid in flavour

Because the zest fraction contains high concentrations of the grapefruit oils of interest, much work used this source rather than whole skin or whole skin plus juice. In a typical experiment, grapefruit zest (Figure 17) was first isolated with a sharp box cutter knife.



Figure 17 Typical zest appearances of the domestic New Zealand grapefruit for this study

Small quantities of pith were unavoidably included in this isolate. The standard quantity of zest used in distillations was 40 g.

In some later experiments, zest + pith (effectively whole skin) or zest + pith + juice (effectively whole fruit) was used as the flavour source. In these experiments the quantities of components other than zest necessarily added moisture, particularly in the case of juice. Thus a valid three-treatment comparison required that water was added to the zest and zest + pith distillation treatments to match the moisture in the zest + pith + juice treatment. This required knowledge of the weight equivalents of pith and juice, in a typical grapefruit compared with standard 40 g of zest. This was experimentally determined using three California grapefruit chosen at random. These fruit were first exhaustively juiced with a domestic manual juicer (lemon squeezer-type). Zest was isolated from the pith as described above. The pith was diced into

cubes about 5 mm on edge. The three fractions from each grapefruit were respectively pooled and randomised (mixed in the case of juice). Weights were recorded and the moisture content of pith determined as follows. A known weight of pith was dried for 4 hours at 110°C, and after cooling in a desiccator reweighed. The moisture content (% w/w) was calculated. The moisture content of the juice was assumed to be 98 % w/w.

Chemicals

Reagent grade limonene (96 % purity claimed) was from BDH, U.K, and anethole (99 %) was from Aldrich, U.S.A. β -Nictotinamide adenine dinucleotide, oxidised, (NAD) and alcohol dehydrogenase (ADH) were from Sigma, U.S.A, types 43410 and A7011 (300 units mg^{-1}) protein respectively. The reference analytical ethanol was 96 % (v/v) from BDH, U.K. The ethanol used in routine distillations was a potable reagent grade. The claimed concentration was 95 % (v/v), but determined to 91 % (v/v) by the enzymatic assay used here (see later). Tris-(hydroxymethyl) aminomethane (Tris) and L-lysine HCl were analytical grades from Applichem, Ottoweg, Germany. Other basic laboratory chemicals used were analytical grade obtained from various sources.

Gum arabic, gum xanthan and polyethylene glycol (PEG-400) were obtained from Sigma, U.S.A., protanal LF-200 and protanal ester PVH-400 were obtained from Norpro, St. Heliers, Auckland. Methycellulose and hydroxymethylcellulose (HPMC) were obtained from DOW Chemical, U.S.A. Methocel K4M food grade was obtained from Swift, Mt. Roskill, Auckland. Triton X-100, Span 20 and Span 80 were obtained from Riedel-dehaën, Germany, ICI, U.S.A., and Sigma, U.S.A., respectively.

Measurement of ethanol concentration

The enzymatic method employs alcohol dehydrogenase (ADH), an enzyme that catalyses the oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD to NADH. The absorbance of the NADH produced is measured at 340 nm (Dawson et al.), in this case using an Ultraspec 2100 pro spectrophotometer (Amersham Bioscience) (Figure 18), connected to a desktop computer to monitor the data.

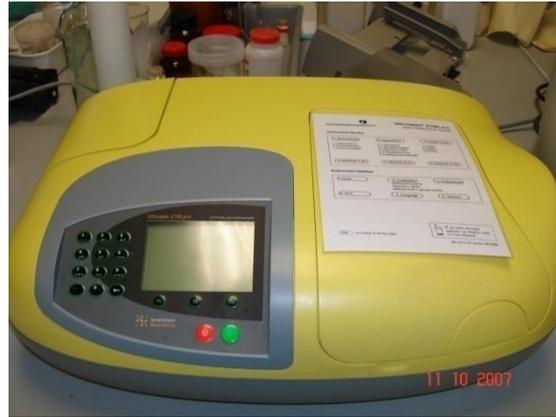
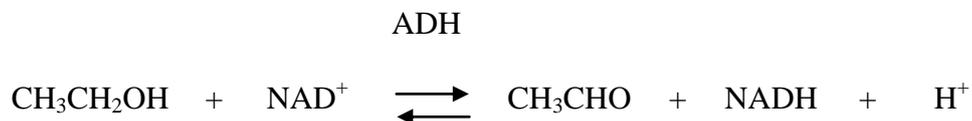


Figure 18 The single-beam spectrophotometer used extensively in this research



Clearly, this reaction is useful only if it goes to completion to the right. This is achieved by buffering the reaction mixture to alkaline conditions to drive the reaction to the right. Note that the reaction generates hydrogen ion. A stock solution of 0.6 M Tris, 0.4 M lysine, adjusted with KOH to pH 9.7, can be stored at 4°C for at least a month (Cornell & Veech, 1983). A stock solution of NAD was prepared by dissolving 0.180 g NAD in 10 mL water (28 mM). This solution is stable for weeks when stored at 0°C (Cornell & Veech, 1983), and solutions are routinely stored frozen. To avoid repeated freeze/thaw cycles the 10 mL solution was distributed in 2 mL aliquots in plastic vials that were held at - 20 °C in a domestic freezer.

The final assay procedure was developed from the method described by Cornell & Veech, 1983. Into a test tube were pipetted 4 mL of Tris/lysine buffer, pH 9.7, and 500 µL of NAD solution. Into 1 cm pathlength, UV-transparent cuvette was added 1.0 mL of the buffer/NAD mixture. To this was added 0.1 mL of the ethanolic sample, plus 1 mL of water. After inversion mixing, the absorbance was read at 340 nm using water as the reference. The reaction was started by the addition of 20 µL of alcohol dehydrogenase (300 units mL⁻¹). The absorbance was monitored at ambient temperature (20 °C) at 1 min intervals until a stable value was reached, typically in 5 min. A reagent blank, containing 0.1 mL of water in place of the ethanolic sample,

was also run. The range of concentrations encountered in this work was far too high for use without substantial dilution. Dilutions yielded absorbance between 0 and 1.5, a range in which linearity with concentration of ethanol has been observed (Cornell & Veech, 1983). Dilutions of the ethanol sample were typically 1:10,000. Under these conditions the concentration of NAD typically exceeded that of ethanol by a factor of 20. Therefore, NAD was never limiting.

The concentration of ethanol of ethanolic sample was calculated using the formula

$$\text{Ethanol (\%)} = 96 \times (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{reference ethanol}} - A_{\text{blank}})$$

followed by a correction for the dilution factor.

In alkaline conditions, oxidized pyridine into products that absorb at 340 nm, and the rate of decomposition becomes particularly rapid at elevated temperatures and at pH values above 10 (Cornell & Veech, 1983). Because of this, NAD is mixed with Tris/lysine within an hour of running the ethanol assay. Under the assay conditions outlined above, the absorbance at 340 nm increases by only 0.005 in 2 hours due to decomposition of NAD (Cornell & Veech, 1983).

A calibration curve was prepared from standard solutions of ethanol diluted with water, in all cases using few dilution steps to minimise errors in accuracy. As will be seen the accurate determination of ethanol was critical in this work.

The standard distillation

Flavoured spirit production comprises distillation and condensation of ethanol, water and volatile constituents from a flavour source dispersed/dissolved in an ethanol/water mixture. The flavour volatile constituents will partition between the volatile distillate (water/ethanol) and the liquid phase in a complex way relating to temperature, vapour pressure, and relative affinities to ethanol and water.

The zest was placed in a 1 L round bottom flask, fitted with a 34/35 mm still head. The flask also contained 500 mL of 91%¹ ethanol and anti-bumping granules. The flask and head were placed in a 200 W-size mantle and set initially to the highest power setting (9). The exposed top of the flask and the still head were wrapped in aluminium foil (not shown in Figure 19).

¹ From this point forward percentage ethanol is always by volume.

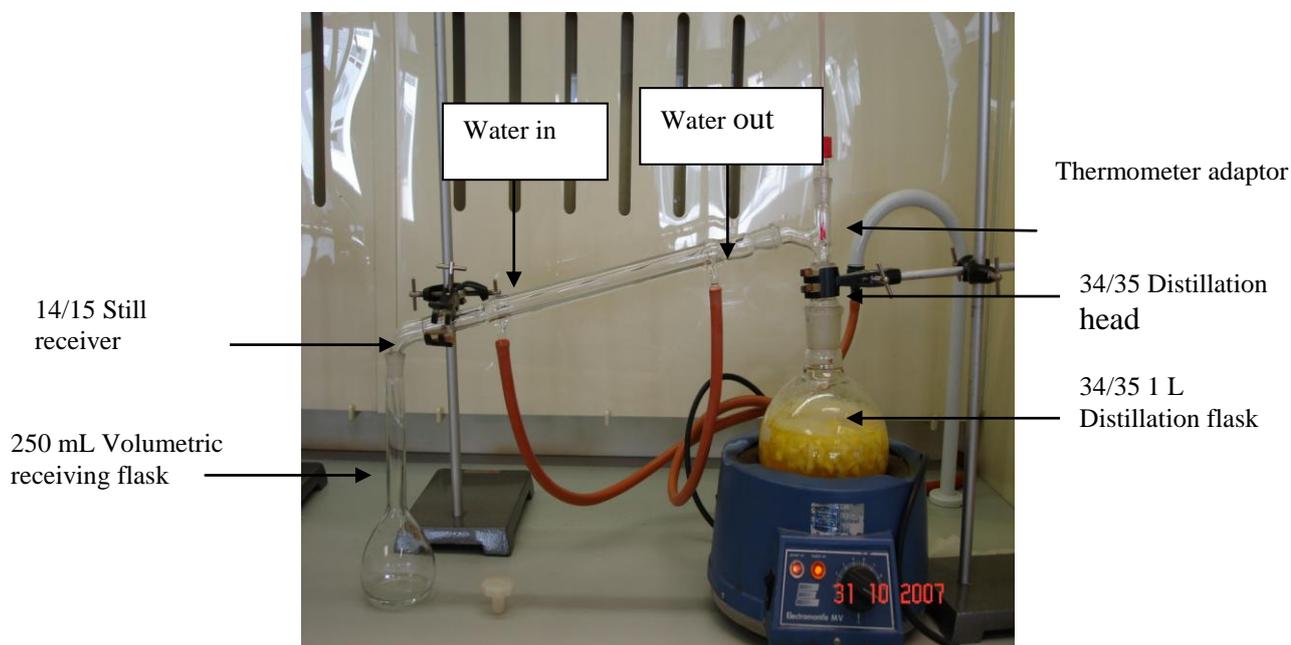


Figure 19 The standard distillation equipment used in this research

When the temperature reached 70°C and distillate appeared, the collection of fractions began. These were 25 mL or 250 mL fractions depending on the experiment. The power setting of the mantle was never changed from 9.

Louching determination by spectrophotometry

The principle of determination of the louching point was based on light scattering in a 1-cm cuvette. Starting with 25 mL of distillate, absorbances of 3 mL aliquots of the progressively diluted ethanol/water mixtures were measured in a spectrophotometer at 450 nm using a (hydrophobic) plastic cuvette. This wavelength was chosen arbitrarily because white light is uniformly scattered by louching. In other words a louching liquid is white in daylight, coloured only by any background pigments in the drink.

During the progressive dilution process – adding water with a burette – the mixture was magnetically stirred, with a 3 mL aliquot taken for measurement after each water addition. After absorbance was measured the 3 mL was added back to the mixture, taking care to leave as little as possible in the cuvette. At or above the critical micelle concentration, light that normally passed through the cuvette with minimal absorbance

was scattered, so greatly increasing absorbance. In this way a curve was generated with axes of percent ethanol concentration (abscissa) and absorbance at 450 nm (ordinate) (Figure 20).

During progressive dilution, 1 mL samples were periodically removed permanently from the each diluted mixture and held in sealed vials. The ethanol concentration in these was later determined by the enzyme method described earlier.

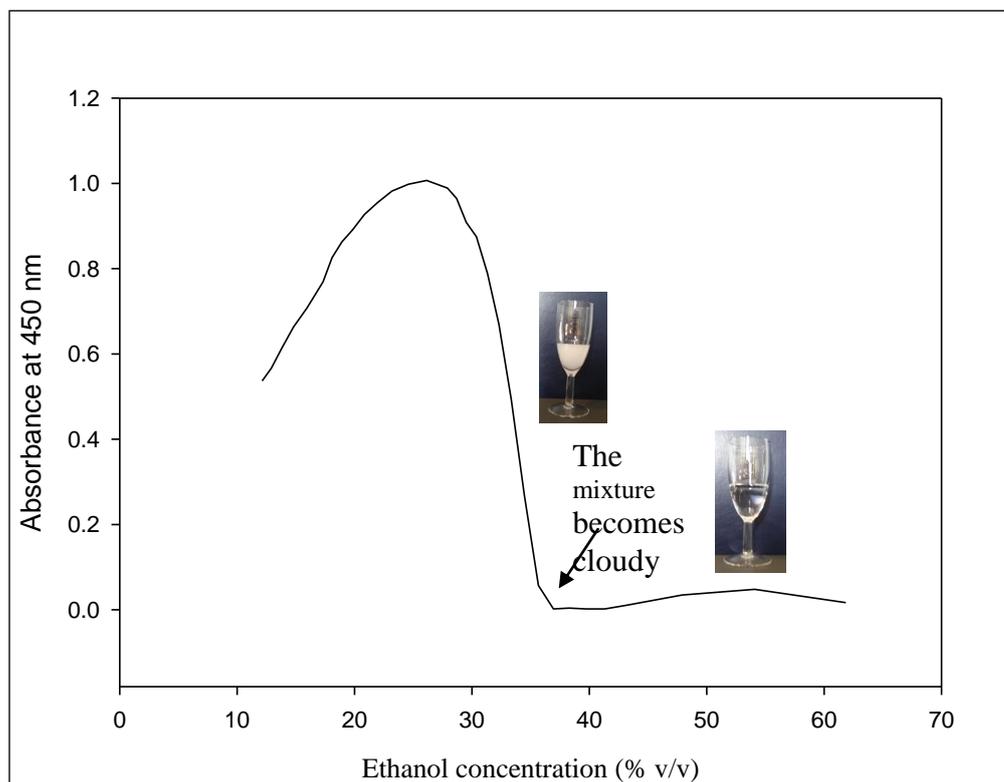


Figure 20 Typical louching phenomenon of grapefruit-flavoured ethanolic mixture. This plot was achieved by progressive dilution with water from a 65 % starting point

Temperature experiments

Louching experiments were sometimes carried out at temperatures other than ambient.

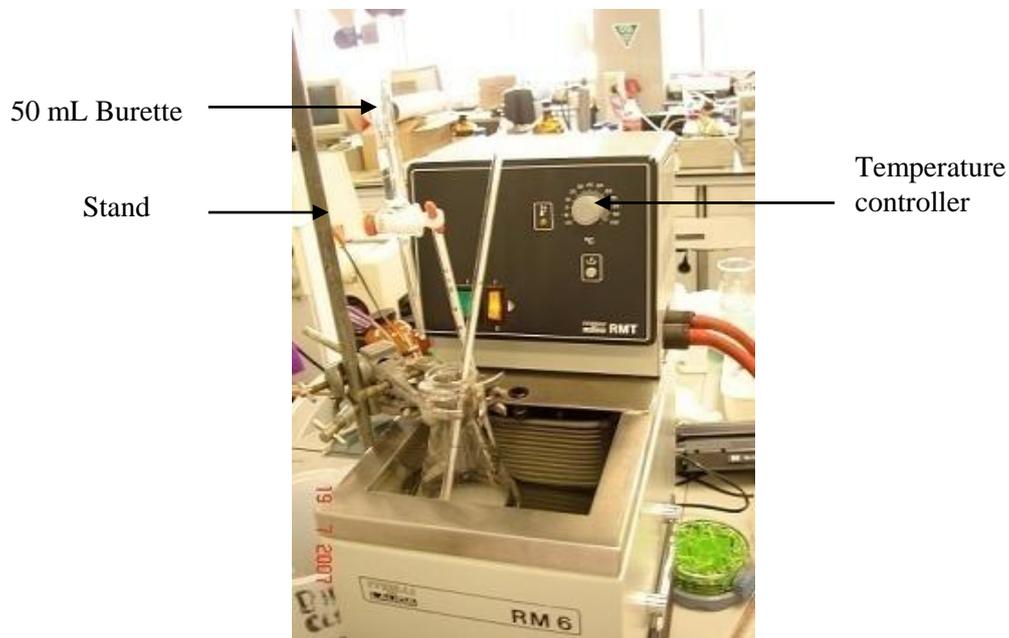


Figure 21 The refrigerated waterbath equipment used in this research. The burette used for water additions is on the left. In these experiments the mixtures were stirred with a thermometer as required.

The progressive dilutions were variously done at four temperatures, 30, 20, 10, and 0°C, to represent storage and serving conditions in practical situations. In these experiments, a 25 mL aliquot of distillate or other liquid under study was placed in a 100 mL conical flask. The flask was placed in a refrigerated waterbath (Lauda Model RM6, Germany), and the temperature inside the flask was monitored.

Gas chromatography

Gas chromatographic analysis was performed using a Shimadzu GC17A gas chromatograph (Shimadzu, Japan) (Figure 22) fitted with a capillary column (ZB-Wax) and a flame ionization detector. The oven temperature was programmed from 120°C (1 min) at an increasing rate of 5 °C min⁻¹ up to 250°C. 1 µL samples of grapefruit distillate-water were injected. The split ratio was 1:5; the carrier gas was nitrogen at a flow rate of 2.67 mL min⁻¹. Relative peak areas were integrated using a Shimadzu program. An internal standard was prepared by diluting 25 mg of menthol

in 100 mL of 91% ethanol. Typically 1 mL of menthol solution was added to 50 mL water-distillate mixture, resulting in a final concentration of menthol of 0.1139 mg mL⁻¹.



Figure 22 The gas chromatography equipment used in this research

Calibration curve of gas chromatography

Eleven concentration levels 0, 0.05 to 0.50 mg mL⁻¹ of limonene or anethole, each dissolved in 91% ethanol, were all spiked with the internal standard. A 10-point calibration curve passing through the origin was created for each analyte by performing linear regression on the area analyte/area menthol vs. concentration of analyte (% w/v). The regression equations and correlation coefficients (R²) were calculated.

Sensory analysis

Focus group interviews were carried out with 10 groups comprising three consumers per group, all selected from AUT staff members and students. Appendix 1 presents the information given to the consumers before the sessions, to explain what the aims were and how the data were to be collected. Importantly they were aware that the sessions were sound- recorded, and that the interviewer (Pornphun Chaipongrattana) was to take notes about what was said. Consent to take part was implicit in their individual participation.

Interviews were performed with three samples: Pernod, grapefruit distillate (no added sugar), and grapefruit distillate (added 3 % sugar). The respective ethanol concentrations were 38, 36 and approximately 36 %. The 15 mL samples were served

to assessors in wine tasting glasses, where each consumer was presented with tray of three glasses. The spirit temperature was approximately 20 °C.

Consumers first assessed aroma and flavour of undiluted (and therefore unlouched) samples. They were instructed to describe what their impressions were. After this phase was completed, the samples were each diluted with 15 mL water (20 °C).

Consumers were invited to assess the aroma, flavour, and to describe their reaction to the louching phenomenon. After these two phases some groups further discussed their impressions.

Notes and sound data were organised into three main categories: aroma, flavour, and impression of louching and summarised in tabular form. Sound data were deleted after transcription.

Data handling

The data collected were analysed using Excel (Microsoft) and SigmaPlot (Systat). Excel was used to calculate the concentration ethanol in the progressively diluted mixtures. The spreadsheet (Figure 23) shows the pattern of data entry and calculated concentration results. It takes into account the 1 mL samples taken for enzymatic-determination of ethanol concentration.

	A	B	C	D	E	F
1						
	Start concn	Action remove mL	Starting and progressive new volume	Water volume added	True new volume	Estimated new concn based on sampling
2						
3	91		25	10	35	65.0
4			35	5	40	56.9
5		1	39	5	44	50.4
6			44	5	49	45.3
7			49	2	51	43.5
8			51	2	53	41.9
9			53	2	55	40.3
10			55	2	57	38.9
11			56	2	58	37.6
12		1	58	2	60	36.3
13			60	2	62	35.1
14			62	2	64	34.1
15			64	2	66	33.0
16			66	2	68	32.0

$91 * 25 / 35$

$56.9 * 39 / 44$

Figure 23 The Excel spreadsheet format used to calculate ethanol concentrations

The basic formula embedded in this spreadsheet was:

$$\text{Calculated ethanol concentration (\%)} = \frac{\text{Initial concn.(\%)} \times \text{Initial mixture volume}}{(\text{Initial mixture volume} + \text{Volume of water added (mL)})}$$

The ethanol concentrations were calculated for all dilutions. These data were supported by the typically three enzymatically determined concentrations taken initially, halfway through and near the end of the progressive dilution. Next, the three calculated data points were plotted against the three equivalent enzymatically-determined points and a polynomial curved fitted. The regression equation and correlation coefficients (R^2) were recorded. The regression equation was embedded in the spreadsheet to calculate the true value of ethanol concentration (Right column in Figure 24).

	A	G	H	K
1	Estimated new concn based on sampling	calculated data points	enzymatically-determined points	Ethanol concentration (% w/v)
2	65.0			61.7
3	56.9	56.9	54.235	54.0
4	50.4			47.8
5	45.3			42.9
6	43.5			41.2
7	41.9			39.7
8	40.3			38.2
9	38.9			36.9
10	37.6	37.6	35.35	35.6
11	36.3			34.4
12	35.1			33.3
13	34.1			32.2
14	33.0			31.2
15	32.0			30.3
16	31.1			29.4
17	30.3			28.6
18	29.4			27.8
19	27.6			26.1
20	25.9			24.5
21	24.5			23.1
22	23.2			21.9
23	22.0			20.8
24	21.0			19.8
25	20.0			18.9
26	19.1	19.1	18.055	18.0

0.948*65

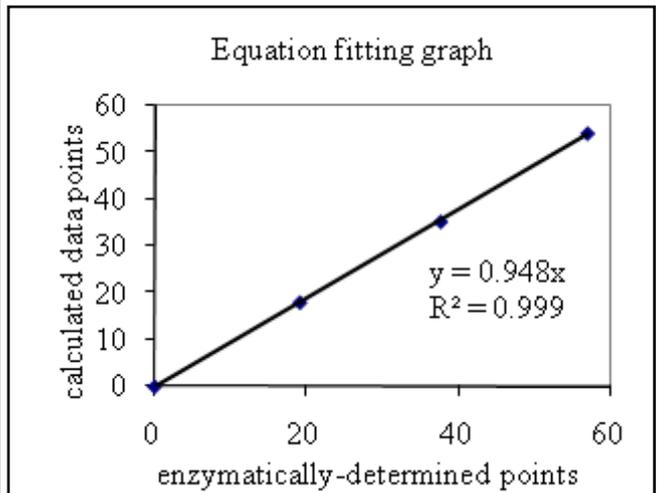


Figure 24 Linear regression formula model

Chapter 3

Louching in Pernod and grapefruit distillate

Louching in Pernod

Method

The basic methodology is described in Chapter 2. In this experiment, the progressive dilutions were variously done at four temperatures, 30, 20, 10, and 0°C, to represent storage and serving conditions in practical situations. In these experiments, a 25 mL aliquot of Pernod – with a declared ethanol concentration of 38 % – was placed in a 100 mL conical flask. The flask was placed in the refrigerated waterbath, and the temperature inside the flask was monitored.

Results and discussion

Figure 25 shows the louching behaviour of Pernod at these temperatures. Each curve in the family necessarily starts at 38 % ethanol.

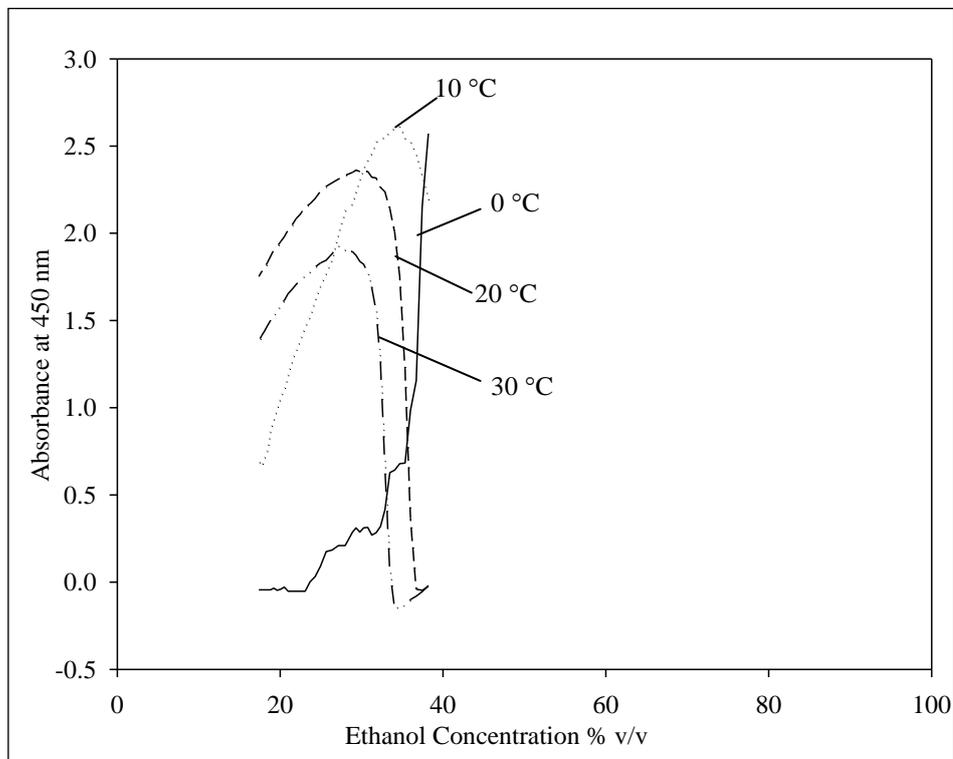


Figure 25 Louching phenomenon of Pernod. These plots was generated by progressive dilution of Pernod with water from a 38 % starting point

At temperatures below 10°C, Pernod immediately became cloudy without the addition of any water. This was confirmed by simply holding Pernod in refrigerator at -4°C. Pernod is typically stored at room temperature and under these conditions (between say 20 and 30 °C) a buyer of this product would see the bottle and its contents as the producer intended, completely clear. At 20°C the smallest addition of water resulted in louching. The louch point was at about 36 %. Because the active louching ingredient, anethole, is more soluble in ethanol than in water, it seems clear that the ethanol concentration in this particular Pernod product is at the very lower limit of ethanol concentration needed to ensure a clear liquid at ambient temperature. This may be for tax reasons because so-called premium spirits with alcohol concentrations higher than 37.2 % attract higher duty (Lea & Piggott, 2003), and therefore cost more at retail.

At 30°C, induction of louching required addition of several milliliters of water. This trend is to be expected because anethole is more soluble in warm water than in cold (Hengde & Kazuhiro, 2005). This is confirmed by the curve at 10°C where louching occurred spontaneously and was enhanced only a little by water addition. In each of the curves at 10, 20 and 30 °C, the initial strong louching peaks then decline with further water addition. The simple explanation for this pattern – which is repeatedly seen in this thesis – is that once the critical micelle concentration point is reached and micelles develop, further addition of water serves only to dilute them and so reduce light scattering.

The seemingly anomalous curve in Figure 25 is that at 0°C, where louching occurred without any water addition and at close to the maximum light scattering. Subsequent addition of water resulted in an initially steep decrease in scattering that slowed below about 35%. In reality the curve may not be anomalous. The missing peak in the 0°C curve could theoretically be present higher alcohol concentrations, say 45 % ethanol. In hindsight this model could have been tested by diluting the Pernod with ethanol and repeating the experiment.

Inspection of the curves in Figure 25 indicates for three curves (10, 20, 30°C) and suggests for four (0°C curve included) that the lower the temperature the higher the absorbance or more accurately light scattering at the peak of louching. One explanation for this could be that micelles are smaller at lower temperatures, and this may in some way be related to increased surface tension at lower temperatures

(Hengde & Kazuhiro, 2005). At a practical level this phenomenon is useful because Pernod is often served as a long drink over ice (Lea & Piggott, 2003).

Finally, the absolute values of absorbance are important in the appearance of the drink. The louching of Pernod is undoubtedly attractive. None of the absorbance peaks was below 1.80, and it remains to be shown what can be achieved with grapefruit distillates.

Louching in ethanolic distillates of grapefruit zest

Method

The basic methodology is described in Chapter 2. Zest of California grapefruit (40 g) was distilled in 500 mL of 91 % ethanol in the standard 1 L flask. Distillate was recovered in 25 mL fractions until 250 mL was recovered. The accurate ethanol concentration of each was determined by the enzymatic method. Subsequently each fraction was diluted with water as required to yield a common starting concentration of 60 %. Each fraction was then progressively diluted at ambient temperature, approximately 23°C, and the absorbance monitored.

Results and discussion

Figure 26 shows the louching phenomenon of ethanolic distillates of grapefruit zest.

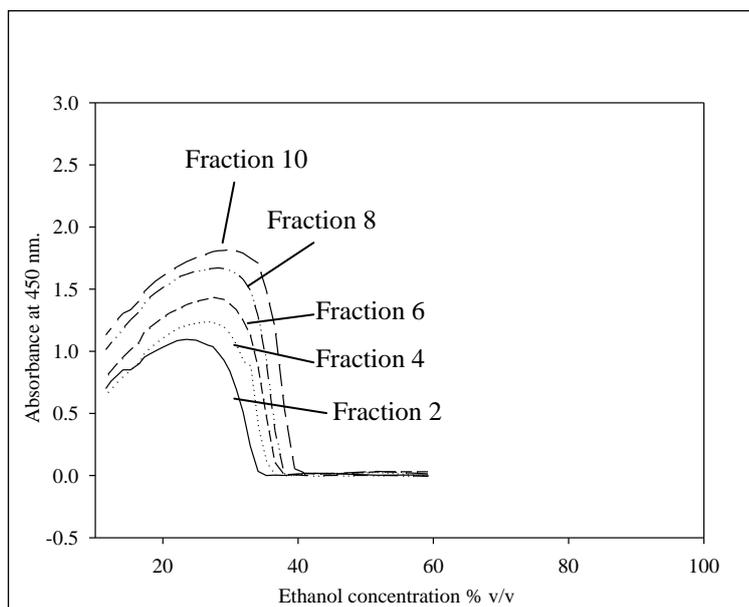


Figure 26 Louching phenomenon of grapefruit zest distillates. These plots were generated by progressive dilution with water at ambient temperature from a 60% starting point. Odd number fractions are not shown to improve clarity.

Fraction 10 had highest louching point (40%) and Fraction 1 (33 % not shown) had the lowest louching point. Therefore the concentration of the compounds responsible for louching terpenes was highest in Fraction 10. The longer the distillation, the more citrus skin terpene was extracted. Terpenes are much more soluble in ethanol than in water, and at first sight it seems odd that Fraction 10 should contain the highest concentration of terpenes, because in a distillation of 60 % ethanol, the concentration of ethanol becomes progressively lower with time. The likely explanation for the highest concentration in Fraction 10 centres on temperature. As distillation progresses, the temperature in the flask steadily increases making the terpenes more volatile. Moreover, as will be shown later, terpenes are more soluble in warmer water:ethanol mixtures, making preferential solubility in ethanol less important. In each curve in Figure 26, the initial strong louching peaks then declines with further water addition. As with Pernod the explanation for this is the dilution of the micelles. The average louching point was around 38 %, similar to that of Pernod, around 36 % at approximately the same temperature (Figure 25). However, the peak absorbance

due to light scattering was higher in Pernod than in grapefruit distillate. Numerically the difference, 2.35 in Pernod and an estimated 1.45 in grapefruit does not seem great. However, it must be realised that absorbance is exponentially derived. Taking the antilog of these values shows that under approximately the same conditions of louching, a 1 cm pathlength of maximally-louched Pernod transmits only 0.4 % of the light while the equivalent grapefruit mixture transmits about eight times as much, 3.5 %. This difference was obvious by inspection. The grapefruit distillates were less milky than louched Pernod. Commercially it seems desirable to generate a strongly louched drink.

The work with Pernod shown in Figure 25 showed that temperature had a major effect on louching, so this approach was extended to grapefruit zest distillate.

Louching in ethanolic distillates of grapefruit zest under three temperatures

Method

The basic methodology is described in Chapter 2. For these experiments 250 mL of distillate was collected rather than fractionated. The accurate ethanol concentration of each was determined by the enzymatic method, and the entire 250 mL was first diluted to 60 % ethanol. For each progressive dilution, a 25 mL aliquot of the 60 % distillate was placed in a 100 mL conical flask. The flask was placed in the refrigerated waterbath, and the temperature inside the flask was monitored. For Pernod, the progressive dilutions were done at 30, 20, and 10°C, to represent possible storage and serving conditions in practical situations.

Results and discussion

Figure 27 shows the louching behaviour of grapefruit zest distillates at these temperatures.

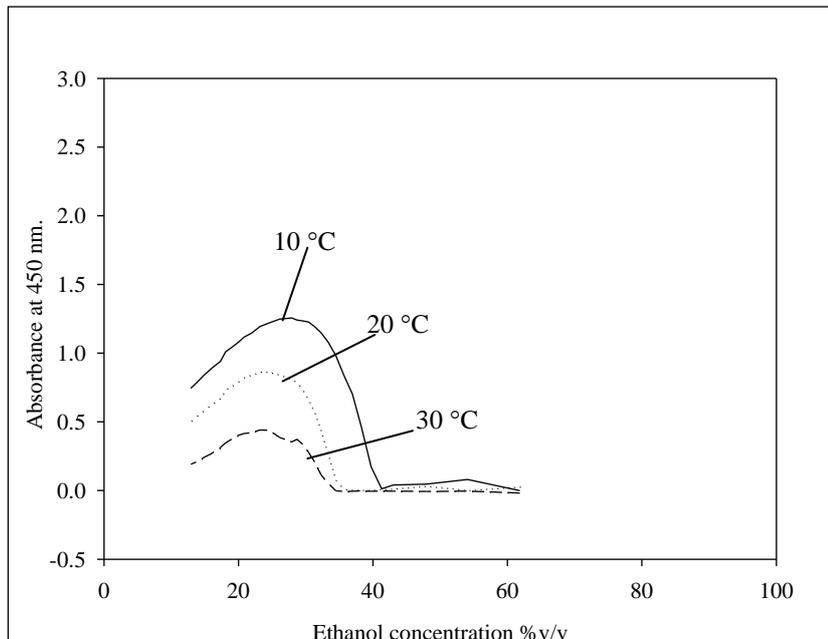


Figure 27 Louching phenomenon of grapefruit zest distillates at three temperatures. These plots were generated by progressive dilution of grapefruit zest distillates with water from a 60 % starting point

At 10°C, the distillate became cloudy at about 41 %, but at 30°C this was reduced to about 38 % which is lower than at 10° C. These louching behaviours of grapefruit distillate parallel those of Pernod, where the louch point occurs at higher ethanol concentration when the temperature is lower. It is clear that the louching ingredient of grapefruit zest distillate (terpenes) is more soluble in warm water than in cold.

As with Pernod, maximum absorbance due to louching increased as temperature decreased. This implies that the micelle particle size is smaller as temperature decreases.

In Chapter 1, it was pointed out that a commercially successful louchable drink would likely retail with an ethanol concentration at or below 40 %. The reason for this is higher alcohol duty as ethanol concentration increases. The zest distillate shown in Figure 26 would display a degree of undesirable louching without water addition at 40 % ethanol at 10°C or lower. Thus the grapefruit distillate is at the very lower limit of ethanol concentration needed to ensure a clear liquid at ambient temperature. The same was however true of Pernod as well.

Inspection of the three curves shows the typical peak in louching with decreased absorbance on further dilution. The explanation of this pattern was described above. Of particular interest was the low scattering, a maximum of 1.3, compared with Figure 25 (2.37, Pernod) and Figure 26 (about 1.45, another grapefruit distillation). The

commercial implications of this were discussed in the previous section. In Chapter 4 ways were sought of decreasing the louch point of grapefruit distillate and at the same time increasing the absolute absorbance due to light scattering. The obvious way to achieve the latter goal is to increase the terpene concentration in the distillate. That is the objective of the next experiment.

Louching in ethanolic distillates of different weights of grapefruit zest

Method

The basic methodology is described in Chapter 2. The weights of grapefruit zest were 30, 40, 50, and 60 g in 500 mL of 91 % ethanol. From each of the four distillations, pooled 250 mL distillates was collected and adjusted to 60 % ethanol after the accurate ethanol concentration of each was determined by the enzymatic method. The progressive dilutions were conducted at ambient temperature.

Results and discussion

Figure 28 shows the louching behaviour of grapefruit zest distillates when the weight of grapefruit zest is varied.

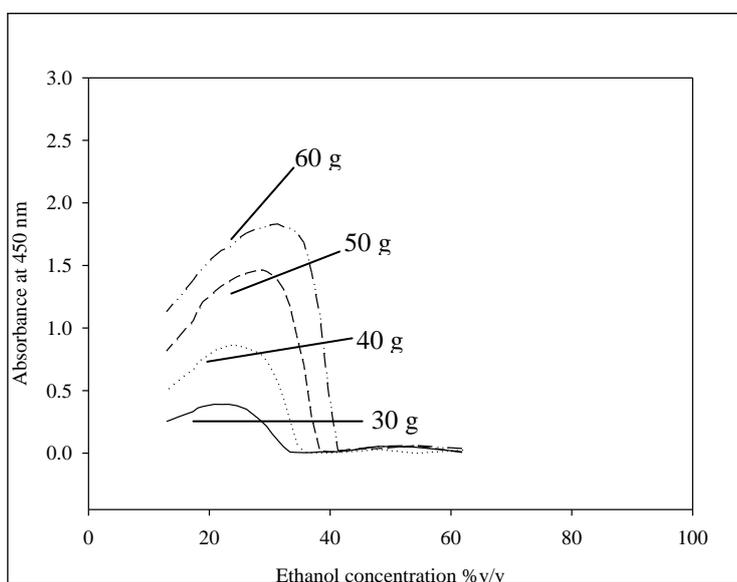


Figure 28 Louching phenomenon of distillates of different weights of grapefruit zest in a constant volume of ethanol. These plots were generated by progressive dilution with water from a 60 % starting point.

The 60 g treatment had the highest louch point, about 42 %. When the weight of grapefruit zest was decreased the louch point also decreased. The lowest weight

treatment, 30 g of grapefruit zest, had the lowest louching point at 34 %. It seems very likely that the greater the mass of citrus zest, the greater the extraction of louchable terpenes.

Comparison of Figure 25 and Figure 28 shows that even at the highest ratio of zest to 91 % ethanol (60 g plus 500 mL), the absolute absorbance was only 1.8. This translates to a transmittance of 1.6 %, an improvement 3.5 % in Figure 26, but still four times higher than for Pernod. In addition, the 60 g-distillate would almost certainly louch spontaneously if the undiluted drink were held at a temperature significantly below ambient.

Conclusion

For Pernod, with a declared ethanol concentration of 38 %, has the louch point is 36% at ambient temperature. At 10 °C or lower Pernod immediately becomes cloudy without any addition of water. It follows that the ethanol concentration in this particular Pernod product is at the very lower limit of concentration needed to ensure a clear liquid at ambient temperature.

At concentrations of grapefruit zest-derived terpenes required to louch at about the same ethanol concentrations of Pernod, the light scattering, as measured by absorbance at 450 nm, was markedly lower than that for Pernod. Its degree of scattering is commercially desirable. Therefore the aim of the next chapter is to explore ways of maintaining louching of grapefruit distillate around 38 % ethanol, and at the same time achieving high light scattering. A broad range of hydrocolloids was tested in an effort to achieve these goals.

The chemical basis of louching is the greater solubility of citrus terpenes, principally limonene, in ethanol than in water (Hengde & Kazuhiro, 2005). Therefore limonene was chosen as a model which simulates the behaviour of grapefruit distillates.

Chapter 4

Louching of limonene and addition of hydrocolloids

Introduction

As discussed in Chapters 1 and 3, the chemical basis of louching is the greater solubility of extracted plant compounds in ethanol than in water. In the case of Pernod the louchable compound is anethole, and in citrus the compound is principally limonene, which makes up 95% of the citrus oil (Fisher & Scott, 1997). In the previous chapter it was found that citrus zest distillate louched around 38 % ethanol, but the absolute absorbance of light – the measure of scattering – was lower than desirable. Scattering could be increased by increasing the ratio of zest to alcohol, but this had the effect of increasing the concentration of alcohol at which louching occurred. For reasons discussed in Chapter 3, this was undesirable from a commercial perspective. In this chapter ways of lowering the louching point are explored.

Limonene is available as a laboratory reagent, and with it ethanolic solutions can be reproducibly prepared without the need for repeated distillations of zest. Therefore limonene was chosen as a model to simulate the behaviour of grapefruit distillates.

Hydrocolloids and emulsifiers can alter the relative solubility of a compound in aqueous and hydrophobic solvents (Chapter 1). Therefore a range of these were tested for their ability to alter the louch point. Before this could be done, a concentration of limonene in 91 % alcohol had to be found that would simulate the typical louching behaviour of the zest distillates.

Louching behaviours of different limonene concentrations

Method

A stock solution of limonene prepared in 91 % ethanol, and a range of final concentrations in 91 % ethanol was prepared by dilution. The final concentration range was 0.05 to 0.5% w/v. Aliquots (25 mL) of these distillates solutions were progressively diluted and the louch point determined for each at ambient temperature

Results and discussion

From the series of progressive dilutions it was found that 0.25 % v/v limonene in 91 % ethanol would usefully produce louching around 40 % ethanol (data not shown). All the experiments in this chapter use this concentration and are referred to as 0.25 %.

Louching behaviour of 0.25 % limonene at different temperatures

Method

Aliquots (25 mL) of 0.25 % limonene in 100 mL flasks were diluted at four temperatures, 30, 20, 10, and 0°C, to represent possible storage and serving conditions in practical situations.

Results and discussion

Figure 29 shows the louching behaviour of limonene at these temperatures.

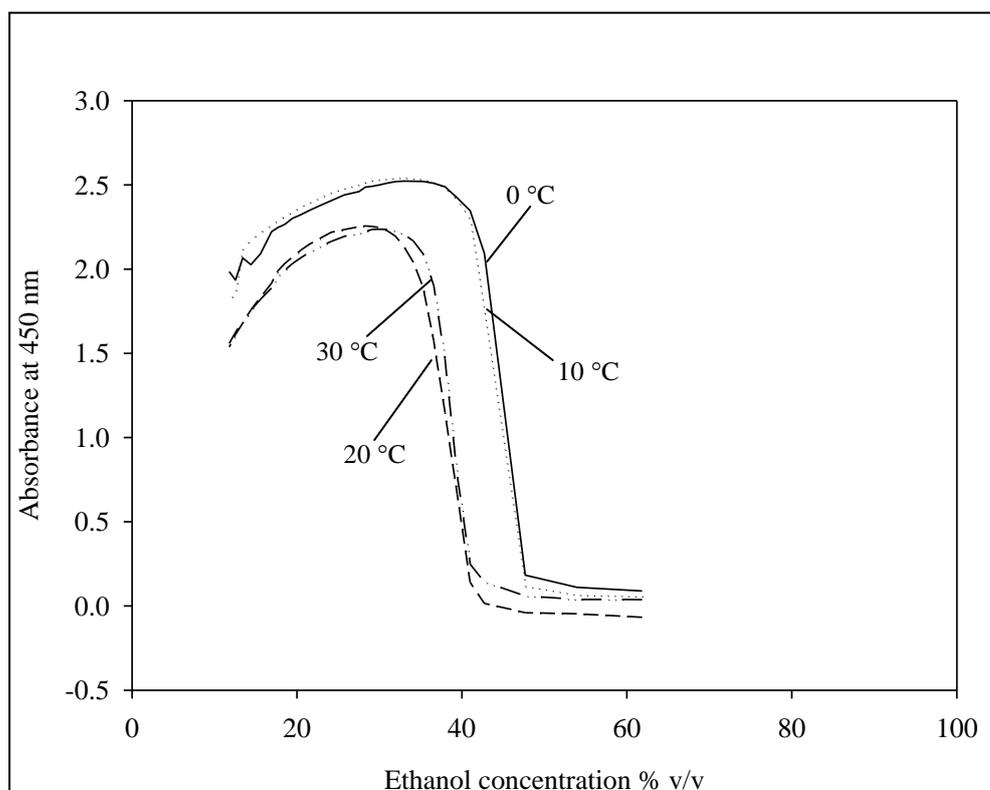


Figure 29 Louching phenomenon of 0.25 % limonene. These plots were generated by progressive dilution of 0.25 % limonene with water from a 60 % starting point

At temperatures below at least 10°C, the mixtures became cloudy at about 48 % ethanol, but at temperatures above at most 20°C, the louch point reduced to about 42 %. These louching behaviours of limonene dilution parallel those of Pernod and grapefruit distillate, where the louch points occur at higher ethanol concentration when the temperature is lower. It seems clear that limonene is more soluble in warm water than in cold, making preferential solubility in ethanol (Hengde & Kazuhiro, 2005) less important. If the experiment is carried out at 15°C, the mixture would become cloudy at about 45 % which is higher than at ambient.

Figure 29 shows the typical curves of louching phenomenon as described in Chapter 3. Limonene alone had the scattering maximum of 2.25, higher than that of grapefruit distillate (1.8) where the louch point was also 42 % in response to a ratio of 60 g zest per 500 mL (Figure 28 in chapter 3). The limonene louch point was similar to that of Pernod (2.35, Figure 25), but in the case of Pernod, the louch point was a commercially useful 36 %.

At this time an interesting observation was made on the louched limonene solutions. After about an hour at ambient temperature it was seen that the cloudiness had disappeared. Loss of cloudiness was never noticed with grapefruit distillate, but it must be admitted that it was not looked for.

What might be the cause? The following models are proposed. When grapefruit zest distillate is mixed with water it becomes turbid, because the lowered alcohol concentration renders the limonene and like compounds insoluble, so generating an emulsion in the form of micelles of limonene dispersed in water/ethanol. All emulsions are inherently unstable for thermodynamic reasons stemming from surface tension (Williams & Phillips, 2003). Eventually the emulsion fails as limonene micelles coalesce and float to the surface. This model was not tested by experimentation.

Another possibility is oxidation of limonene to carvone (Belitz & Grosch, 2004) (Figure 30), by exposure to air. Carvone is insoluble in cold water but slightly soluble in warm water (Wikipedia, retrieved on 22 May 2008), in the same way that limonene is. The relative solubility of the two compounds in water is not known, but inspection of the molecules reveals that carvone will be more polar. According to this model, as carvone concentration increases solubility in water/ethanol would increase, causing

cloudiness to disappear. Neither was this model was tested by experimentation, but it would be testable by gas chromatography designed to resolve the two compounds.

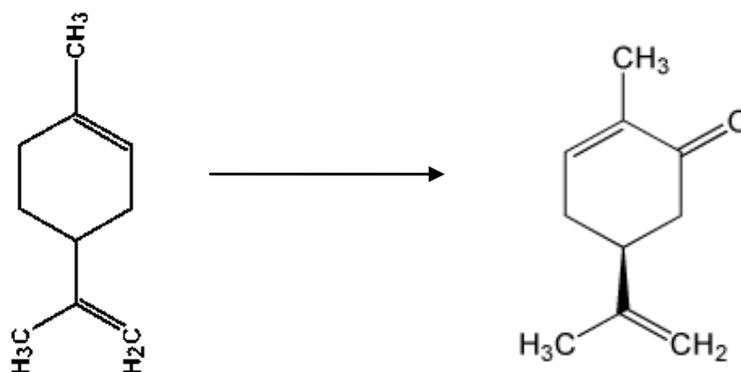


Figure 30 The structure of limonene and its oxidation product, carvone

Effect of assorted hydrocolloids, emulsifiers and other compounds on the louch point of limonene

In this experiment, nine hydrocolloids or similar compounds and three surfactants were chosen to explore the solubility of limonene in ethanol/water mixtures. The structures and properties of the agents were described in Chapter 1. They are Protanal LF-200 (sodium alginate from brown sea algae), Protanal Ester (PVH-A) (alginic polysaccharide esters), polyethylene glycol (PEG 400), β -cyclodextrin, (a cyclic polymer of alpha-D-glucopyranose), hydroxypropyl methycellulose (HPMC), methylcellulose, Methocel K4M food grade, sucrose, gum arabic (polysaccharide-protein complex), xanthan gum (extracellular polysaccharide from *Xanthomanas campestris*), Span 20 and Span 80 (surfactants based on sorbitan fatty acid esters) and Triton 100 (an emulsifier based on substituted sorbitol).

Method

The agents were usually added in two concentrations, 0.05 and 0.10 % w/v, and compared to the control, 0.25 % limonene in 91 % ethanol alone. Progressive dilutions of the 25 mL aliquots were done at ambient temperature.

Results

Figure 31 shows the louching phenomenon of 0.25 % limonene treated with 0, 0.05, 0.1, or 0.5 % w/v gum arabic.

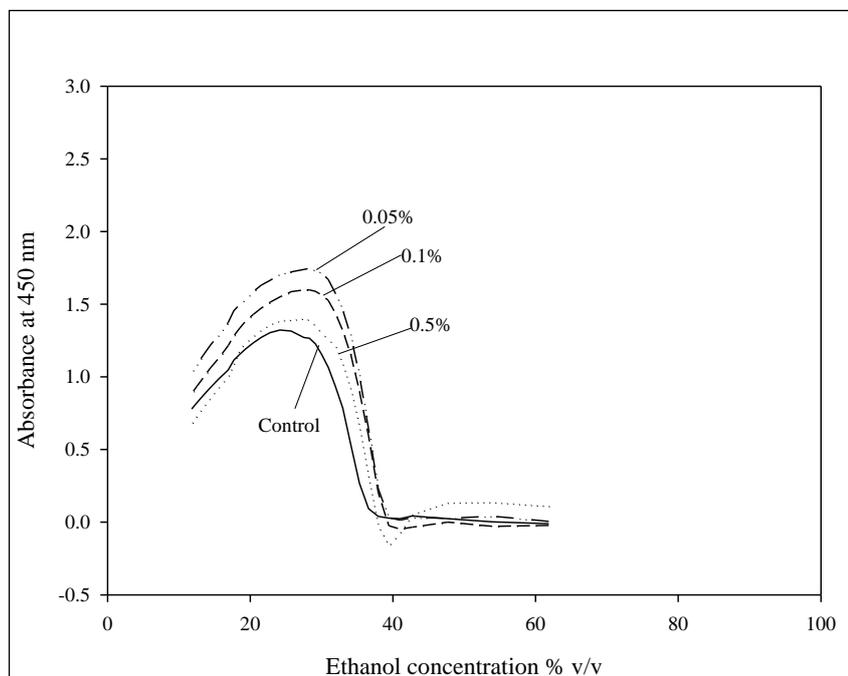


Figure 31 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05, 0.1 and 0.5 % gum arabic

The control (0.25 % limonene alone) had a louch point of about 37 %. At the outset it was seen that the louch point was very much lower than would be expected from Figure 39, where the point was 42 %. Later work in this chapter confirms that the louch point was slightly variable from one experiment to another. While error is inherent in any preparation, there may be unidentified subtleties of technique that cause these differences, for example the rate of addition of water.

In the presence of gum arabic, the louch point increased to about 40 % which is three percentage points higher than for the control. Gum arabic increased the light scattering of limonene, peaking at 0.05 and 0.1 % rather than 0.5 %. However, the absorbance was never as high as that achieved by Pernod (Chapter 3).

Figure 32 shows the louching phenomenon of 0.25 % limonene treated with 0, 0.05, 0.1, or 0.5 % w/v xanthan gum.

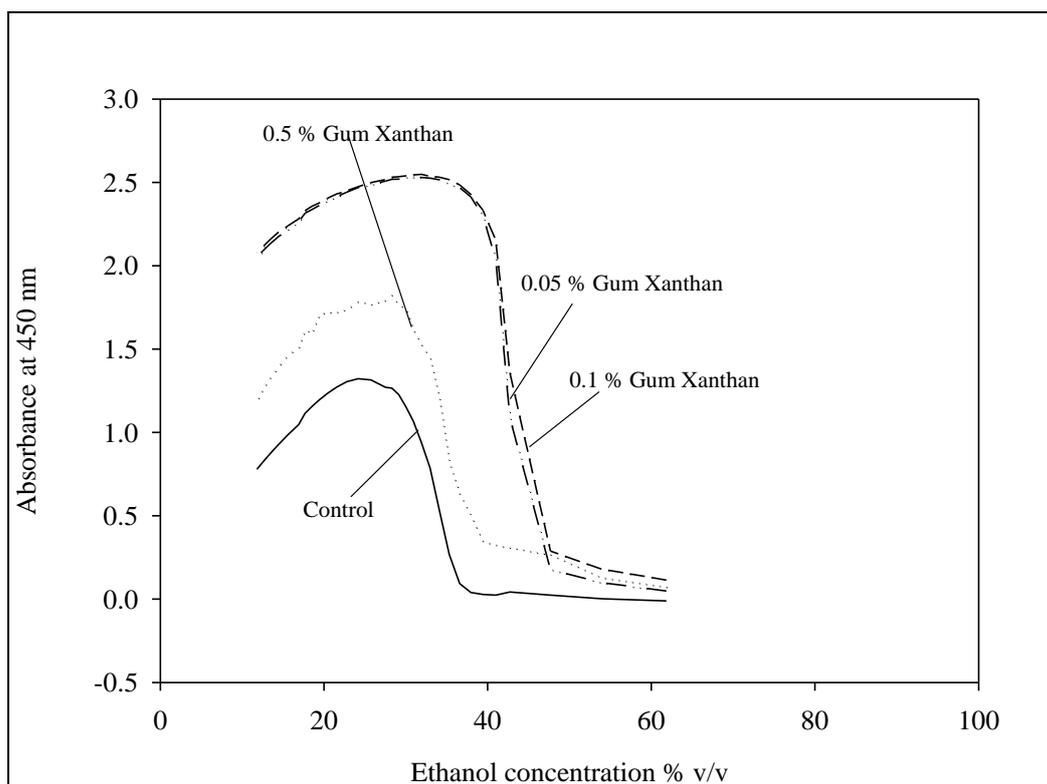


Figure 32 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05, 0.1 and 0.5 % xanthan gum

In the presence of xanthan gum, the louch point increased to about 44 % on average which was higher than control (39 %). However, inspection of Figure 32 shows that for two concentrations, where useful increases in absorption were obtained, the louch point was about 10 percentage points higher. Even before the louch point was attained, light scattering of limonene treated with xanthan gum increased significantly. For instance, even at the starting point (60 %), absorbances slightly higher than the control value were maintained to the louch point. The peak absorbance, 2.5, achieved with 0.05 and 0.1% xanthan gum, was as high as that achieved by Pernod (Chapter 3). However, increasing the amount of xanthan gum to 0.5% lowered absorbance to about 1.8, still higher than control. Thus xanthan gum increased the light scattering but also increased the louch point.

Figure 33 shows the louching phenomenon of 0.25 % limonene treated with 0, 0.05, or 0.1 % w/v Protanal LF-200.

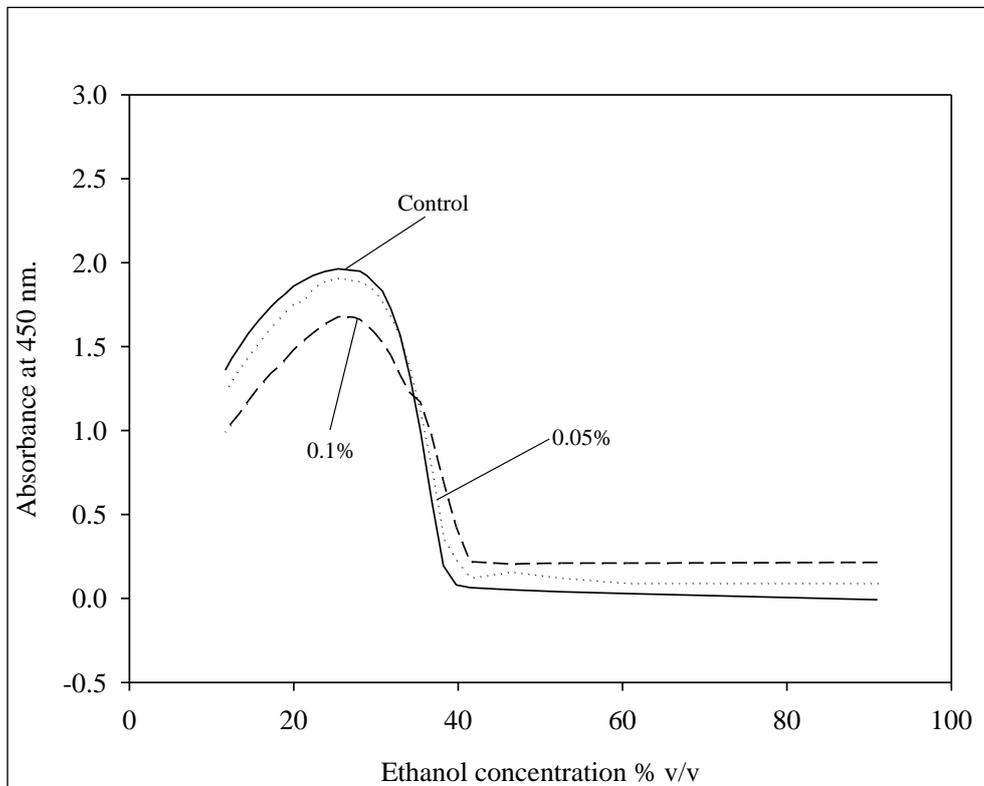


Figure 33 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05 and 0.1 % Protanal LF-200

Protanal LF-200 increased the louch point of limonene to about 42 % which is three percent points higher than control. Also, at the starting ethanol concentration (60 %), the absorbance was slightly increased in the presence of Protanal LF-200. In contrast to the previous two additions (gums arabic and xanthan), the scattering of light was lower in the two treated dilutions than in the control. Thus, Protanal LF-200 did not increase the light scattering, and did not lower the louch point either.

Figure 34 shows the louching phenomenon of 0.25 % limonene treated with 0, 0.05, or 0.1% w/v Protanal Ester (PVH-A).

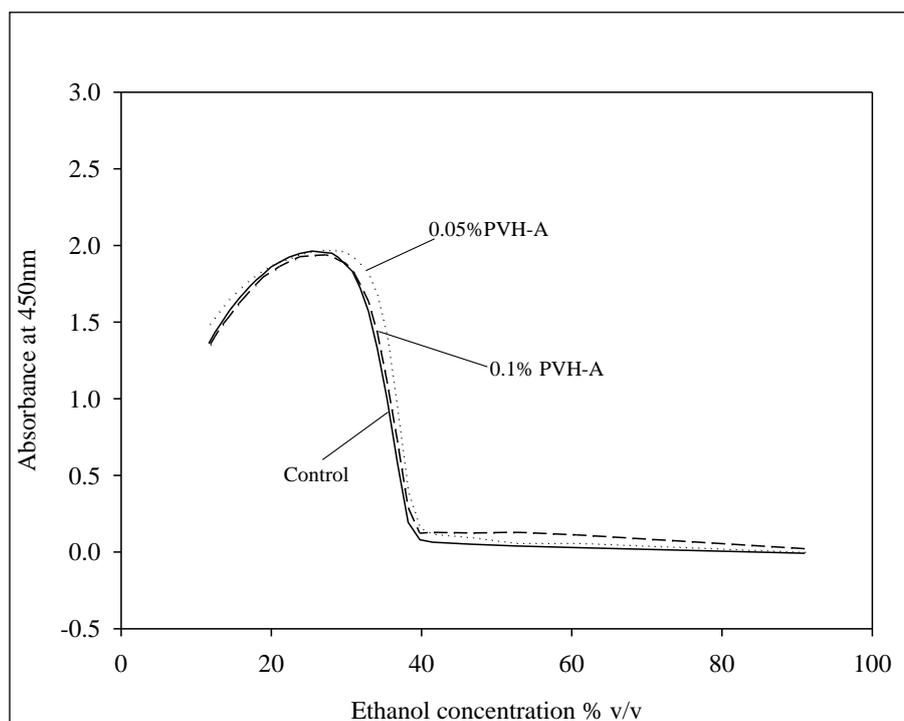


Figure 34 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05 and 0.1 % Protanal Ester (PVH-A)

Protanal Ester did not cause a difference in the louch point and the light scattering.

Figure 35 shows the louching phenomenon of 0.25 % limonene treated with 0, 0.05, or 0.1 % w/v polyethylene glycol 400 (PEG-400).

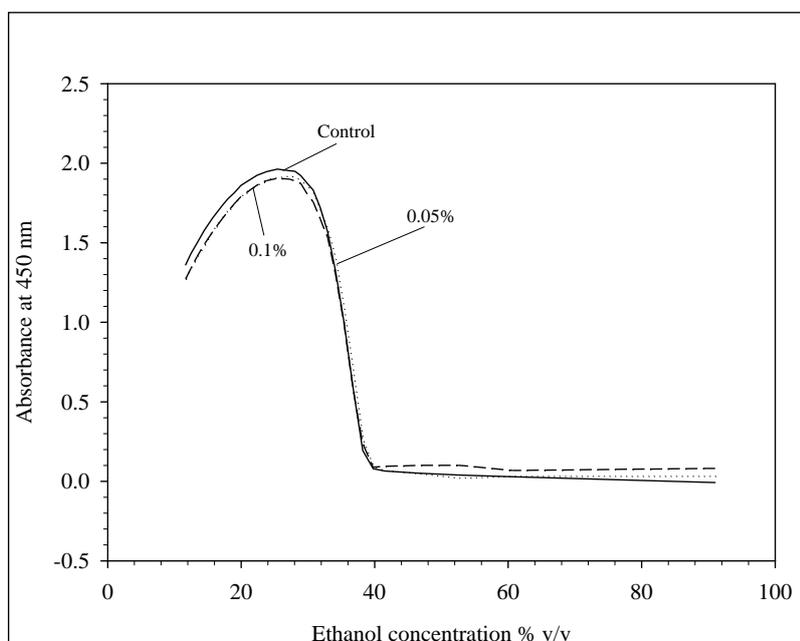


Figure 35 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05 and 0.1 % polyethylene glycol 400 (PEG-400).

As with Protanal Ester, polyethylene glycol 400 did not cause a difference in the louch point and the light scattering.

When a very different class of compound, β -cyclodextrin, was tested, the theme of no change continued (Figure 36), although at the starting point (60 %), β -cyclodextrin at 0.1 % slightly increased the light scattering.

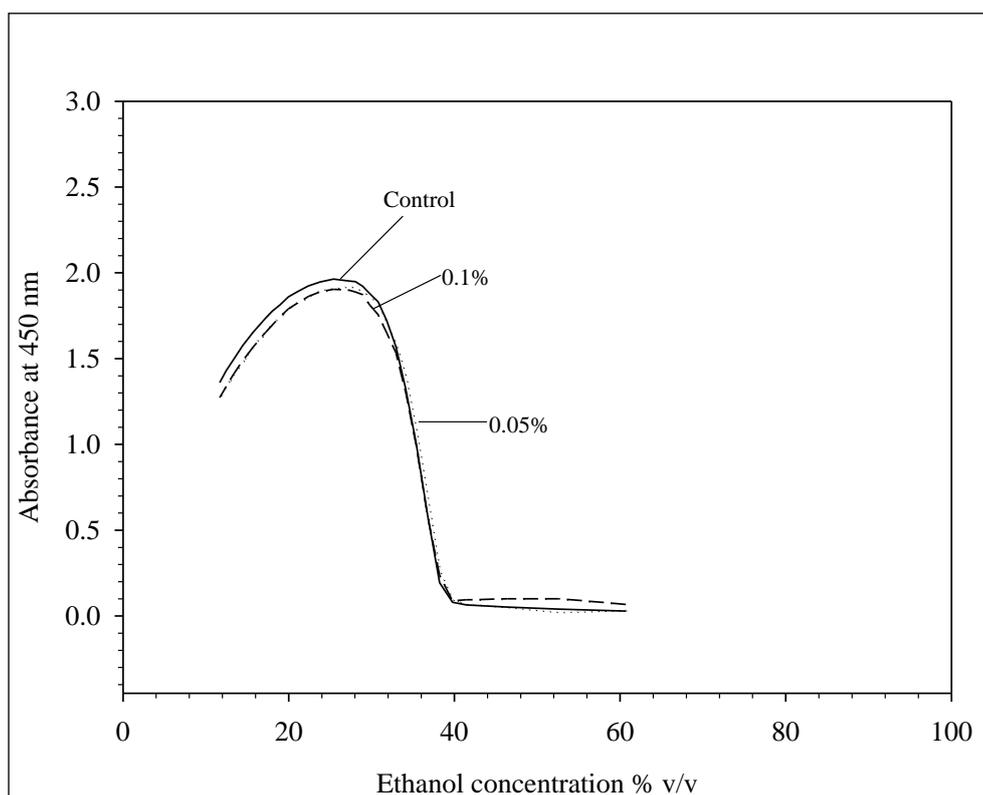


Figure 36 Louching phenomenon of 0.25 % limonene in the presence of 0, 0.05 and 0.1 % β -cyclodextrin.

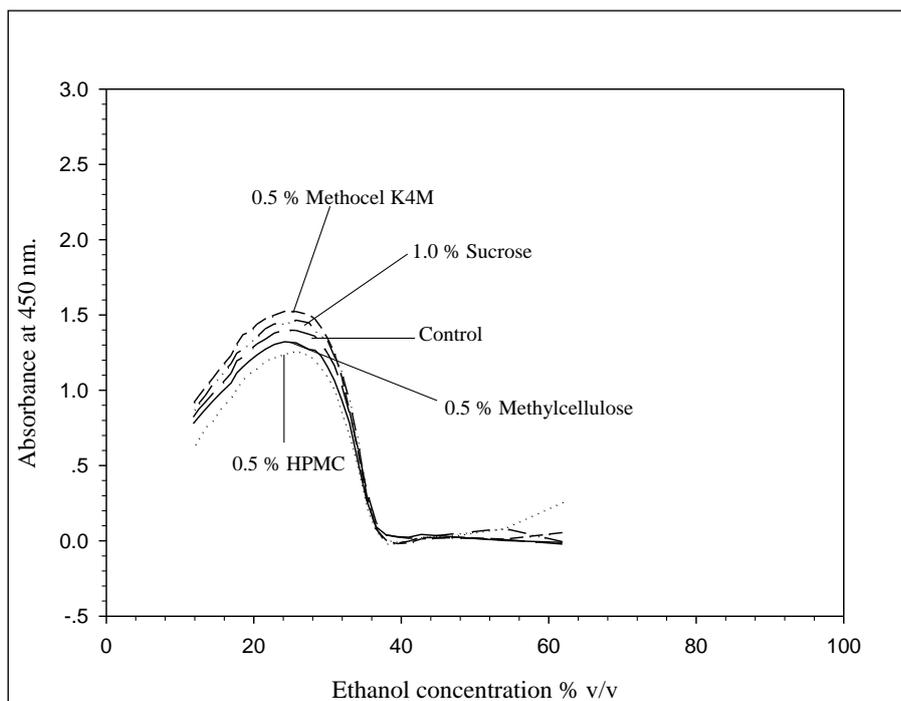


Figure 37 Louching phenomenon of 0.25 % limonene treated with 0.5 % w/v hydroxypropyl methylcellulose, methylcellulose, Methocel K4M, and 1 % w/v sucrose.

Figure 37 shows the louching phenomenon of 0.25 % limonene treated with 0.5 % w/v hydroxypropyl methylcellulose, methylcellulose, Methocel K4M, and 1 % w/v sucrose.

The control had at louch point at about 39 %. None of the treatments increased the louch point. However, Methocel K4M and sucrose increased the absorbance due to light scattering, peaking at 1.5 and 1.4, respectively, but the control was not much lower, 1.3. The absorbance was never as high as that achieved by Pernod (Chapter 3). HPMC seemed to increase the light scattering at the initial point (60 %), but when the addition of water was continued, the absorbance decreased to the same value as other treatments and become cloudy at the same point (39 %).

While at least xanthan gum has some surface activity, none of the above compounds are notable surfactants. In contrast, the relatively low molecular weight Spans and Tritons are very surface active.

Figure 38 shows the louching phenomenon of 0.25 % limonene treated with 0, and 0.25 % Span 20 and 80, and Triton X-100. The results were very different from those shown in Figures 29 to 36, so much so that dilutions had to start at 91 % ethanol.

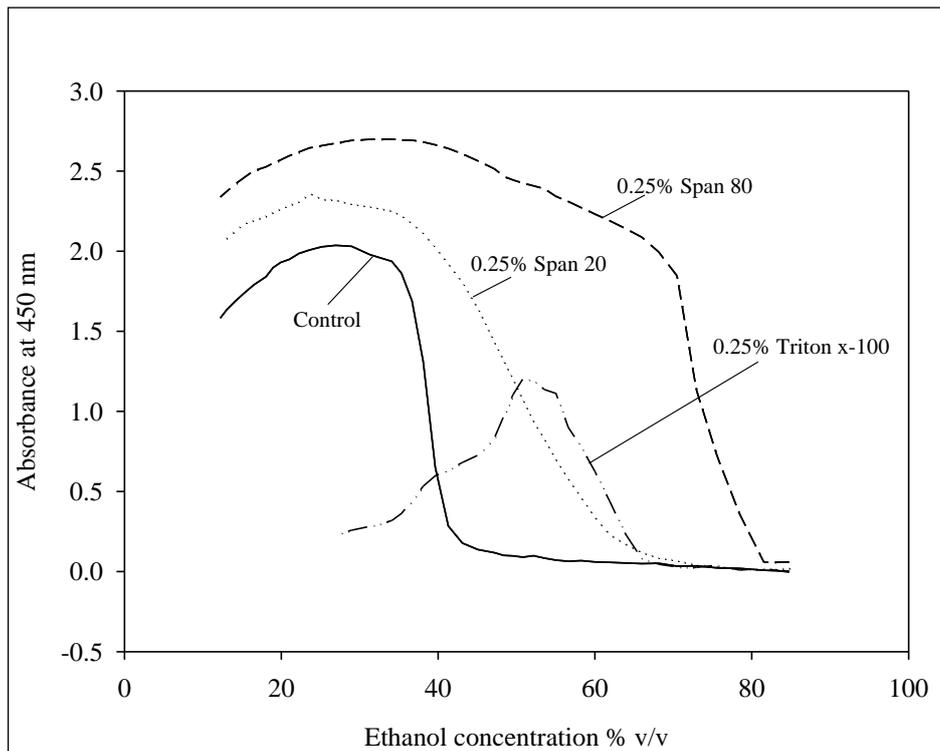


Figure 38 Louching phenomenon of 0.25 % limonene in the presence of 0, and 0.25 Span 20, 80, and Triton X-100.

Span 80 had the louch point at 81% immediately after a small volume of water had been added. Span 20 had the louch point at about 64 % which was close to that of Triton X-100 (65 %). Thus all of the additions resulted in louch points at least 20 percentage points higher of than for the control (41 %). However, Span 20 and 80 greatly increased the light scattering, peaking at 2.7, and 2.4, respectively, even higher than that achieved by Pernod, 2.3 (Chapter 3). Thus, these surfactants increased the light scattering but also increased the louch point a lot. In contrast, Triton X-100 failed on both counts, making the situation much worse than the control. In this case, a non-food grade Triton X-100 was chosen because I wanted to find more range of hydrocolloids that could improve the solubility of limonene.

Discussion

The aim of the experiments reported in the chapter was to investigate the effects of various hydrocolloids and surfactants on the louch point of grapefruit distillate, and the absolute absorbance due to light scattering. The hope was that compounds could be identified that decreased the louch point and/or increased the light scattering. Ideally both would be preferred. Of course not all the compounds could be used

freely in commercial products, but at this stage of product development, a broad range was considered to gain insights into louching behaviour.

Table 4 The summary of louching point and light scattering of hydrocolloids and low molecular weight surfactants.

	Louch point (ethanol % v/v)	Light scattering (absorbance)
Hydrocolloid and others		
Gum arabic	Increase	Increase
Xanthan gum	Increase	Increase
Protanal LF-200	Increase	No change
Protanal ester PVH-A	No change	No change
PEG 400	No change	No change
β -Cyclodextrin	No change	No change
HPMC	No change	No change
Methycellulose	No change	No change
Methocel K4M	No change	No change
Sucrose	No change	Increase
Low molecular weight surfactant		
Span 20	Increase	Increase
Span 80	Increase	Increase
Triton X-100	Increase	No change

Table 1 shows that none of these hydrocolloids or surfactants lowered the louch point. In fact the surfactants (Span 20 and 80, and Triton X-100) increased the louch point at least 20 percentage points higher than for limonene alone (40 %). Although the hydrocolloid and others compounds also increased the louch point, the increases were much smaller. On average, the increase in louch point was by about two percentage points.

While the surfactants increased light scattering, edible versions of them (if available) can be completely ruled out of contention, because the primary goal was to lower the louch point. Surfactants act by encapsulating hydrophobic molecules such that the hydrophilic parts of their structure protrudes into the aqueous phase and lowers the interphase tension. In the more complex case of a water, ethanol, limonene and surfactant mixture it is not obvious why the micelles develop at high ethanol concentrations nor is it known what the structure of the micelles might be. Certainly a range of complex structures have been described for micelle structure in complex

mixtures (Belitz & Grosch, 2004). Moreover, the hydrophilic-lipophilic balance (HLB) of these three surfactants will affect the way they behave. HLB values of Span 20 and Span 80 are 8.6 and 4.3 respectively (Banker & Rhodes, 2002), favouring oil-in-water and water-in-oil emulsions respectively. The louch point for the Span 20 occurred in more aqueous mixtures than for Span 80, and this is almost certainly related to the HLB values in some way.

Of the hydrocolloids and other compounds, only gum arabic and xanthan gum increased the absorbance peak, but in both cases at the expense of increasing the louch point and in the case of xanthan gum very significantly. Xanthan gum can be ruled out. Gum arabic, which is preferentially soluble in water than in ethanol, certainly increased light scattering, with only a small increase in the louch point. Thus, it may have practical value, particularly as it will increase viscosity as is often a desirable measure in high alcohol drinks, citing Cointreau as an example.

The other compounds in the category 'hydrocolloid and others' were less interesting and there is little to comment on except that several of those hydrocolloids could be used as thickening agents without affecting louch.

β -Cyclodextrin, the 7-membered glucose ring was added speculatively but had no effect on louch probably indicating that limonene was not trapped by the hydrophobic cyclodextrin cavity, although the cavity is large enough to hold it. The cavity diameter of β -cyclodextrin is 0.78 nm, and the widest dimension of limonene is not more than 0.50 nm, based on a C to C bond length of 0.154 nm, a tetrahedral bond angle of about 109° and a C to H bond length of 0.11 nm (Yu et al., 2007). It is proposed that in the ethanol concentration range tested (60 to 10 %) limonene is more 'self-soluble' in the form of micelles than 'soluble' in the cavity. However, there may be an energy barrier to overcome that could be achieved by heating (Banker & Rhodes, 2002). This was not tested.

Sucrose is usually added into beverages in order to give the sweetness and to improve mouth feel (Coulter & Davies, 1994). Figure 37 shows that at 1.0 % added sucrose, the louch point was the same as for the control. Also, the absorbance peak increased a little, but not so much as to equal that of Pernod. Addition of sucrose in further development would not effect the ethanol concentration at the louch point. That is a useful result.

The work with grapefruit to this point has used only the zest because the skin is a waste product in New Zealand, and the use of the whole fruit has been avoided. As noted earlier in this chapter the researcher's taste perception was that the flavour of zest distillate lacked intensity. It was thought that better flavour results might be obtained by using the whole grapefruit (zest, pith, and juice) rather than zest only. However, the effect on louch point and light scattering is unknown. These issues are explored in Chapter 5.

Chapter 5

Louching of whole grapefruit and the effect of water addition on distillation

Introduction

In the previous chapter it was found that none of the hydrocolloids or surfactants would enhance the solubility of limonene. Moreover, the informal taste assessment work done in Chapter 3 showed that the flavour from grapefruit zest alone lacked intensity. Of course zest alone was chosen because grapefruit skin is a waste product in New Zealand. Only the juice is used commercially (Wright, 2007). It was proposed that using the entire fruit might give better results both for flavour and possibly light scattering. In the experiments reported here, zest + pith (effectively whole skin) or zest + pith + juice (effectively whole fruit) was used as the flavour source.

The quantities of components other than zest necessarily added moisture, particularly in the case of juice. The basic methodology is described in Chapter 2 and is here modified to take moisture into account. The approximate moisture content of a typical California grapefruit is shown in Table 5.

Table 5 Weights of pith and juice and moisture content in a typical California grapefruit compared with 40 g of zest (mean of 3 fruit)

Fraction	Wet weight (g)	Moisture content (% w/w)
Zest	40	Not required
Pith	112	80.0
Juice	212	98 (estimated)

Louching in ethanolic distillate of various grapefruit fractions

Method

A valid three-treatment comparison required that water was added to the zest and zest + pith distillation treatments to adjust for moisture in pith and juice. Moisture in the zest + pith + juice in a typical grapefruit compared with standard 40 g of zest is shown in Table 6. The volume collected was 250 mL for each treatment.

Table 6 Volumes and weights of zest, pith, juice and water used in the three-treatment comparison flavour source

	Flask A Zest alone	Flask B Zest + pith	Flask C Zest + pith + juice
91% Ethanol (mL)	500	500	500
Grapefruit zest (g)	40	40	40
Pith (g)	0	112	112
Water added (mL)			
(Moisture due to pith)	89.6	0	0
Juice (mL)	0	0	212
Water added (mL)			
(Moisture due to juice)	207.8	207.8	0

Results

Figure 39 shows the louching phenomenon of ethanolic distillate of Flask A

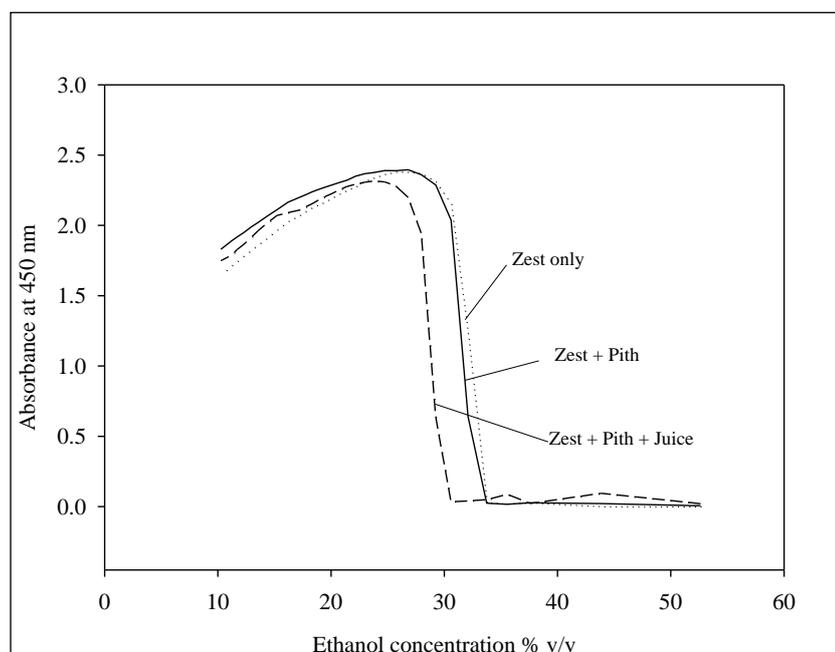


Figure 39 Louching phenomenon of distillate of various grapefruit fractions at ambient temperature

With zest alone the louch point was 36 % and, interestingly, the maximum absorbance was 2.4 at 450 nm, the highest attained so far where the louch point was below 36 %. At once this suggested that water addition to the distillation flask was useful. Inclusion of pith to the mix (Flask B) did not cause any difference in the louch point (36 %) or the light scattering (2.4). However, for Flask C (zest + pith + juice), the louch point may have decreased between one and two percentage points (Figure 39). Because of time constraints the comparison experiment was not repeated, so the reduction in louch point may not have been statistically significant. However, the use of whole fruit certainly did not increase the louch point. Inclusion of pith + juice did not cause any difference to light scattering.

Assume for the moment that addition of pith or pith plus juice caused no significant change in louch point or light scattering. This is simply explained because limonene, purportedly the louch-active agent in grapefruit, is found in grapefruit zest only.

However, informal smelling and tasting indicated that the distillate from whole grapefruit had a more intense flavour than that from zest alone. In grapefruit juice, two compounds, namely, nootkatone and p-1-menthene-8-thiol (structures shown in Chapter 1) have been identified as making an important contribution to juice aroma. The thiol has a very low odour threshold of $0.1 \times 10^{-9} \text{ g L}^{-1}$ (Belitz & Grosch, 2004).

Water in the distillation mix may be responsible for the increase in light scattering noted above. Therefore the next experiment focuses on the effect of water addition to the distillates.

Effect of water addition on ethanolic distillation of zest

As noted above, the aim of these experiments was to determine if addition of water to the ethanolic distillation of zest could extract more louchable compounds than could be achieved by 91 % ethanol alone. This hypothesis was drawn from the previous experiment which indicated that water addition increased scattering with no increase in the louch point. Also, in Chapter 3 it was shown that later distillation fractions – which have higher water content – were more flavoured in informal sensory trials. Thus there was the possibility that water additions could give more intense flavour in the distillate.

Method

The basic methodology is described in Chapter 2. For these experiments, where the ratio of water: ethanol was varied (Table 7), distillate was recovered in 100 mL fractions until 500 mL was recovered. The accurate ethanol concentration of each fraction was determined by the enzymatic method. Subsequently Fractions 1, 3 and 5 were diluted with water as required to yield a common starting concentration of 60 %. Each fraction was then progressively diluted at ambient temperature, approximately 23°C, and the absorbance monitored at 450 nm.

Table 7 Quantities of zest, ethanol and water before distillation

	Flask			
	1	2	3	4
Grapefruit zest (g)	40	40	40	40
Ethanol, 91 % (mL)	300	400	500	600
Water (mL)	500	400	300	200
Ethanol:water ratio	0.52	0.83	1.32	2.15

Results

The curves obtained for these progressive dilutions are shown in Appendix 1 for Fractions 1, 3 and 5, while the key attributes of those curves are shown in Table 8. Only Fractions 1, 3, and 5 were tested because if do all fractions, the graphs will be out of scales.

Table 8 Louching phenomena of zest distillate at different ratios of ethanol and water

Treatments (Ethanol:water ratio)	Louch point (ethanol %)	Peak light absorbance at 450 nm
0.52		
Fraction 1	37	2.10
Fraction 3	29	0.10
Fraction 5	29	0.10
0.83		
Fraction 1	37	2.00
Fraction 3	29	0.30
Fraction 5	29	0.00
1.32		
Fraction 1	39	2.15
Fraction 3	No louch point	0.10
Fraction 5	No louch point	0.10
2.15		
Fraction 1	36	2.05
Fraction 3	36	2.00
Fraction 5	No louch point	0.00

Fraction 1 of all treatments resulted in a distinctive louch point and a stronger light scattering, compared to other fractions. The average louch point of Fraction 1 in all

treatments was about 37.3 %. The light scattering was above 2 but was not as high as that achieved by Pernod (2.35). For treatments 0.52, 0.83 and 1.32, representing increasing ethanol concentrations, the peak absorbance due to light scattering dramatically decreased in Fractions 3 and 5 (and almost certainly Fraction 4) to lower than 0.3. The most distinctive result was the highest alcohol treatment, Treatment 2.15. The louching phenomenon occurred in Fractions 1, 3 and almost certainly Fraction 2 (not tested). The louch point was 36 %, equal to that of Pernod and the light scattering was above 2, which was not as high as that achieved by Pernod (2.35). Fraction 5 of all treatments either did not louch at all or showed very flat peaks (Table 8). It seems clear that few or none of louchable compounds were extracted into the final fractions (500 mL). In case of Treatment 0.52, the lowest ethanol concentration, the distillate became cloudy during condensation. A likely explanation for this is as follows. As distillation progress, the temperature in the flask steadily increases making water more volatile such that the louch phenomenon occurred immediately. The proportion of ethanol: water of 2.15 gave the best results. In hindsight the comparison in Table 8 should have extended to a fifth flask, where no water was added to 91 % ethanol. This would have resulted in an ethanol:water ratio of 10.1, which was the ratio encountered in previous chapters. However, the results shown in Table 8 for Treatment 2.15 were the best obtained so far, so water has been included in subsequent fruit-based experiments by virtue of the fact that whole grapefruit were used. Nonetheless the ratio used was not necessarily 2.15. In the following experiment where distillations of zest and whole grapefruit were compared, the ratio was 2.15.

Comparative distillation of zest and entire grapefruit with an ethanol:water ratio of 2.15

Method

The weights of materials to be distilled are shown in Table 9, where the ratio of ethanol:water was 2.15, whether the water was added as liquid or derived from grapefruit pith and juice. For these experiments, 300 mL of unfractionated distillate was collected. The ethanol concentration was measured by the enzymatic method.

Each treatment was then progressively diluted at ambient temperature and absorbances recorded.

Table 9 Quantities of ingredients used to generate an ethanol:water ratio of 2.15 before distillation

	Flask A	Flask B
Ethanol 91 % (mL)	600	600
Zest (g)	40	40
Pith (g)	0	50
Juice (mL)	0	165
Water (mL)	200	0

Results and discussion

When two very different distillation treatments were tested, there were slight differences in both the louch point and the absorbance due to the light scattering (Figure 40).

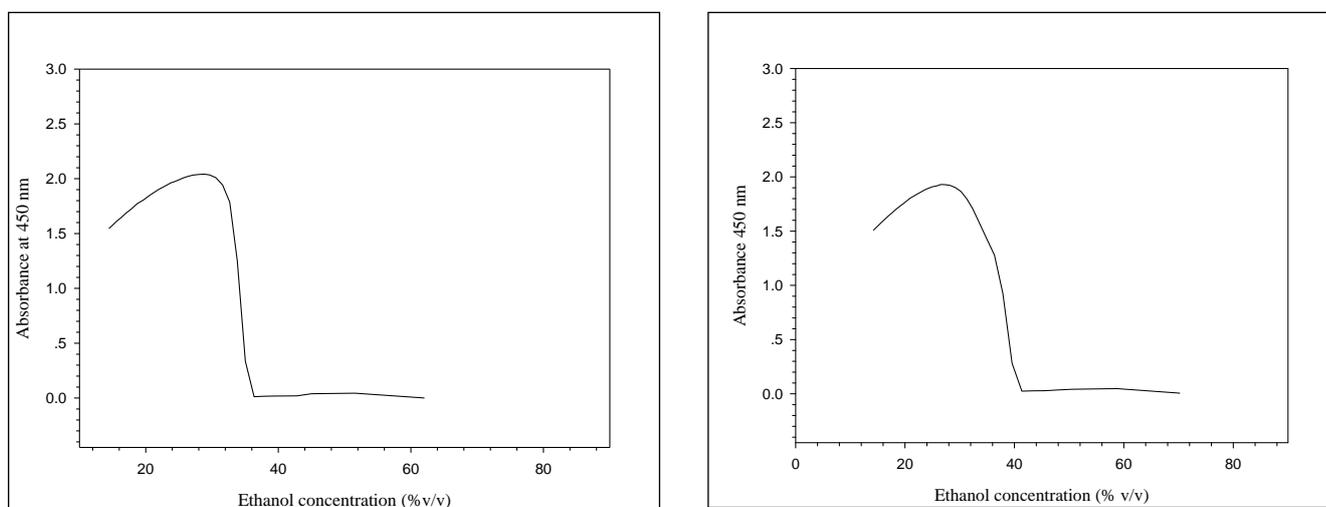


Figure 40 Louching phenomenon of zest in ethanol:water of 2.15 (left) and whole grapefruit (right) distillates at ambient temperature.

In the zest treatment of, the louch point was about 38 % and the peak absorbance was 2.1. However, in the whole grapefruit treatment the louch point was slightly increased to 40 %, and peak absorbance may have been slightly lower at 2.0.

On the face of it there was no advantage in using whole grapefruit instead of zest alone, but as noted earlier, the informal odour assessment showed that – perhaps unsurprisingly – the former yielded a more flavourful spirit as judged by odour.

To this point, all the work was done with the imported California fruit. The intended raw material for distillation was always to be the New Zealand fruit, and as this became available a direct comparison became possible.

Louching in New Zealand and California grapefruit distillate

Introduction

As discussed in Chapter 2, California grapefruit were chosen because the work began in February and local fruit were not available until July. Anecdotal evidence and a knowledge of fruit physiology indicated that New Zealand fruit harvested later than August would be sweeter, and would probably be comparable in physiological development to the commercial California product. Therefore a direct comparison of the louch behaviour of the two varieties was done in October 2007.

At the outset it must be noted that the following experiment lacks statistical meaning because the scale of distillation required that one or at most a few fruits were used. In the event only one typical New Zealand grapefruit was compared with one typical California equivalent. The idea was to see if there were any major differences which would point the way to statistically valid trials which could realistically be carried in the laboratory. To make the comparison as valid as possible, the ethanol:water ratio of 2.15 was used for both fruit.

Method

The basic methods in this experiment were the same as for the whole fruit treatment in the previous experiment, where the ratio ethanol: water was 2.15. Distillate was recovered in 100 mL fractions until 300 mL was recovered. The accurate ethanol concentration of fraction each was determined by the enzymatic method.

Subsequently each fraction was diluted with water as required to yield a common starting concentration of 60 %. Each fraction was then progressively diluted at ambient temperature and absorbance recorded.

Results and discussion

The louching phenomena for New Zealand and California grapefruit distillates are shown in Figure 41.

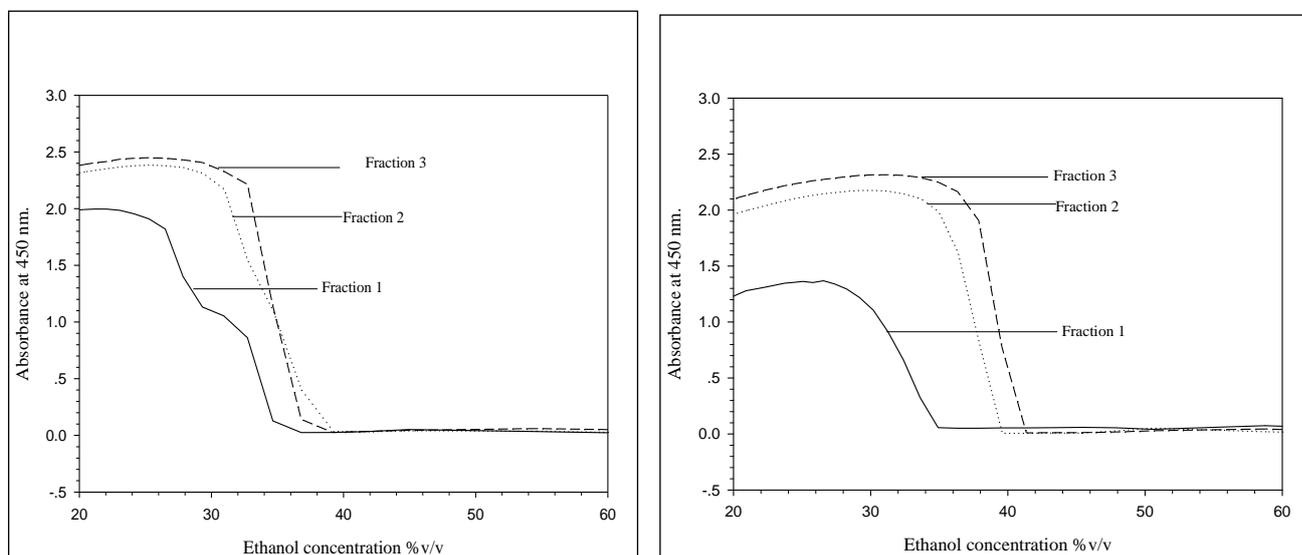


Figure 41 Louching phenomenon at ambient temperature of New Zealand grapefruit (left) and California grapefruit (right) distillates

For the New Zealand grapefruit, Fraction 3 had the highest louch point, just under 40 %, and Fraction 1 had the lowest point louch point (~ 36 %). The average louch point was around 39 % (Figure 40, left). The highest peak absorbance due to the light scattering was for Fraction 3 at 2.5.

Within the limitations of fruit selection, the results show that there are no marked differences in the louching profile of New Zealand and California grapefruit, and certainly not enough to warrant a full statistically valid comparison. Therefore, California grapefruit would be acceptable to use instead of New Zealand grapefruit while the latter were not available.

Conclusions

Addition of water in the ethanolic distillation extracted more louchable compound than achieved by 91 % ethanol alone. The proportion of ethanol:water of 2.15 gave the best results, and increased the light scattering with no increase the louch point.

Using whole grapefruit gave a more intense flavour than from zest alone. Inclusion of pith and juice did not cause any difference to the light scattering and louch point.

From this point, for further development of grapefruit distillate, the entire grapefruit will be used for better flavour results.

Either fruit would give the same louching phenomenon in both louch point and the peak absorbance due to the light scattering. However, the problem remains that anethole (from Pernod) gave better light scattering while the louch point is still low. Therefore, the next chapter addresses this issue by the way of louching and direct quantitation of louchable components in grapefruit distillate and Pernod by gas chromatography.

Chapter 6

Louching of anethole compared with limonene

Introduction

In Chapter 5, it was found that the addition of water in the ethanolic distillation improved the light scattering of grapefruit distillate but not at the expense of increasing the louch point. However the scattering was never as high as that achieved by Pernod. To find out why, experiments were performed to directly compare louching of authentic limonene and anethole and to quantify the concentration of louchable matter in commercial Pernod and in New Zealand and California grapefruit distillates.

Louching in authentic anethole and limonene

Method

Aliquots (25 mL) of 0.25 % w/v anethole and limonene in 91 % ethanol were progressively diluted at ambient temperature (23 °C) to represent the louching of anethole and limonene alone. These starting concentrations were equivalent to 0.0165 M anethole and 0.0154 M limonene.

Results and discussion

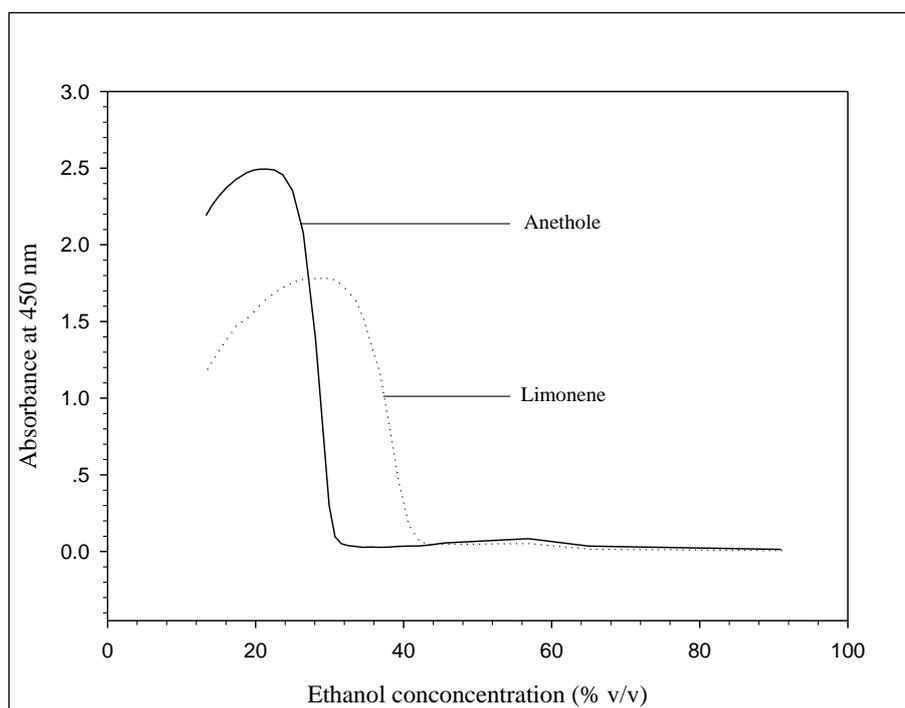


Figure 42 Louching phenomenon of 0.25 % anethole and 0.25 % limonene in 91 % ethanol

The 0.25 % limonene solution had a louch point at about 42 % ethanol (Figure 42). At the outset, it was seen that the louch point was very much higher than that of 0.25 % Anethole. Moreover the peak absorbance due to the light scattering of the 0.25 % anethole was 2.5, but in case of 0.25 % limonene, the louch point was below 2.0. As noted in Chapter 3, these absorbances represent very different transmissions of light because absorbance is on a logarithmic scale. The following reason is advanced as to why this should be so.

Table 10 The melting and boiling points¹ of *trans*-anethole and d-limonene

	Boiling point (°C)	Melting point (°C)	Crystal shape
<i>trans</i> -Anethole	81.5	21.4	Leaf
d-Limonene	176	-174	Not known

¹Handbook of Chemistry and Physics (48th Edition), page C-168, 1967

Table 10 reports the melting and boiling points of *trans*-anethole and d-limonene. The melting points are of particular interest because Pernod is always served cool, if

not ice cold. Close inspection of Pernod drink over ice shows the presence of flat crystals, presumably those of anethole, because the melting point (21.4°C) is well above that of an iced drink. The literature (Handbook of Chemistry and Physics, 1947) confirms that the crystals of anethole precipitated from ethanol are ‘leaf’ like, as might be expected from the planar structure of the molecule (Figure 43) where the aromatic rings would stack due to van der Waals forces. Thus louching of Pernod or anethole would generate flat crystals that might be randomly oriented in aqueous suspension. These would almost certainly scatter light better than a liquid-liquid emulsion/dispersion of limonene. Limonene remains a liquid even in an iced drink because its melting point is far below 0°C.

The results in Figure 42 suggest that the concentration of anethole (or compounds that louch in a similar way to anethole) in Pernod as sold at 40 % ethanol would be about 0.11 %. The reasoning behind this argument is as follows. The louch point of authentic Pernod is around 35 % ethanol (Chapter 3), compared with 32 % ethanol for 0.25 % anethole in 91 % ethanol. Therefore, the estimated concentration of anethole in Pernod at its louch point would be $35/32 \times (0.25 \% \times 32/91) = 0.096 \% \text{ (w/v)}$. As sold at 40 % ethanol, the concentration of anethole in Pernod would be about $0.096 \% \times 40/35 = 0.11 \% \text{ (w/v)}$.

The equivalent concentration of limonene at its louch point of 42 % ethanol would be about $0.25 \% \times 42/91 = 0.12 \% \text{ (w/v)}$.

These calculations were then tested against gas chromatographic determinations of limonene and anethole in grapefruit distillates and authentic Pernod.

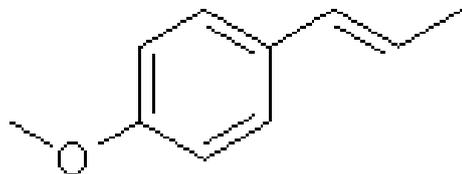


Figure 43 Structure of anethole

Concentration of louchable compounds in Pernod and entire grapefruit distillates

Method

This experiment determined the concentration of louchable compounds in Pernod (anethole) and in entire grapefruit distillates (thought to be limonene). The basic methodology is described in Chapters 2 and 5. In the case of grapefruit distillate at 60 % ethanol, a direct injection was made. However, in the case of Pernod, a 10-fold dilution in 91 % ethanol was made before injection. This was done to dilute the corn syrup sugar declared to be an ingredient in Pernod. Underivatised sugars thermally decompose and contaminate gas chromatographic equipment. The internal standard menthol was added after a 10-fold dilution.

Results

The calibration curves of each authentic compound are shown in Figure 44 and Figure 45.

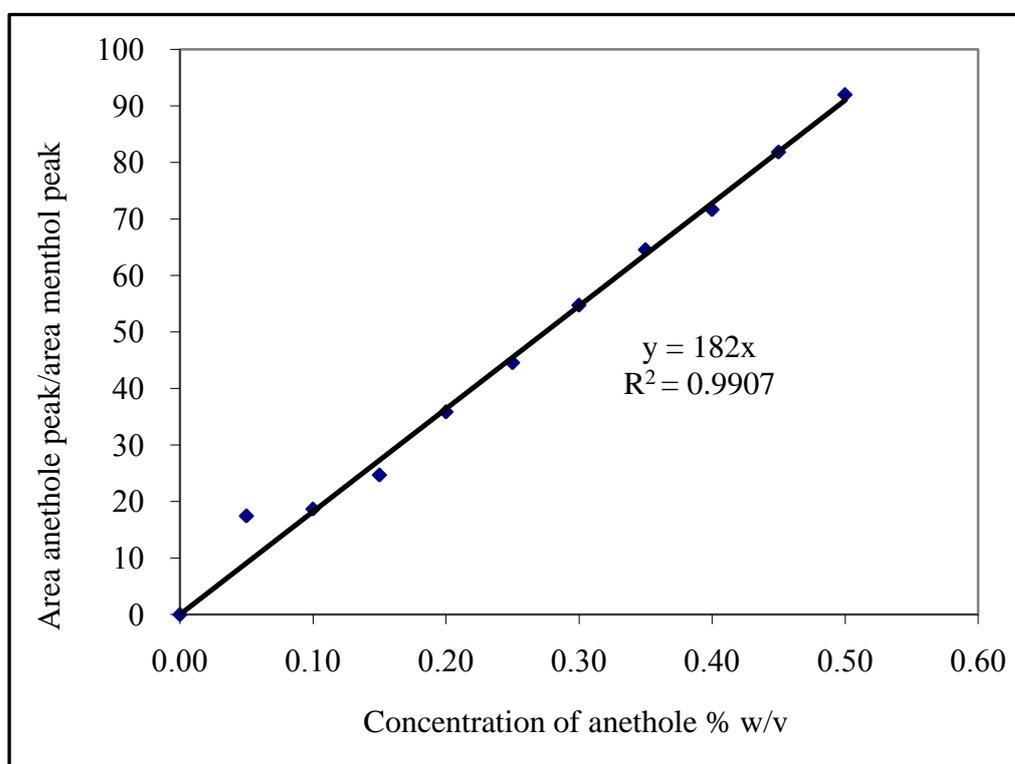


Figure 44 Calibration curve of anethole in 91 % ethanol using menthol as the internal standard

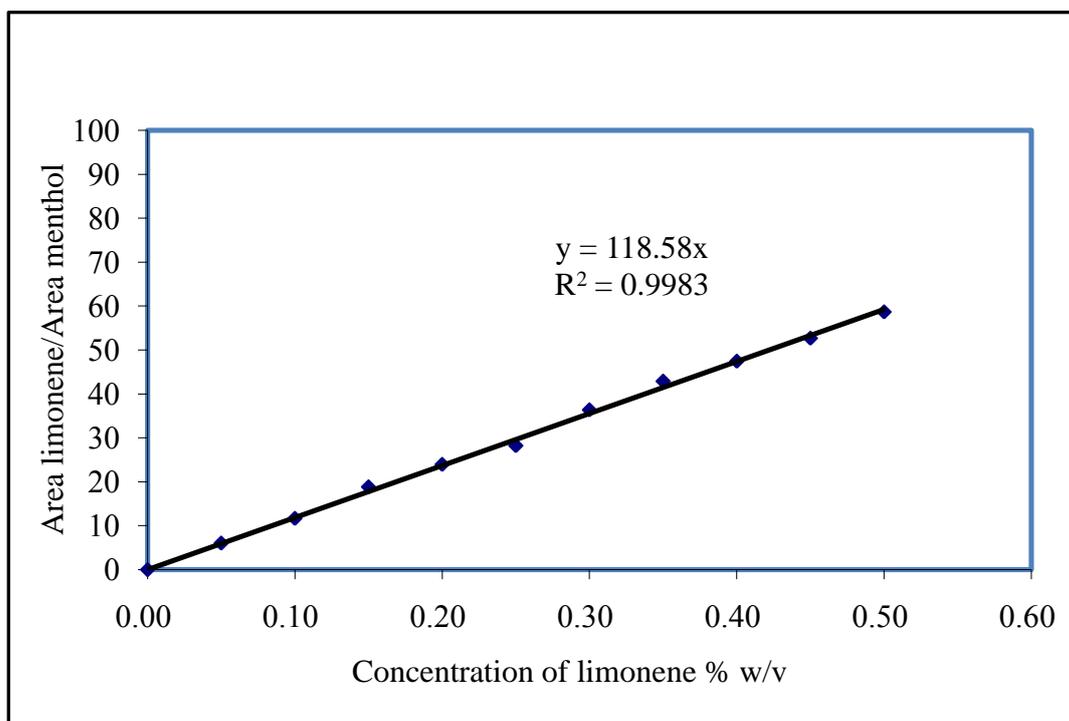


Figure 45 Calibration curve of limonene in 91 % ethanol using menthol as the internal standard

The typical gas chromatogram of diluted Pernod is shown in Figure 46. The undiluted grapefruit distillate is shown in Figure 47.

The calibration curves were prepared with a menthol internal standard concentration that was by admitted mistake about 10-fold lower than that of the analytes. With this mismatch the error in determinations will be higher than if the peak areas were more comparable. However, the correlations were excellent and the subsequent results were accepted as valid.

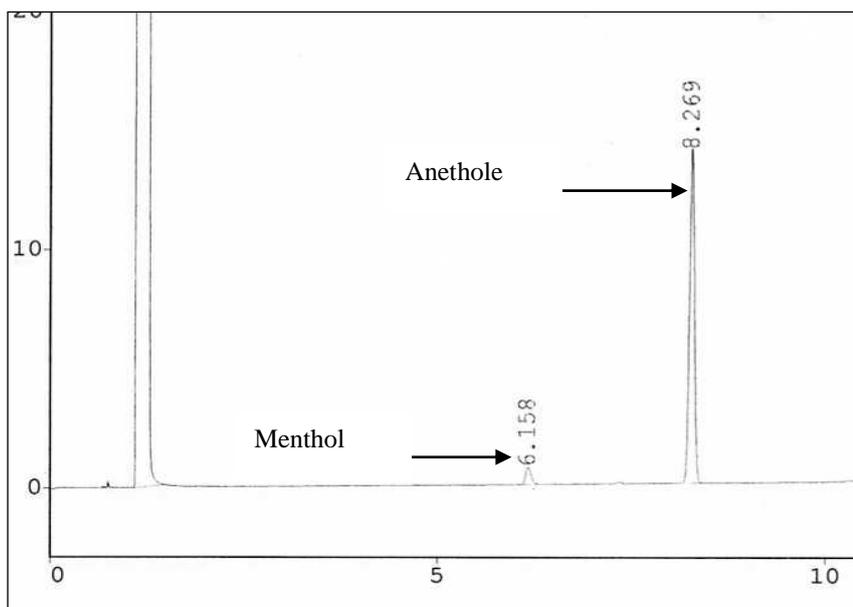


Figure 46 Chromatogram of diluted Pernod

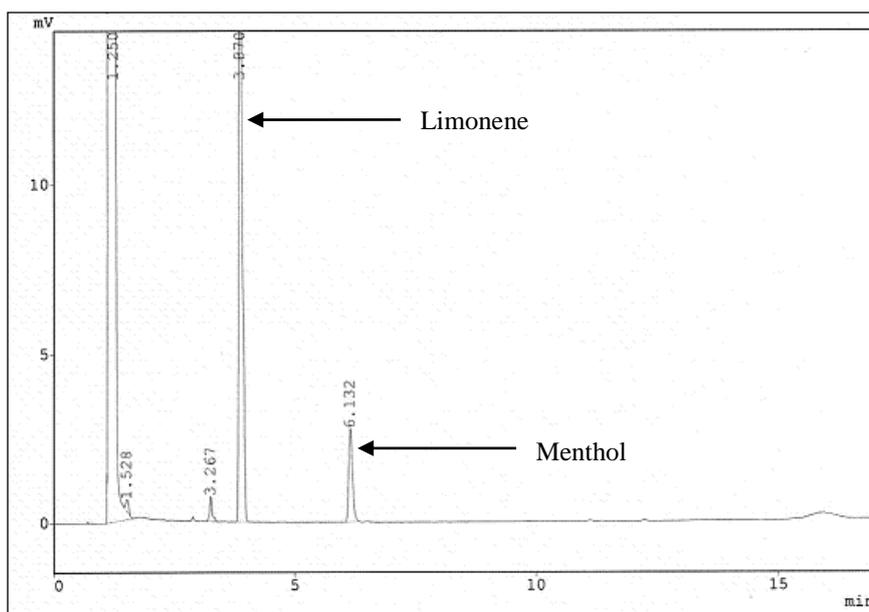


Figure 47 Chromatogram of undiluted grapefruit distillate

Ethanol was eluted just after the first minute. The Pernod chromatogram was dominated by anethole (6.0 minutes), but a significant range of other compounds was detected, mainly eluting before anethole (peaks not clear on Figure E as vertically scaled). The dominant peak in grapefruit distillate was clearly limonene (3.87 minutes), with other unidentified peaks. The molecular weights of anethole and limonene are similar, so the longer elution time for anethole can probably be ascribed to its obviously greater polarity due to the ether group.

The identified volatile compounds in the products and their concentrations are shown in Table 11.

Table 11 Anethole concentration in authentic Pernod, and limonene concentrations in distillates of entire New Zealand and California grapefruit, all data adjusted to 40 % ethanol

Origin and (compound)	Peak absorbance at 450 nm	Concentration ² (% w/v)
Pernod ¹ (Anethole)	2.60	0.11 ± 0.02
New Zealand grapefruit distillate (Limonene)		
Fraction 1 ³	2.41	0.31 ± 0.04
Fraction 2	2.40	0.20 ± 0.06
Fraction 3	2.00	0.12 ± 0.04
California grapefruit distillate (Limonene)		
Fraction 1	2.39	0.11 ± 0.01
Fraction 2	2.35	0.11 ± 0.05
Fraction 3	1.40	0.08 ± 0.05

¹ The Pernod data are corrected for the 10-fold dilution

² Means and standard deviations of at least 3 replicates

³ Each fraction was 100 mL

Based on one New Zealand grapefruit and one California grapefruit, the data show that the former contained more distillable limonene. The means for the three fractions were 0.21 and 0.10 % respectively, and the equivalent mean peak absorbances were 2.27 and 2.05. Realising that the absorbance scale is logarithmic, the results of absorbance and determined concentrations are consistent. The more interesting comparison is between grapefruit distillates as a group and Pernod. A lower percent concentration of anethole resulted in a much higher absorbance, probably for the reasons discussed with respect to Table 10.

The louching experiment for Pernod in Figure 42 led to an estimation of 0.11 % (w/v) anethole which was identical to the gas chromatographic determination within the limits of experimental error. This is very good evidence that anethole is the totally dominating louchable compound in Pernod. In the case of grapefruit distillate, Figure

42 showed that the estimated concentration of limonene was about 0.12 % (w/v) which is slightly lower than that shown for two grapefruit by gas chromatography. The grand mean for these three fractions from two grapefruit was 0.16 % (w/v), again good agreement within the limits of experimental error.

Returning to the subject of grapefruit distillates, in Chapter 2 it was shown that as distillation progressed, the temperature in the distillation flask steadily increased, which would make the terpenes more volatile. Therefore, the highest quantity of limonene might be expected to be in Fraction 3. [The boiling point of limonene is 176°C (Table 10)]. However, Table 11 shows that New Zealand grapefruit performed in the reverse manner in that Fraction 1 had the highest limonene concentration, which then slightly decreased as the distillation progressed.

Because of time constraints, the comparison experiment was not replicated to gain statistical validity. If the results in Table 10 were representative of the two varieties, the cause of this variation might lie in fruit structure. Boiling point is one thing, but for limonene to become volatile it first must be released from the oil glands in the zest. The physical structure of the glands in the two varieties might be substantially different. This matter was not explored further.

Conclusion

The concentration of anethole in Pernod (0.11 % w/v) was slightly lower than that of limonene in distillates from two typical grapefruit. However, the light scattering of diluted Pernod, as measured by absorbance at 450 nm, was markedly higher than that of diluted grapefruit distillate. It was argued that the cause of this was due to the difference in melting points such that a solid-in-liquid dispersion (anethole in ethanol/water) would scatter light more than a liquid-in-liquid dispersion (limonene in ethanol/water).

In spite of this comparative deficiency the grapefruit distillate nonetheless does louch. The aim of the next chapter was to explore the hedonic responses of consumers to the final grapefruit distillate. The focus group interview was chosen as the sensory evaluation.

Chapter 7

Focus group interviews

Introduction

In Chapter 5, the method for generating a louchable spirit from entire grapefruit was developed. Although such a spirit did not louch as well as Pernod, it could still have market potential on the basis that it could be made from a distinctive New Zealand cultivar of grapefruit, and could be made as a 40 % spirit that would louch on dilution. There is no competing drink on the market.

This chapter reports a focus group assessment and comparison of Pernod and California grapefruit distillate. The reasons for choosing this assessment method were discussed in Chapter 1. At the time the assessment was done, the New Zealand fruit were not available so the California equivalent had to be used. Because many alcoholic drinks have added sugars, including Pernod it was decided to add another test treatment to the assessments. Thus the drinks to be assessed and compared were Pernod, grapefruit distillate and grapefruit distillate with 3 % added sucrose.

Method

The methodology is described in Chapter 2, and the questionnaire is shown in Appendix 2 and Appendix 3. In outline, focus group interviews were made with 10 groups comprising three volunteer panelists per group, with all panelists being AUT staff or students. The respective ethanol concentrations of the three treatments – Pernod, grapefruit distillate, and grapefruit distillate with 3 % added sucrose – were 38, 36, and approximately 36 % (v/v). The distillates were prepared from whole grapefruit rather than zest alone. Panelists were advised of the broad nature of the three drinks. The 15 mL undiluted samples were simultaneously presented at room temperature in wine glasses. Each group independently assessed for undiluted and diluted aroma (orthonasal evaluation), and for diluted (15 mL of water) flavour and texture by in-mouth evaluation. Discussion within a group occurred spontaneously and was allowed.

Result and discussion

The full responses of the 10 groups are in Appendix 4. Table 12 summarises the main outcomes.

Table 12 Summary of focus group interview results

	Pernod		Grapefruit distillate		Grapefruit distillate 3 % added sucrose	
	Undiluted	Diluted	Undiluted	Diluted	Undiluted	Diluted
Aroma	Aniseed Medicine Sumbuca Delicate	Fruity syrup Antacid Cough-syrup like Meat cooking ingredient	Refreshing Clean Citrusy Gin aroma	Grapefruit Fresh Sugary Fruity	Lemonish Chemical-like Citrusy Lack of grapefruit odour	Less fruity Chemical-like Lack of grapefruit odour Sugary More pleasant aroma
Appearance	Artificial colour	Lemon juice like Dislike milky appearance Colour looks dense	Clear like vodka Prefer add colour such as orange, yellow, or pink	Lemonade Pearly-look Soap water like	Clear Prefer add colour such as orange or yellow	Lemonade Pearly-look Soap water like
Flavour		Liquorice Strong repulsive		Burning Lack of grapefruit flavour Slightly burning in mouth Clean taste Gin taste Bitter		More mouth feel Chemical feel finish Lack of grapefruit flavour Sour and bitter Prefer bitterness Need more sweetness Gin taste
Comment		Do not like the cloudy yellow	Feel like want to drink it			Prefer 3% added sugar because more body (smoother), taste higher in alcohol. Fiery in body

Both undiluted grapefruit distillate treatments were clear and colourless. Most panelists expressed the view that the distillates should be coloured pink, orange, or another colour reminiscent of grapefruit in order to make it more interesting than just a drink that simply resembled vodka. The opinion was expressed that if colour were added to the grapefruit distillate, it would make consumers believe that the grapefruit distillate had more body, and also more fruity taste. Significantly, Pernod contains declared colouring agents E104, Quinoline Yellow and E124, Ponceau 4R (Brilliant Scarlet), which some panelists found to be ‘artificial’ and ‘like medicine’ (cough-syrup).

Table 12 also shows aroma profiles obtained by orthonasal evaluation of the three undiluted three drinks. The two grapefruit distillates were perceived by most panelists as ‘refreshing’, ‘clean’, ‘fruity’, and ‘citrusy’ in aroma. However, the drinks were sometimes ‘too strong in alcohol aroma’, giving rise to a ‘chemical’ impression. In the case of Pernod, the aroma was perceived as delicate and aniseed-like but some panelists perceived this as ‘cough-syrup medicine’.

The louching phenomenon of Pernod and grapefruit distillates was liked by most of the panelists. In case of both grapefruit treatments, consumers referred to the louching colour as a ‘pearly-look’ which was attractive. However, some of panelists found the appearance of the grapefruit distillates was like ‘soapy water’. Pernod became dense yellow after louching, but most panelists expressed a dislike of the cloudy yellow colour.

There were obvious differences in comments reported for louched grapefruit distillate and 3 % added sucrose grapefruit distillate. Both of distillates were said to lack grapefruit flavour and there was a common perception of a strong chemical finish. Moreover, the grapefruit distillates had a bitter aftertaste, probably due to the nootkatone from grapefruit zest that is responsible for its bitterness. The 3 % added sucrose treatment reportedly had a smoother taste than the straight distillate. In the comparison between grapefruit distillates as a group and Pernod, the latter was found to be more dominant in all its attributes because Pernod has an intense aniseed flavour, an obvious yellow colour, and a more intense light scattering effect as was anticipated from the greater absorbances at 450 nm reported repeatedly in this thesis.

Conclusion

No great differences were found in aroma, appearance and flavour profiles between the two grapefruit distillate treatments. Consumers preferred the grapefruit distillate after dilution but there was a perceived need to have a more intense grapefruit flavour, colour, and sweetening. In spite of the use of the whole grapefruit rather than zest – to increase flavour – the panelists felt the drinks fell short of their expectations when Pernod was used as the anchor reference.

Final remarks

The experiments showed that grapefruit skins distillate in 91 % v/v alcohol yield an alcoholic solution which when diluted just below 40 % exhibited the opalescence properties of pastis. The results showed that the major terpene (these are responsible for the phenomenon) in grapefruit skin is limonene, which is available as a laboratory reagent. The grapefruit distillate develops opalescence at and around 40 % v/v. The importance of this concentration is as follows. Spirits are traditionally sold around this concentration and if a significantly higher alcohol concentration were needed to maintain the clarity of the undiluted drink, the market may not accept it, and the alcohol duty would be unacceptably high.

At same concentrations of grapefruit zest-derived terpenes required to louch at about the same ethanol concentration of Pernod (38 % v/v), as measured by absorbance at 450 nm, was markedly lower than that for Pernod. Thus, the aim was to have a limonene concentration as high as possible- to generate a strong opalescence on dilution – while at the same time not exceeding an alcohol concentration, of 40% v/v.

A broad range of hydrocolloids was determined to alter the relative solubility of terpene (mainly limonene) in water/ethanol mixture. However, the results showed that none of hydrocolloids or surfactants lowered the louch point.

Grapefruit zest was used because the skin is a waste product in New Zealand. Moreover, the informal taste assessment work indicated that the flavour from grapefruit zest alone lacked intensity. It was thought that better flavour result might be obtained by using the whole grapefruit (zest, pith, and juice) rather than zest alone. The addition of pith or pith plus juice caused no significant change in louch point or light scattering. However, informal smelling and tasting indicated that the distillate from whole grapefruit had more intense flavour than that from zest alone.

Limonene, anethole, pernod and grapefruit distillate were determined by gas chromatography. Comparisons between New Zealand and California grapefruit were determined in order to measure the amount of limonene in both kinds of grapefruit. Therefore, the results show that New Zealand grapefruit contain more limonene compounds than California grapefruit.

The 0.25 % limonene solution had a louch point (42 % v/v) which is much higher than 0.25 % anethole. Moreover, the peak absorbance due to the light scattering of 0.25 % anethole was 2.5, but in case of 0.25 % limonene, the peak absorbance was markedly below 2.0. One possible for this is the difference in melting points such that a solid-in-liquid dispersion (anethole in ethanol/water) would scatter light more than a liquid-in-liquid dispersion (limonene in ethanol/water).

More interesting is the comparison between grapefruit distillate and Pernod . A lower percent concentration of anethole resulted in a much higher absorbance, probably for the reasons discussed earlier. Although grapefruit distillate did not louch as well as Pernod, it could still have market potential on the basis that it could be made from distinctive New Zealand cultivar of grapefruit.

The sensory perception of aroma, appearance, and flavour in commercial Pernod and grapefruit distillate was assessed. The results showed that the grapefruit distillates were perceived by most panelists as refreshing, clean, fruity, and citrusy in aroma, but lacking grapefruit flavour and there was a common perception of a strong chemical finish. For Further developments are necessary to enhance grapefruit flavour while retaining the louch point at or below 40 % v/v.

Appendices

Appendix 1: The curves obtained from water addition on ethanolic distillation of grapefruit zest

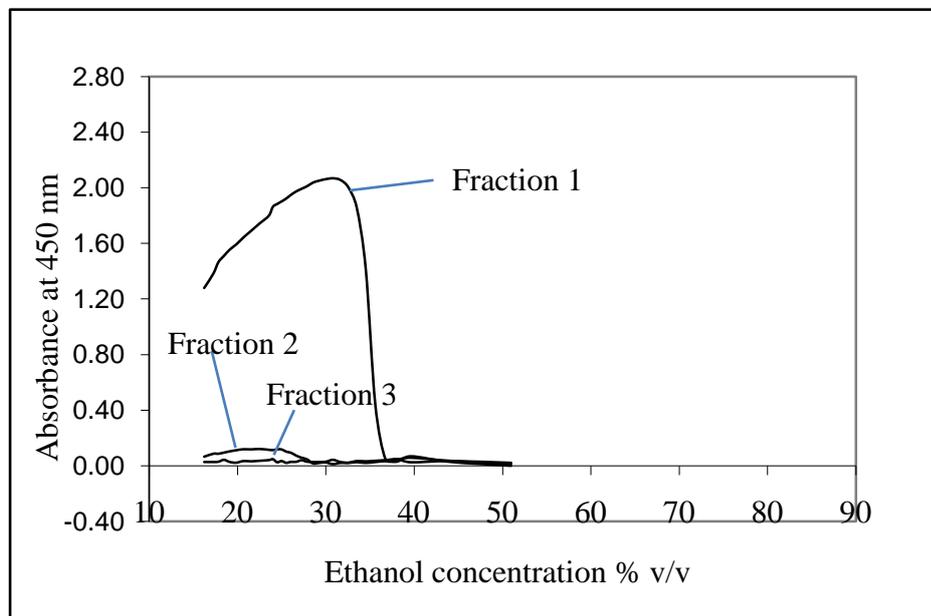


Figure 1 Louching phenomenon of ethanolic distillation of grapefruit zest which treated with 0.52 ethanol:water

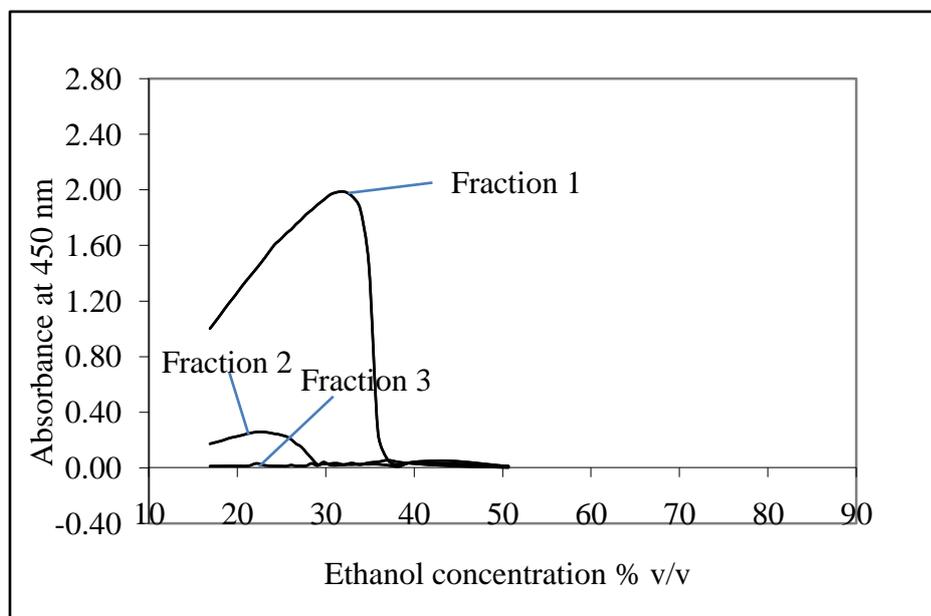


Figure 2 Louching phenomenon of ethanolic distillation of grapefruit zest which treated with 0.83 ethanol:water

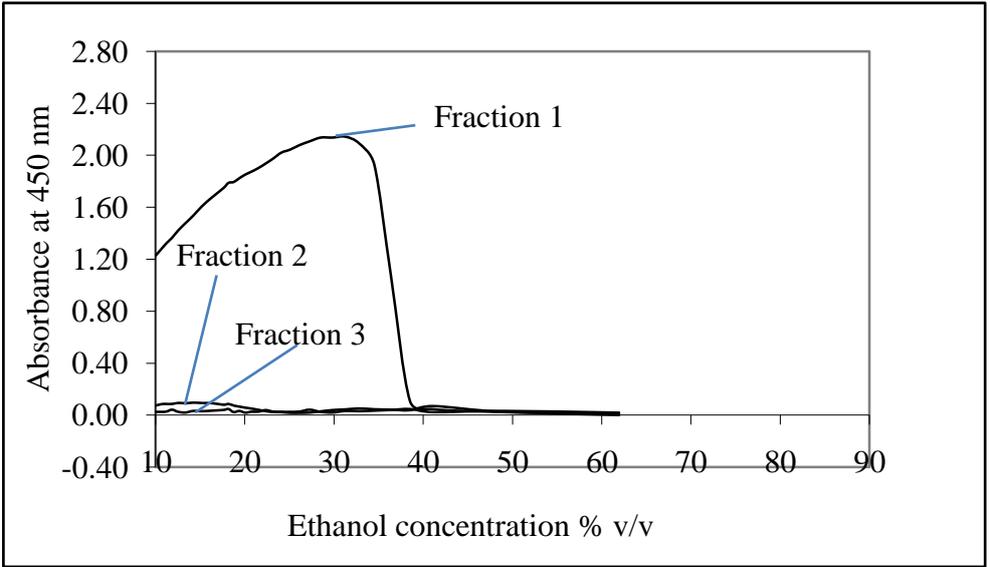


Figure 3 Louching phenomenon of ethanolic distillation of grapefruit zest which treated with 1.32 ethanol:water

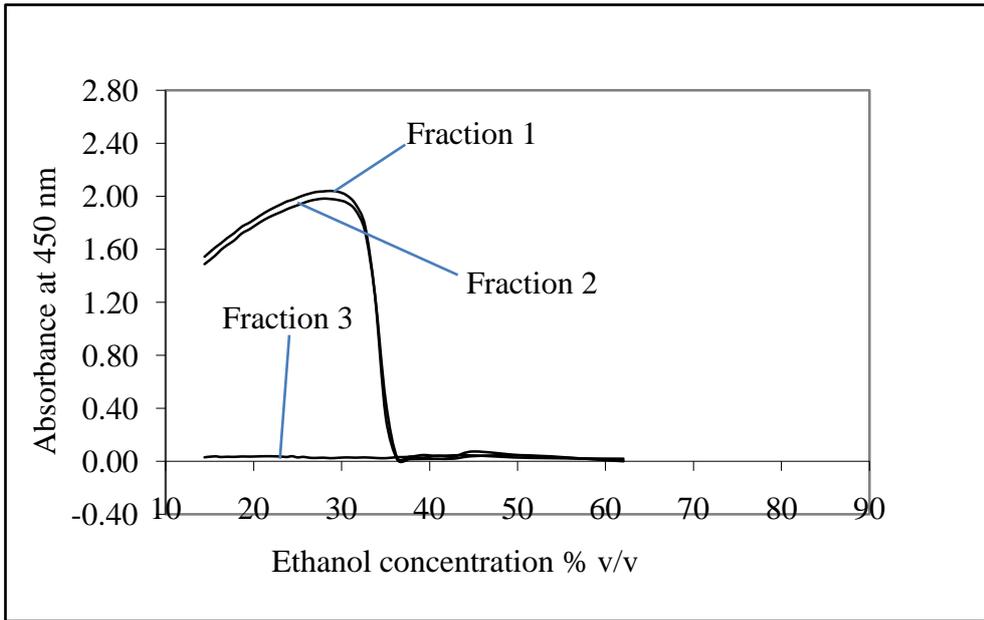


Figure 4 Louching phenomenon of ethanolic distillation of grapefruit zest which treated with 2.15 ethanol:water

Appendix 2: Focus group information



I invite you to take part in a focus study as a part of my Master of Applied Science thesis project.

I want to know whether the development of grapefruit-flavoured spirit with the opalescence properties of pastis might be accepted by consumers.

You have to be 18 year or older to take part.

Your participation in this study is entirely voluntary and you may withdraw at any time.

I want to record your verbal responses so that I can analyse them at my leisure. (After transcription, the recording will be deleted, and names will not be ascribed to the responses.)

You will first be asked if you want to take part.

You will be presented with three spirits samples at 40 % alcohol by volume, the normal spirit strength.

You will first assess their aromas and colours.

Then, as you observe, I will dilute these samples in a standard way, after which you will be invited to assess the aroma and flavour. If you choose to taste the diluted spirit (you decide), you do not have to swallow. You are provided with paper cups for that purpose.

After assessing the spirits we will discuss your reactions.

Pornphun Chaipongrattana 021 204 0424

(Supervisor: Associate Professor Owen Young, WS514, ext 8150 or 027 414 7402)

Grapefruit spirit tasting					
Group Number					
Gender	M	<input type="checkbox"/>	F	<input type="checkbox"/>	
Age Group	18-29	<input type="checkbox"/>	30-39	<input type="checkbox"/>	
	40-49	<input type="checkbox"/>	50-70	<input type="checkbox"/>	

Appendix 3: Topics for the focus group interview

1. Aroma of undiluted.
2. Colour of undiluted
3. Reaction to louching
4. Aroma of diluted.
5. Colour of diluted
6. Sweetness of diluted
7. If this product were sold in the market, would you like to buy it? If not why?

Appendix 4: Focus group interview results

Table 1 Focus group interview results of Group 1

	Undiluted	Diluted
Pernod	Aniseed flavour, Sambucca, most don't like it	Prefer with crush ice on the drink. Liquorice aftertaste, fruity syrup
Grapefruit distillate	Refreshing, clean, citrus flavoured, fruity, feel like want to drink it.	Appearance: Like lemonade Aroma: Grapefruit, fresh Flavour: very strong, weak in grapefruit taste, slightly burning in the mouth, clean taste but lack of fruity taste, smell lovely.
Grapefruit distillate 3 % added Sucrose	Lighter than no added sugar, lime-flavoured, stronger odorant, more chemically in the drink odorant.	Appearance: Prefer diluted with water Aroma: less fruity than no added sugar. Flavour: Chemically, no fruity smell, bitter taste on the background, couldn't taste any sweetness but smell great.

Table 2 Focus group interview results of Group 2

	Undiluted	Diluted
Pernod	Strong	Aroma: smell like antacid, cough-syrup like, meat-cooking ingredient, look like lemon juice
Grapefruit distillate	Orange, citrus, very nice, could have been red-colour	Sweeter "sugary". Fruity smell
Grapefruit distillate 3 % added Sucrose	Orange, citrus	Bitter taste on the background, sour and bitter, prefer bitterness

Comments: Louching is interesting. If sold in the market, would buy grapefruit spirit.

Table 3 Focus group interview results of Group 3

	Undiluted	Diluted
Pernod	Aniseed flavoured, medicine, lovely delicate	Colour looks dense the other two. Far-dense.
Grapefruit distillate	Fruit-like, added colouring because clearness look like vodka, probably in yellow like a orange colour.	Appearance: Louch is attractive, pearly-look, not usually distinct. Aroma: look like soap water or dishwater Flavour: grapefruit strong, smoother in mouth.
Grapefruit distillate 3 % added Sucrose	Citrus, fruit-colour, probably yellow	Aroma: Chemical smell, slight off taste (quite-chemical feeling finish) Flavour: prefer this one as more body (smoother), taste higher in alcohol, fiery in body.

Comments: If sold cheaper than commercial product, would buy one.

No, if current drink but would buy if the drink gets developed

Table 4 Focus group interview results of Group 4

	Undiluted	Diluted
Pernod	Smells, very strong, repulsive	Still don't like cloudy yellow
Grapefruit distillate	Artificial colour, e.g. Banana colouring Nice, fruity, citrusy, feel like burning, like the clear colour	Aroma: fruity but not much flavour Flavour: effect of alcohol is good but not much flavour on the drink. No citrus taste
Grapefruit distillate 3 % added Sucrose	Similar fruity smell to no added sugar, more chemical, like the clear colour	Flavour: not feel strong as before diluted with water but the taste still stronger than no added sugar. No citrus taste

Comments: Do not want to buy this product if sold in the market. However, if add more citrus and fruit taste, then would buy it. Also if price is good but definitely need more fruity taste in the drink.

Table 5 Focus group interview results of Group 5

	Undiluted	Diluted
Pernod		Like cloudiness of the drink
Grapefruit distillate	More grapefruit smell, more colour would be more attractive	More appeal, taste and aroma better than added sugar
Grapefruit distillate 3 % added Sucrose	More subtle so suggest higher quality	Subtle taste, softened taste

Table 6 Focus group interview results of Group 6

	Undiluted	Diluted
Pernod	Prefer yellow colour rather than clear, aniseed smell	Still like cloudy yellow better than cloudy white, still smell nice, not lessened by water, strong in flavour, would taste better if sweeter to balance, unusual dry
Grapefruit distillate	Smell like gin aroma, could have grapefruit colour	Smell weaker after diluted, taste harsh and bitter, little more sugar would make it smoother.
Grapefruit distillate 3 % added Sucrose	Feel the same as no added sugar, not much difference between added and no added sugar	Taste smoother than no added sugar but still needs more sugar.

Comments: would buy this product in order to keep a variety of spirits in cupboard.

Table 7 Focus group interview results of Group 7

	Undiluted	Diluted
Pernod	Aniseed-like smell, medicine like	Don't like the cloudiness reaction became less strong in aniseed smell. Better taste towards the end.
Grapefruit distillate	Smell sweeter than added sugar, citrus smell. Would be nice if in grapefruit colour and bring out more citrus smell	Don't like the cloudiness reaction. Little bit of grapefruit taste but still get bitter taste.
Grapefruit distillate 3 % added Sucrose	Alcohol smell, very faint smell	Strong smells after diluted with water. Also stronger in taste, burning sensation in mouth, strong bitterness and smoother when added more water but less flavour

Table 8 Focus group interview results of Group 8

	Undiluted	Diluted
Pernod	Aniseed-flavour	
Grapefruit distillate	Fruity stronger, like more colouring	More flavour, like colour
Grapefruit distillate 3 % added Sucrose	Subtle	After diluted, gives more pleasant smell and taste. Like colour very much

Table 9 Focus group interview results of Group 9

	Undiluted	Diluted
Pernod	Like light ginger colour	Like the cloudiness
Grapefruit distillate	Fruit like smell	Like the flavour
Grapefruit distillate 3 % added Sucrose	Fruit like smell	Prefer taste rather than no added sugar

Table 10 Focus group interview results of Group 10

	Undiluted	Diluted
Pernod	Don't like smell, smell like medicine	Doesn't taste good
Grapefruit spirit no sugar	Would be nice if in brandy-coloured	Don't like the cloudiness, like the taste which is smoother than added sugar. Don't like the taste because of its gin taste
Grapefruit spirit added 3% Sugar	Like the smell (lemonish), stronger in lemon aroma	Desn't give a good appearance after diluted with water. Too strong taste and also stronger than no added sugar.

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