The Effect of Dispersion Mechanisms on Aroma Delivery

by

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ABSTRACT

Dispersion of aroma compounds in food matrices is a common process in the production of many food products. However, the degrees of dispersion on the distribution and subsequent release of these compounds during consumption may have considerable consequences for perception of these flavours.

This thesis investigates the effects of a range of dispersion techniques on the delivery and release of aroma compounds from several solid and semi-solid matrices which commonly contain added flavourings. Dispersion was achieved on three main scales ranging from molecular, through micro regions to physical separation or layering. The effects of different levels of mixing were assessed by measuring aroma release *in vitro* and *in vivo* via APcI-MS.

Having defined the stages of mixing, systems were developed to measure the influences of different dispersion techniques on aroma delivery. Layering to physically separate homogenous aroma-rich layers showed no significant effects on aroma release or perception from gelatine sucrose gels. It seems that mastication is very effective in re-mixing these systems and, *in vivo* there is no difference in aroma release. Although the degree of mixing could be controlled using a static mixer system for yoghurts, no effects of the different levels of mixing were observed on aroma release. Stirred yoghurts showed that mixing could influence equilibrium headspace concentrations but overall release *in vivo* was inconclusive. Sample selection in these systems may be important for influencing perception. Visual and textural cues may be more important for perception, in these mixing examples, than aroma release.

Using co-solvents as dispersion agents significantly increased the ease of dispersion of a range of aroma compounds. Static headspace analysis confirmed that all the carrier solvents influenced the partition of aroma compounds and *in vivo* release from model confectionery systems. Increasing solvent concentration

increased the solubility of a range of aroma compounds in the liquid phase. Release from gels was also influenced by the presence of solvents with hydrophobic aroma compounds showing patterns of release similar to those of hydrophilic compounds when dispersed using solvents.

Finally, this work studied release from phase separated systems where the aroma compound was present as a microdroplet. Release was very intense and rapid and was investigated in aqueous solutions and gelatine-sucrose gels. In this case the release mechanism was not the conventional air-liquid partition but was based on direct release from the air-liquid interface. Release was dependent on both hydrophobicity and vapour pressure.

1 INTRODUCTION

1.1 Flavourings and Aroma Delivery

1.1.1 What is Flavour?

Food recognition and acceptance is determined by the consumer's response to the different characteristics of the food product provided by the interaction of all of the senses. Foremost amongst these is the perception of flavour.

Flavour is a term used to describe the overall perception of a food that the consumer experiences during eating. It is a multimodal stimulus which involves contributory factors from aroma, taste, mouthfeel, texture and other tactile responses (British-Standards-Institute 1975). Aroma perception is dependent on detection of volatile compounds at the nasal receptors in the olfactory epithelium and subsequent olfactory signals sent to the brain (Fisher and Scott 1997; Taylor 1998). Flavour perception is not only dependent on aroma but, as the definition suggests, implies a combination of the entire input of the senses to produce an integrated response to the food. Taste perception is based on detection of non-volatile compounds by the tongue. Four main categories have been described in the literature; salt, bitter, sour and sweet. A fifth, umami (a savoury taste caused by monosodium glutamate (MSG) and monophosphate nucleotides) has also been described (Goldstein 1999). Texture, "mouthfeel" and trigeminal components of perception are detected by nerves in the oral cavity, which send signals to the brain related to the structure, temperature and irritation caused by food in the mouth (Green 1996).

Interaction of the senses is now recognised as a key aspect for overall flavour perception. Many studies have shown interactions of different sensory components on perception. Citric acid and sucrose have been shown to suppress

the 'burn' of capsaicin and piperine (Lawless and Stevens 1984). Tuorila *et al.* (1993), showed that sucrose enhanced perceived fattiness and fat enhanced sweetness in a yoghurt system, and Nahon *et al.* (1996), showed complex interactions between the interaction of sweetness and aroma in soft drinks.

Neither the taste nor the textural aspects of foods have been considered in this study and thus will not be discussed in any further depth. For more information on these aspects of perception the reader is directed to Laing and Jinks (1996). Subsequently any discussion of flavour will be referring solely to the aroma portion of the perceived response.

1.1.2 Aroma Release Mechanism

The actual process by which the aroma compounds are released so that they are available for perception is a complicated process and depends on a number of factors. These include the properties of aroma compounds and food matrix components. Aroma compounds are generally low molecular weight (<400) organic compounds that possess a range of physicochemical properties. Many of the properties will differ with the most important for foods being hydrophobicity (measured as Log P), solubility, vapour pressure (volatility), molecular weight and size (Taylor 1998). All of these will contribute to the dispersion of aroma molecules throughout a food and subsequently on how their release from that food. It has also been noted that some aroma molecules may also have taste functions with examples of these being vanillin and menthol.

The release mechanisms for volatile (aroma) compounds from foods in the mouth are well established (Taylor 2002). During mastication of solid foods, many changes take place. These include hydration and dilution with saliva, increases in surface area, and dissolution of non-volatile components (Taylor 1996). Aroma compounds present in food are released into the saliva phase. From there they are able to partition into the gas phase (Friel and Taylor 2001).

Subsequently, they are diluted and delivered to the olfactory pathways by the exhalation air stream (Buettner and Schieberle 2000b).

The mechanism of aroma release is dependent on a range of factors including; the type of matrix (Druaux and Voilley 1997), the nature of the aroma compound (Linforth *et al.* 1999c), the interactions between the aroma compound and matrix components (Solms 1986), mass transfer (Harrison and Hills 1997a; Marin *et al.* 2000), breathing and saliva flow rates (Piggott and Schaschke 2001) and other aspects of physiology (Buettner and Schieberle 2000b). These factors are all important in influencing the amount of aroma that reaches the olfactory receptors.

1.1.3 Aroma Perception Mechanism

Perception of aroma compounds is vital to the overall 'flavour' of a food product. For instance, individuals with anosmia (inability to detect nasal stimuli) or conditions that reduce nasal detection, such as a 'blocked nose', show reductions in their abilities to perceive flavours (Gudziol 1995). The initial stage of aroma perception begins when the consumer is able to smell the food. Aroma compounds, in the gas phase, are transported into the nasal cavity where they are detected by the receptors. This is known as the orthonasal route for aroma delivery. By contrast, when a food is eaten, the food is broken down and the components are released into the oral cavity as described in Section 1.1.2. The aroma compounds, once in the gas phase, will be transported to the back of the throat by mastication where they are subsequently carried in the airstream, from the mouth and throat, into the nasal cavity by the process of exhalation during breathing. This is known as the retronasal route of transport and is the process by which aroma compounds contribute to perception during eating (Linforth *et al.* 2002).

Detection by olfactory receptors occurs at the nasal epithelium. There are over 1000 different receptor proteins, with trans-membrane domains, present in the epithelium that are capable of detecting the wide range of different aroma compounds (Buck and Axel 1991). These receptors have relatively high specificities, but due to the similar nature of many of the aroma compounds, it is suggested that competition for binding sites may occur. Once an aroma compound has bound to a receptor, secondary messenger pathways are activated leading to depolarization of the receptor cell and generation of a nervous signal (action potential) (Breer 2001). These signals move via the olfactory bulb to higher brain centres where perception occurs.

The release of aroma compounds from foods has been show to be a dynamic process dependent on a range of activities described in Section 1.1.2. Subsequently delivery of aroma compounds will occur over a range of time periods depending on the nature of the food matrix and aroma compound. This results in perception of aroma compounds being significantly influenced by the timing and rate of release. Thus changes in timing can significantly affect the overall 'flavour' perceived.

1.1.4 'Flavours'

"Flavours" are not made up of single pure volatile compounds but are mixtures of a range of individual compounds. The balance of these components is vital to achieving the desired perceived response during eating. This response will be based on the concentrations of aroma delivered to the olfactory receptors rather than the initial levels present in the food. Changes in components of the food can lead to alterations in the levels of aroma compounds that reach the nasal epithelia and thus may disrupt the flavour perception. For example, removal of fat, a reservoir for many aroma compounds, can disrupt the pattern of aroma compound release and subsequent delivery to the nasal receptors may be altered (Brauss *et al.* 1999). Therefore, mechanisms and theories of aroma release are

fundamental to the understanding of the behaviour of aroma compounds and the influence they will impart on perception.

The flavour industry is an ever expanding industry with many new applications being developed every year. The Leatherhead Food Research Association estimated that the global market for flavours in 1998 was $4800m (£3000m) and was still increasing. The ability to produce balanced flavours is essential. There is an ever increasing demand for not only flavours that mimic natural products but also development of novel and interesting flavours. Variation in properties of food matrices mean that different flavours need to be developed for each of the systems to provide the desired response. An example would be the addition of flavour to a low fat yoghurt compared to a high fat one. Studies into these differences have shown that the profiles of release will differ significantly (Brauss *et al.* 1999) and the consumer acceptance is also altered (Tuorila *et al.* 1995).

Flavours are of paramount importance in the food industry and therefore it is no surprise that considerable investment is made each year by flavour companies. A Swiss flavour company, Givaudan, in 1999 invested SFr84m (£35m) on product research and development. Research in developing flavours is only one-side of the story. Knowledge on how the components of flavours will behave in different food systems can considerably enhance the development of new products, and significantly improve the efficiency of creating existing products.

1.2 Aroma Compound Release Theory

Aroma compounds are present in all foodstuffs however, the main features of interest are the mechanisms of release from their respective matrices. Static equilibrium release of aroma compounds forms the basis of the bulk of the historical research. These studies involved measuring the headspace concentrations above food samples to assess the partition of aroma compounds

between the sample and the gas phase above it (Nawar 1966; Hansch *et al.* 1968; Wientjes 1968; Buttery *et al.* 1971; Nawar 1971). Whilst important, this technique does not give a profile of the release of aroma compounds in dynamic situations. Subsequently, developments of systems to measure the effects of release in dynamic systems have been developed. These allowed the effects of changes in release over time to be measured. Most recently, due to the development of 'in nose' monitoring techniques (Taylor and Linforth 1996; 1999), *in vivo* release has been investigated and is able to give a better indication of changes in aroma release from human subjects. These techniques provide information on the effects of physiology on release. Correlations with sensory data can also be used to explain the effects of changes in release on perception.

1.2.1 Release in Static Systems

Static systems have been widely studied to assess the behaviour of aroma compounds at equilibrium. Static conditions imply that there are no outside influences on the sample and the sample will eventually reach equilibrium with its surroundings. Under these conditions aroma release from the sample is dependent on the partition coefficient (K). This describes the ratio of aroma compound partition between one phase and another. The most commonly described version of K is the K_{gl} which applies to the partition between a liquid and its surrounding gas phase. Equation 1.1 shows that the K_{gl} is defined by the concentration of aroma compounds detected in the gas phase (C_g) divided by the concentration remaining in the liquid phase (C_l). Most data are for K_{aw} where the gas is air and the liquid is water.

$$K_{gl} = C_g / C_l \qquad \textbf{Equation 1.1}$$

At equilibrium the partition coefficient represents the maximum concentration of aroma compound that will be present in the gas phase relative to the liquid phase.

Values of K_{aw} are mentioned extensively throughout the literature (Chaintreau *et al.* 1995) however, there is no definitive data set of K_{aw} values for all aroma compounds.

Correlated to the partition coefficient is the activity coefficient (Equation 1.2). This describes the activity of the molecules in solution and is dependent on the vapour pressure of a compound within a particular system. The saturated vapour pressure refers to the equilibrium pressure exerted by a substance in a closed system at a specified temperature (Perring 1999). Vapour pressure is directly related to the amount of a substance in the gas phase and can be used to calculate other thermodynamic parameters that can also be used to indicate the volatility of a compound.

$$\gamma_\infty = K_{aw} (P_t / P_i^*) \qquad \textbf{Equation 1.2}$$

where P_i^* is the saturated vapour pressure of a substance, *i,* at a given temperature, P_t is the total pressure in the system and γ_∞ is the infinite dilution activity coefficient

Aroma compounds, when present in aqueous systems are generally in a state of near ideal infinite dilution. In this system, Henry's Law applies, where the partial pressure of a solute above the solution is proportional to its molar concentration in the liquid (Buttery *et al.* 1971).

$$H = P_i^* \gamma_\infty \qquad \textbf{Equation 1.3}$$

Static release provides important data on the effects of different physicochemical properties on partition. Changes in partition coefficient can explain the fundamental chemical effects of matrix components on the potential for release. Friel *et al.*, (2000) used static release to study the effects of sucrose

concentration. Nawar (1971) showed the influences of sugars, glycerol and acids on partition. Buttery (1969) showed that the volatile concentration in the headspace, for a range of alkanals and 2-alkanones in a homologous series, increased as the carbon chain length increased. K_{aw} is dependent on the system used to measure the values. Calculated values, using modelling techniques based on physicochemical properties of the aroma compounds, have shown good correlations to the measured values (Marin *et al.* 1999). Determination of compound release from a food system will therefore depend on its physicochemical characteristics.

Whilst important for studying aroma behaviour, static release gives no indication of the complexities of release during eating of a food. Eating is a dynamic process involving multiple changes in sample environment and as such will be a non-equilibrium process. Static equilibrium situations are more likely to occur for orthonasal perception; for example, sniffing of a beverage. The profile of release is a key aspect, and will contribute significantly to the overall perception of a food. It is this temporal aspect that is so often overlooked, and is impossible (or at least very difficult, time-consuming and inaccurate) to monitor with traditional headspace measurement techniques. Therefore studies using dynamic systems need to be considered.

1.2.2 Dynamic Dilution Release

Studying aroma release from dynamic situations provides a better indication of the events happening in in-mouth conditions. In dynamic situations, equilibrium is disrupted by changes in either the gas or liquid phase such that the position of equilibrium is altered. The driving force for the change in release will be determined by the mass transfer of the aroma compounds across the air-water interface (Fick's Law). Fick's law states that "the net diffusion rate of a gas across a fluid membrane is proportional to the difference in partial pressure,

proportional to the area of the membrane and inversely proportional to the thickness of the membrane".

All systems, due to entropic considerations, will attempt to reach equilibrium between phases. Dynamic release will therefore be defined by kinetic factors which will in turn produce a drive towards a re-equilibration of the system (van Ruth *et al.* 2002a). Theories of mass transfer suggest that depletion of aroma from the gas phase will cause a subsequent movement of aroma from the liquid however, this level of partition will be dependent on the volatile nature of the aroma compound (Marin *et al.* 2000). This type of data can be analysed by traditional headspace techniques measured at a range of time points (van Ruth and Roozen 2000), or more rapidly via continuous on-line monitoring using APcI-MS (Marin *et al.* 2000; Harvey and Barra 2003). Data from dynamic release gives a better correlation to 'real' release during eating by careful control of external parameters. The commonly used model mouth systems generally measure dynamic release (Deibler *et al.* 2001; van Ruth *et al.* 2002a). However, the interactions that occur between human physiological characteristics and aroma release cannot be accounted for and hence true correlation with perception cannot be achieved.

1.2.3 *In vivo* Aroma Release

An even more complex situation is observed for aroma release in mouth. A range of other parameters will influence the partitioning and mass transfer of volatiles. Breathing produces a tidal air flow that delivers aroma from the mouth into the nasal regions and thereby depletes the gas phase concentration leading to a dynamic pattern of release. Other factors including mastication, hydration, swallowing, and saliva flow rates will also influence the degree of food breakdown and aroma release (Buettner and Schieberle 2000a; van Ruth and Roozen 2000; Buettner *et al.* 2002b; Hodgson *et al.* 2003b; Wright and Hills

2003; Wright *et al.* 2003; van Ruth and Buhr 2004). Methods to study aroma release *in vivo* are discussed later (Section 1.7)

The time-dependency of aroma release is another factor to be considered in aroma release. Direct mass spectrometry techniques allow quantitative measurement of the amount of aroma that passes to the nose during the course of consumption and can measure aroma release in 'real time' (Taylor and Linforth 2003). Analysis of release profiles produced allows the process of aroma release to be studied (Linforth *et al.* 1999b).

1.3 Models for Flavour Release

The mechanism of aroma release has been described as a dynamic process and as such will depend on a number of kinetic factors. These factors will be influenced by their surrounding environment. A number of models therefore exist in relation to aroma release depending on the matrix concerned. A variety of mathematical models are discussed in more detail in the following reviews (de Roos 2000; Linforth 2002; Harvey and Barra 2003; Wright and Hills 2003).

1.3.1 Mass Flux of Aroma Compounds

Transport of volatiles across a gas-liquid interface is described by the mass flux from the liquid to the gas phase (Figure 1.1) (Darling *et al.* 1986).
The flux of aroma to the liquid side of the interface is given by:

$$J_L = k_L (C_{BL}\text{-}C_{IL}) \qquad \textbf{Equation 1.4}$$

The flux away from the gas side of the interface is given by

$$J_G = k_G(C_{IG}\text{-}C_{BG}) \qquad \textbf{Equation 1.5}$$

Where J is the mass flux, k relates to the mass transfer coefficient, C represents the aroma concentration and L, B, G and I refer to the liquid, bulk, gas and interface respectively.

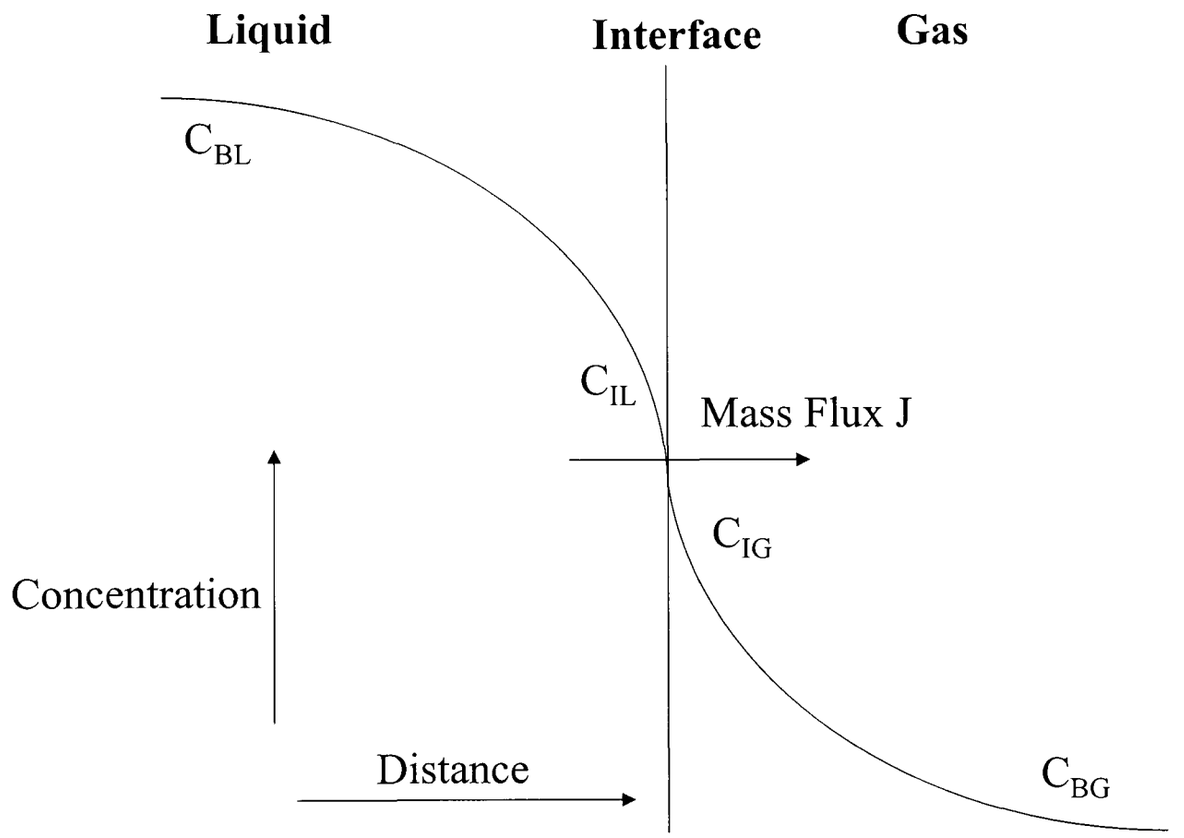


Figure 1.1 The transport of aroma from liquid to gas phase governed by the mass flux (J) (redrawn from (Darling *et al.* 1986)).

In most situations, aroma diffusion in the gas phase is extremely rapid. Therefore the gradient between the bulk and interfacial regions will be very small and can be ignored. Consequently, aroma concentration at the liquid interface (C_{IL}) determines the concentration in the bulk gas phase, producing the following equation

$$J_L = k_L [(C_G / k_{GL}) - C_L] \qquad \textbf{Equation 1.6}$$

Equation 1.6 describes the interfacial mass transfer across a gas-liquid interface and this is the primary factor controlling aroma release. This suggests that

volatility of the aroma compound and its resistance to mass transfer will determine the concentration gradient at the interface.

Mass flux is controlled by the rate of diffusion within the sample. Correlating mass flux to Fick's law of diffusion demonstrates the importance of the rate of diffusion on the rate of mass transfer. Hence, decreasing the rate of diffusion, leads to an increased concentration gradient between the phases and a higher level of movement into the gas phase. A slower rate of diffusion results in a slower rate of surface regeneration at the liquid interface, and thus greater aroma depletion.

1.3.2 Stagnant Layer Theory

The stagnant layer theory, proposes a simple mechanism for the release of aroma compounds. It is based on the assumption that the boundary layers between the two phases are stagnant and therefore mass transfer occurs via molecular diffusion (Hills and Harrison 1995). Diffusion can occur via two processes, either molecular or eddy diffusion. Molecular diffusion describes the simple, random movement of molecules within a system, whereas eddy diffusion is a convective process associated with turbulent movement of molecules.

Movement of aroma compounds into the gas phase causes a subsequent reduction in the concentration gradient within the liquid phase. This process leads to a lengthening of the concentration gradient requiring an increase in the distance aroma compounds will need to diffuse in order to reach the gas phase. Hence a reduction of aroma release is observed. In this case, the mass transfer coefficient will depend on the level of diffusion (diffusion coefficient) and the reciprocal of the thickness of the stagnant layers (Hills and Harrison 1995). These authors showed that this model applies well to foods where the surface layers are well defined and mixing of the interfacial layers is low. For example,

this mechanism is suggested to explain aroma release from a boiled sweet where dissolution of the matrix is the main step for aroma release.

1.3.3 Penetration Theory

A different theory is proposed for systems which do not have stagnant interfaces. The Penetration theory assumes that the interfaces are continually moving, with changing interfaces between the component phases (sample, saliva and gas). In contrast to the stagnant layer theory, eddy diffusion plays a significant role. This will allow aroma compounds from the sample to rapidly access the interfacial region where they will come into contact with the gas phase. Molecular diffusion then acts to transport the aroma compounds from this region into the gas phase. Due to the rapid nature of eddy diffusion it is thus a consequence that the rate of aroma release is limited by the transfer at the saliva-gas interface (Harrison and Hills 1996; Harrison and Hills 1997b; Harrison *et al.* 1997; Harrison *et al.* 1998; de Roos 2000; Linforth 2002).

Darling *et al.* (1986) modelled isopentyl acetate release from galactomamman and sucrose solutions into the headspace. Their model was based on the penetration theory of interfacial mass transfer. They concluded that the rate of interfacial surface regeneration was dependent upon several factors, including matrix composition and the concentration gradient within the phases, which was found to be dependent upon the extent of mixing of the system.

Release from emulsions has also been modelled using the penetration theory of mass transfer, assuming that resistance to mass transfer across the emulsion-air interface is the rate-limiting step for release (Harrison and Hills 1997a; Harrison *et al.* 1997).

1.3.4 Interfacial Mass Transfer

Marin *et al.* (1999) has shown that removal of interfacial stagnant layers via agitation of the sample leads to a homogenous distribution of aroma compounds in the liquid and gas phases. This leads to an instantaneous equilibration between the gas and liquid phases. They demonstrated that in a dynamic system (depletion of the gas phase) the release of the aroma compounds was determined by their partition coefficient if the K_{aw} was lower than 10^{-3}. For compounds with higher K_{aw} values, mass transport in the gas phase became significant and the Reynolds number also played a role.

This reinforces the importance of the aroma compound vapour pressures on the rates of aroma release from homogenous systems. It also demonstrates why saliva rates and breathing rates and volumes can have significant effects on aroma release and flavour perception. These effects will be especially apparent for compounds with higher volatilities.

1.3.5 Overview

Mass transport of aroma compound in liquid phase is therefore an important parameter for controlling aroma release. Diffusion rates play an important role in many of the theories and reduction of diffusion rates can significantly alter the rate of release. For instance, molecules that restrict aroma movement will reduce the rate of release. In homogenous systems under the influence of rapid mixing, the effect of diffusion will not be as key due to the steep concentration gradients maintained between the gas and liquid phases (Marin *et al.* 1999). In heterogenous matrices where the type of diffusion will have more of an influence the Penetration theory may be more applicable. Although there are many mathematical models for aroma release there are few instances where the models have been validated with experimental data.

Examples of systems where models have been validated include work by Harrison and Hills (1996) who demonstrated the relevance of their model for aroma release from gels by studying the *in vitro* release of food dye. They showed that the driving force for dye release was dependent on the melting point of the gels. Andriot *et al.* (2000) used a multi-faceted approach to show good correlation between in vitro headspace concentrations and sensory assessments with data obtained from the model of Harrison and Hills(1997b) for the binding of methylketones with β-lactoglobulin. These data suggest that the models will have some importance when studying aroma release from model systems.

1.4 Defining Dispersion Systems

Aroma release from many food systems has been studied extensively (Taylor and Linforth 1996; 1998), however the potential of different methods of dispersing and delivering aroma on flavour perception are not well understood. Release has mainly been studied from homogenous systems, due to difficulties involved with producing and defining inhomogeneous systems. However, most food products have some degree of inhomogenity and this will lead to partition of flavourings into different microenvironments depending on the nature of both the matrix and the aroma compounds (de Roos 2003). Aroma compounds will partition between different phases depending on their properties. Addition of a flavouring dissolved in vegetable oil to a hot sugar melt results in a biphasic system with oil on top of a sugar phase. In this system, phase partitioning between oil and sugar phase is much slower than the rate of volatilisation from the non-viscous oil phase. The consequence is that volatilisation occurs from the oil phase rather than from the total product phase (de Roos 2003). This will also be the case in low water systems such as boiled sweets. Schober and Peterson (2004a), suggest that aroma compounds come out of solution in these low water systems. Formation of "pocket-like cavities" into which the aroma compounds partition leads to implications for aroma release. These data suggest that the micro-environment of an aroma compound will be most influential in determining its release.

Addition of flavours to foods is a common process in the food industry and methods of dispersing the flavours are an important consideration. Many industrial processes seek to produce homogenous products whereby the ingredients (including the flavouring) are dispersed throughout. Mixing not only affects the volatile components of a food mixture but also influences the mixing of non-volatile components. Mixing may affect the structure which has consequences for product rheology. A balance must be maintained between dispersion of ingredients and desired rheological properties of the final products. Over-mixing of ingredients can lead to a reduction in the quality of the final product as well as a cost in financial terms due to increased time and energy requirements. Under-mixing can also lead to poor quality final products or unmixed regions of food components. This implies that the level of mixing needs to be controlled. Measurements of the degree of mixing are thus important for predicting, not only the dispersion of the aroma components, but also for determining the final structure of the food.

This research aims to look at the different degrees of dispersing and delivering of aroma compounds and the consequences that this may have for aroma release and perception. In this situation, the term dispersion is used to describe how homogenously mixed the aroma compounds are throughout a particular matrix. This means that the better the mixing, the more homogenous the sample will be. As foods are not homogenous, the aim is to investigate how the different methods of dispersing aroma compounds throughout food matrices affect the way in which these compounds are released and subsequently what the individual perceives.

In this study the definition of dispersion has been separated into three areas for ease of investigation although there is some overlap between each of the areas. These areas are:

Higher scale mixing via physical separation of aroma via layering and dispersion on a larger length scale. (Chapter 4)

Addition of aroma compounds in carrier solvents to disperse aroma throughout matrices (molecular scale) (Chapter 5).
Determination and production of phase separated regions of aroma compounds (droplets) (Chapter 6)

1.4.1 Scale of Scrutiny

The "Scale of Scrutiny" is a term used to describe how closely a mixture is scrutinized. This is best demonstrated by Figure 1.1. The square on the far left shows a completely mixed and homogenous sample. As the level of magnification is increased (moving from left to right) the pattern becomes more distinct and separate black and white regions appear. Further increases in magnification show more and more distinction between the regions as a smaller area of the mixture is assessed (Rielly 1997).

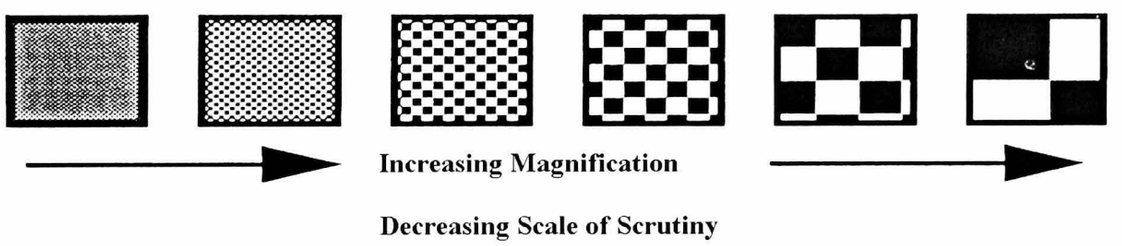


Figure 1.2 Schematic representing the effects of increasing sample magnification on the scale of scrutiny (as taken from (Rielly 1997)).

The scale of scrutiny is an important aspect of this thesis as it helps to explain the different levels of mixing that were investigated. In order to define the mixing of a system a statement about the scale of scrutiny must be made (Danckwerts 1952). This indicates that care must be taken when using the term homogenous as this will depend on the level of magnification being discussed. For example, at the highest level of magnification the atomic level, then no food system will be completely homogenous. Microemulsions are an example of a system which is

macroscopically homogenous, yet heterogeneous on a molecular scale (Yaghmur *et al.* 2002a).

1.4.2 Higher Scale Dispersions

At this level of mixing the important factor is the actual physical separation of aroma into homogenous macro-regions such that there may be large differences in aroma concentration between different regions of the matrix. Industrial processes generally tend to develop mixing systems which rapidly create homogenously mixed samples. However, this may not be the best arrangement for producing products with optimal release characteristics. For example, localised flavour, provided by delivery from encapsulants, has shown increased levels of release (Harvey and Barra 2003). Levels of mixing can be measured using chemical engineering principles related to flow rates, pipe dimensions and types of mixing blade. These can be applied to aroma release profiles to ascertain an idea of the level of mixing on dispersion and release.

This category of dispersion mechanisms involves a range of mechanical or physical techniques to produce these samples. This is the equivalent of an intermediate scale of scrutiny (see earlier). A range of techniques have been investigated during the course of this research to produce mixing on this scale. Layering, stirring and static mixing have all been used to create a range of differently mixed products.

1.4.3 In-line Static Mixer System

Static mixers, as the name suggests, do not have moving parts to facilitate the mixing process. Instead they consist of a varying number of specially designed mixing elements within a length of tubing. Industrial processes generally involve the pumping of fluids through the mixing elements. Increasing the number of

mixing elements increases the level of mixing that occurs. In the laminar regime, each mixer element reduces the striation thickness (scale of segregation) thereby producing a more homogenous sample. Equation 1.7 shows the number of mixer elements required to reduce the striation thickness. This applies to a static mixer which has 2 alternate paths at each element.

$$\delta_o/\delta = 1/2^M \qquad \textbf{Equation 1.7}$$

where δ_o is the original striation thickness, δ is the final striation thickness and M is the number of mixing elements.

Static mixers are highly efficient for blending fluids, as doubling the number of elements, squares the amount of mixing that occurs (as measured by a decrease in striation thickness) (Godfrey 1992; Rielly 1997).

Important uses of these types of mixers are for mixing viscous materials often with varying properties (Strieff 1979), and rapid liquid-liquid dispersions. They are commonly used on a large scale in sewage treatment plants. Static mixers have been used to homogenously mix fruit purees into yoghurt (Maiocchi 2003) and to ensure thorough mixing of chemicals into drinking water (Ortiz and Ducoste 2004). Several studies have been conducted on the efficiency of mixing using static mixers and their ability to produce homogenous mixtures (Visser *et al.* 1999; Fang and Lee 2001; van der Hoeven *et al.* 2001).

The main differences between static mixers and other types of mixer are the stationary nature of the elements and the ability to run the systems as a continuous process. This is ideal for processes such as sewage treatment which was mentioned earlier. The importance of using a static mixer system for this study was that the mixing intensity can be readily altered simply by using varying numbers of mixing elements. For further information on static mixers see Godfrey (1992).

Principles of mass transfer will again apply to the release of flavour from these types of products although the interesting factors may be more in the initial release of flavour as subsequent mastication and mixing in mouth of the samples may provide the same environment as non-layered products (Prinz 1999). However, chewing has shown an increase in the surface area exposed to the gas phase thereby increasing volatile release (van Ruth and Roozen 2000).

1.4.4 Carrier Solvents

Carrier solvents are defined as "food additives used to dissolve, dilute, disperse or otherwise physically modify a food additive without altering its technological function in order to facilitate its handling, application or use" (Preston 1998). Carrier solvents are used in numerous food and food-related products in order to provide a number of functions. These include dissolution of aroma compounds, dispersion in food matrices, humectants, flavouring itself (especially in the case of ethanol), improving flavour handling and acting as a preservative. They can also be used as dilution agents as aroma compounds are generally too concentrated to be used 'neat' (Wright 2002). For this research, the key characteristics are the effects of carrier solvents on flavour addition and dispersion, with special consideration of solvent ability to increase flavour solubility within food matrices.

Different carrier solvents are required due to the wide range of solubilities observed for different aroma compounds. They can be used to dissolve oil soluble compounds in hydrophilic systems or alternatively water-soluble compounds in oil-rich environments. Most volatile compounds have low solubilities in water due to their hydrophobic nature (Gunning *et al.* 1999) and this will affect where they will partition in foods. From this definition, it is easy to see that many substances fulfil these criteria. However, the number that are permitted for use in foods is relatively low.

Owing to their status as food additives, very few compounds are acceptable as carriers and those that are, have significant restrictions upon their usage. Approved carriers include ethanol, propylene glycol, glycerol and triacetin (Sharma *et al.* 1998; Wright 2002). There is also concern over the uses of solvents with some being unsuitable for a number of applications for either practical or ethical reasons (e.g. ethanol is prohibited in Kosher foods) (Preston 1998).

Although of commercial importance, there is very little published data on the effects of carrier solvents on dissolution and release of aroma compounds in food systems. Recently Schober *et al.* (2004b; 2004a) investigated the effects of propylene glycol, miglyol and 1,8-cineole on the release of L-menthol from a boiled sweet matrix. They showed that differences relating to the hydrophobicity of the solvents could affect the release of L-menthol, with hydrophilic solvents having a two-fold increase in the intensity of release. Numerous other authors have investigated confectionery style systems in relation to aroma release and perception but none have studied the influence of the carrier solvents (Hills and Harrison 1995; Ingham *et al.* 1995b; Harrison and Hills 1996; Harvey 1999; Linforth *et al.* 1999b; Ong *et al.* 1999; Linforth *et al.* 2001; Lubbers and Guichard 2003).

Alcoholic beverages, due to their commercial importance, have been widely investigated (Voilley *et al.* 1991; Conner *et al.* 1994; Conner *et al.* 1998; Withers *et al.* 1998; Escalona *et al.* 1999; Ebeler 2001). Ethanol is present in a range of concentrations in these systems although generally in much higher concentrations than carrier solvent levels. This can give insights into the importance of ethanol on aroma compound solubility and release. Connor *et al.* (1998) showed that higher ethanol concentrations affected headspace partitioning and others have shown that the presence of ethanol can affect the perception (Fischer *et al.* 1994; Perpete and Collin 2000). Co-solvents are generally much more widely studied in pharmaceutical systems using a range of models to

predict the effects of altering solvent concentrations (Gould *et al.* 1984; Yalkowsky and Rubino 1985; Rubino and Yalkowsky 1987; Banerjee and Yalkowsky 1988; Pinal *et al.* 1990; Li and Yalkowsky 1998; Jouyban-Gharamaleki *et al.* 1999; Li *et al.* 1999; Yalkowsky 1999b).

As volatile aroma compounds are, in the main, hydrophobic compounds, they have low solubilities in water. The hydrophobicity of a compound can be estimated by its octanol-water partition coefficient (Log P) which gives a value of how the volatile will partition in a mixed system of octanol and water (Taylor 1998). As most foods contain at least some water, it is important that these compounds can be dispersed and diluted in foods.

Carrier solvents provide both of the above functions by reducing the actual concentration of the aroma compounds by dilution and by increasing solubility of aroma compounds in the appropriate matrix. It is therefore important to assess the behaviour of these solvents on aroma release and perception as well as their applicability to certain systems.

1.4.5 Phase Separated and Heterogenous Dispersions

It has been observed that a burst of flavour may be important for stimulating the optimum perceptual response to an aroma (Blake 2002) with numerous studies suggesting a continued release of aroma can lead to adaptation (Davidson *et al.* 1999; Hollowood *et al.* 2000; Breslin 2001) and a subsequent reduction in perception. It has also been shown that taste-aroma interactions also play an important part in perception with numerous studies showing a decline in sugar leading to reduced perception even in the presence of a continued level of aroma (Davidson *et al.* 1999).

This section discusses the areas of research based on the localisation of aroma at high concentrations, such as phase separations or droplets. Obviously there is

some overlap in this area with both the higher and lower ends of the mixing scale but due to the importance of this area it warrants investigation under its own title. It has been noted within our lab that droplets of aroma compounds are subject to migration in liquid systems and upon interaction with the gas-liquid interface are subject to volatilisation rather than partition (Linforth 2004, Personal Communication).

In many systems, the levels of moisture within the system may be low or the compatibility of the aroma compounds with the matrix may be reduced to such a level that concentrated regions of aroma may form. This may especially be the case in samples such as dried products or hard candies. In these systems, the low water content may cause insolubilities of aroma compounds and these may form regions within the matrix structure where they are concentrated (de Roos 2003; Schober and Peterson 2004a). Insoluble regions within a matrix, such as pulp in an orange juice, may preferentially contain aroma compounds. These regions may produce concentrated aroma regions that may increase the levels of these compounds during consumption, subsequently influencing the perception (Rega *et al.* 2002).

Encapsulating flavours is a popular way of trapping aroma within a processed food to prevent loss or chemical change. Data from studies on encapsulants has shown that they can produce higher aroma release intensities during the early consumption of chewing gum than when the aroma was solubilised in the gum base (Harvey and Barra 2003). Studying the behaviour of concentrated aroma regions may provide important information on the links with release from encapsulated aromas.

1.5 Solubility, Solvents and Solute Effects

1.5.1 Principles of Solubility

Generally solutions can be described as homogenous liquid phases consisting of more than one substance, where one of the substances will be denoted the solvent and the others solutes. The earliest investigations into studying the solubility of solutes produced the well known chemical rule "like dissolves like". In many cases, the presence of similar functional groups is sufficient to produce this effect; however this conclusion has limited applicability with numerous exceptions for chemically dissimilar compounds. In fact, insolubility can be observed in spite of similar functional groups, e.g. polyvinyl alcohol in ethanol (Reichardt 1979).

It is therefore, the interaction between the solvent and solute molecules that will determine the mutual solubility. The main properties of a solution can be described in terms of the interactions between the solvent and the solutes. A solute will dissolve in a solvent if the solute-solvent intermolecular forces are energetically more favourable than those of the solute-solute or solvent-solvent. Intermolecular forces in solution are responsible for the attraction or repulsion between molecules and are much weaker than chemical bonds. The important intermolecular forces which determine solubility behaviour are detailed below:

- ion-dipole interactions are exhibited when an ion is solvated in solution by a polar solvent and explains the solubility of salts in water and other polar solvents but not in non-polar solvents;

- van der Waals forces are a collective group of attractive forces;

dipole-dipole;

dipole-induced dipole;

dispersion forces (induced dipole-induced dipole);
hydrogen bonding;

- Hydrophobic interactions (repulsive interaction between a polar molecule and a non-polar molecule but an attractive interaction between two non-polar molecules so as to exclude polar molecules coming in between the molecules).

These interactions, which control the solubility and partitioning behaviour of solutes in a range of media are discussed further in the following texts (Reichardt 1979; Grant and Higuchi 1990; Atkins 1994; Yalkowsky 1999b; Franks 2000). It is clear that at infinite dilution any solute-solute interactions can be ignored.

1.5.2 Solvation in Water

Water is the most common solvent in food systems with almost all food related products containing some available water molecules. The solvation of a hydrophobic solute by water can superficially be described by the hydrophobic effect. The hydrophobic effect can be defined as the disaffinity of oil with water, and describes the insoluble nature of oil in water (Southall *et al.* 2002). The hydrophobic effect is not discussed in depth here and the reader is directed to the following references for more information (Sharp and Madan 1997; Dill *et al.* 2002; Southall *et al.* 2002; Urbic *et al.* 2002).

The common explanation for a solute to dissolve in water is described. For a solute to become dissolved in water, a number of stages need to occur. First the H-bonding network has to be disrupted in order to form a cavity within the water structure. This cavity must be able to accommodate the solute. Subsequently, dispersion will then occur to enable the compounds to gain access to the cavities and these will be applicable to all solutes. Finally, interactions between similar regions will occur and final solubility will be dependent on these solute-solvent interactions. However, this mechanism is questioned by Ruelle and Kesselring

(1998a). They suggest that the H-bonding structure of water is not a static structure and these bonds are being rapidly broken and reformed between other donor and acceptor groups. This implies that the H-bonded structure of water is mobile. Because of this, 1% of the time the solute will not be in solution and this may result in effects on partition behaviour.

Aroma compounds are generally hydrophobic which means that their solubilities in water tend to be low. This is a generalisation as many compounds do have high aqueous solubilities (methyl acetate, ethanol), however, it is the more hydrophobic compounds that are of most interest in this study. The hydrophobic nature of a compound is commonly indicated by the Log K_{ow} (Log P) value. This represents the degree of partition of aroma compounds between a water phase and an associated immiscible oil phase (normally octanol).

1.5.3 Effects of Co-solvents (carrier solvents)

Carrier solvent is the term commonly used to describe those solvents that are used to deliver aroma compounds to foods and flavourings. However, the properties they demonstrate are actually those of co-solvency and strictly speaking they should be called co-solvents. This is a better term to use when describing the effects that they have on compound solubility in aqueous systems.

Co-solvents are generally organic molecules that are, for the most part, miscible with water. They are mainly liquids but can also include a range of solids dissolved into water, e.g. sugars. The main principle is that they can facilitate the solubilisation of non-polar solutes into an aqueous solution. This is achieved by a reduction in the interactions between water molecules and thus allowing more access for solutes to be solubilised in the aqueous phase or a reduced ability of water to "squeeze out" nonpolar solutes (Yalkowsky 1999b). The polarity of solute can be determined by comparison to that of the co-solvent; therefore nonpolar solutes will be less polar than the co-solvent and so on.

The effects of co-solvents on the release of aroma compounds from food matrices have not been investigated to any great extent. Most of the previous work has been carried out in predicting the effects of altering co-solvent composition for the solubility and release of pharmaceutical products (Section 1.4.4) Recently some research was carried out by Schober and Peterson (2004b), on boiled sweets which suggested that the hydrophobicity of the carrier solvent could affect the aroma release. They showed that a hydrophilic solvent (propylene glycol) had a higher level of release compared to a hydrophobic solvent (1,8 cineole) for the release of the hydrophobic compound L-menthol.

Ethanol is a well-used co-solvent due to its high solubility for non-polar solutes as well as its miscibility with water. Yalkowsky (1999b) suggests that ethanol is twice as powerful a co-solvent as propylene glycol and four times more powerful than glycerol. Ethanol in food systems however, can cause some complexities as it can be present as either a solvent, solute or as an aroma compound. Much of the work done with ethanol concerns its use in alcoholic beverages and the work on these is discussed elsewhere (Section 1.4.4).

As carrier solvents are commonly used in flavouring preparations and other food stuffs it is of interest to investigate the influences that the type of solvent used may be having on the overall behaviour of the aroma compounds. These effects are not limited to the solubilisation and dispersion of aromas within a matrix but may have an impact on the levels and rates of release.

1.5.4 Effects of Sugars

The effect of sugars on the partitioning behaviour of volatiles has been studied extensively. The initial studies were made by Nawar (1966) who used gas chromatography to investigate the effects of glucose on the headspace concentration of food volatiles. Further studies showed that a wide range of

effects could be seen depending on the sugar and aroma compound investigated. Wientjes (1968) showed an increase in the partial vapour pressure for some strawberry volatiles and a decrease for others when invert sugar and fructose were added. Sugars may affect the release of volatiles, through binding and the 'salting-out effect' (relative changes in the partition coefficient due to changes in solute concentration in the solution). Typically, the addition of salt or acid to a system will enhance the headspace concentration of polar volatiles (Nawar, 1971). This work showed that increasing sucrose concentration increased the concentration of acetone but correspondingly reduced the concentrations of heptanone and heptanal. The same authors also showed that at 60% sucrose, different modes of aroma addition affected the headspace concentration and they suggested that this would be due to interactions between sucrose and water molecules. Glycerol 50% and gelatine 10% also reduced the headspace volatile concentrations for all the volatiles. Since these first studies, many more investigations have been carried out showing both increases and decreases of volatile headspace concentrations for a range of sugars and aroma compounds (Voilley *et al.* 1977; Kieckbusch and King 1979b; Nahon *et al.* 1996; Nahon *et al.* 1998a; Nahon *et al.* 1998b; Nahon *et al.* 2000) .

Voilley *et al.* (1977) observed an increase in headspace concentration of acetone and 1-octanol when increasing concentrations of sugars were added to an aqueous solution. They explained these changes on the basis of mole fraction and activity coefficient. In some cases, changes in the mole fraction and activity coefficient (with increasing solute concentration) effectively cancel each other out and there is no change in headspace concentration. Decreases and increases in headspace can also be explained by these mechanisms.

Friel and co-workers (2000) showed increases and decreases in headspace concentration for 40 volatile compounds in 65% sucrose solutions. They used QSPR (Quantitative Structure-Property Relationship) modelling to predict the effects of volatile partitioning and found that 3 physicochemical descriptors provided a good explanation of the data ($(\text{Log P})^2$, LUMO energies and a

connectivity term). These authors illustrated the importance of the aroma compound physicochemical properties on their partition coefficients from high concentration sucrose solutions. Later, Hansson *et al.* (2001a) showed that increasing the sugar concentration from 0-60% significantly increased the headspace concentrations of five volatile compounds but the effects for glucose syrup were less pronounced. They suggested that the reduced effects of glucose syrup were due to the higher concentration of free water compared to sucrose. Changes in a formulation that affect sugar content may therefore have profound effects on the release of the volatile aroma compounds in that food.

Sugars present in solution will disrupt the rigid water structure and subsequently moderately increase the hydrophobic nature of the solution. This will have implications for the solubility and subsequently the activity and partition coefficients of aroma compounds (Yalkowsky 1999b). Hydrogen bonding of water with equatorial hydroxyl groups could result in the formation of hydrophobic regions in which aroma compounds may become entrapped (Franks 1983).

In commercial terms, sugars are a key part of the flavour response observed by consumers. They influence perception through taste effects and through aroma effects related to the "salting in" and "salting out" of aroma compounds. This may disrupt the balance of flavours and so research into predicting the effects on different aroma compounds is imperative. The influence of differing amounts of carrier solvents on this sugar-aroma interaction may also provide valuable information on partitioning in real systems.

1.6 Effects of Food Matrices

Dispersion of aroma compounds is dependent on the types of food matrices investigated. Foods are generally made up of a range of numerous components including carbohydrates, lipids and proteins. These components will have

significant effects not only on the partitioning, but also on the release behaviour of aroma compounds. Effects may occur due to; reduced diffusion due to increased viscosity, increased binding to sites on components, or changes in the solubility of the aroma compounds.

Viscosity is generally increased by the presence of macromolecular components or a reduction in the liquid phase of a system. Many components are actually added to enhance the viscosity in many food systems. The effects of these mechanisms will be to reduce the levels of diffusion and mass transfer of aroma compounds (Yven *et al.* 1998).

Binding of aroma compounds can occur for both proteins (Landy *et al.* 1995; Lubbers *et al.* 1998; Burova *et al.* 2003) and carbohydrates such as hydrocolloids (Roberts *et al.* 1996; Yven *et al.* 1998) and starch (Solms 1986; Langourieux and Crouzet 1994; Boutboul *et al.* 2000). This may occur to differing degrees, from weak adsorbtion, due to Van der Waals forces, to specific chemical binding (covalent bonds).

Proteins are capable of significant aroma binding, and their presence in a food lessens the effective free concentration available for release and hence can significantly alter the overall flavour available for perception (Overbosch *et al.* 1991). This binding is dependent on the physicochemical conditions of the system (e.g., pH, temperature, ionic strength, water content) which will determine the state of the protein, and hence the groups available for aroma binding. The form of the protein (denatured or natural) will influence the degree and type of aroma binding that may occur. It is likely in many food systems that the proteins will be denatured (by heating processes) and therefore studies on denatured proteins may give better indications of aroma binding.

Fats can affect the solubility and partitioning behaviour of aroma compounds. Fat is an important flavour solvent. Aroma compounds will differ in their fat solubility and therefore the effects of the fat will be mainly dependent on the compound

hydrophobicity. Hydrophilic compounds will be unaffected by the presence of fat. Therefore the presence of fats will have differential impacts on aroma compounds release depending on their fat solubility. Buttery, Guadagni and Ling (1973) showed that the gas-emulsion partition coefficient decreased with increasing chain length. Delahunty *et al.* (1996) showed that hydrophobic volatiles will preferentially partition into the fat phase in cheese, and that this may slow down their volatilization during eating. Therefore, by changing the fat content of foods, one would expect changes in volatile composition, volatile intensity and rate of release (Plug and Haring 1993). Harrison and Hills (1997) modelled aroma release from emulsions and their suggestion that the emulsion-gas interface is the rate-limiting step for aroma release supports the previous findings. De Roos (2003) reported that volatilisation of aroma compounds was lower from vegetable oil than from a sugar slurry. These systems suggest that the alteration of fat contents will play a considerable part on the release of hydrophobic aroma compounds. These changes will need to be considered when systems containing fat are investigated.

Overall, aroma compounds possess a range of functional groups and individual compounds will be affected by the food matrix to differing degrees, this may alter the balance of a flavour as well as causing a decrease in the overall intensity. The use of model systems will therefore provide a good indication of the basics of different types of mixing and the effects on release and will help to minimise the effects of non-volatile components. It is also the aim to take these principles and re-apply them to real food products.

1.6.1 Xanthan Solutions

Xanthan is a commonly used thickener, produced by a bacterium, *Xanthomonas campestris*. It is completely soluble in hot or cold water, and provides very high viscosity solutions at low concentrations. It is commonly used to stabilize aqueous dispersions, suspensions or emulsions in products such as salad dressings (Odake *et al.* 1998) by way of increasing the viscosity of the sample.

The effects of hydrocolloids on aroma release and perception are complex. Sensory studies generally agree that the perceived flavour intensity decreases as the viscosity increases (Hollowood *et al.* 2002; Cook *et al.* 2003b). The effect is not seen at concentrations below c* (the biopolymer concentration when the chains begin to entangle) but above c* there is a clear decrease in flavour intensity. Because of this observation, it has been hypothesised that the increased viscosity hinders mass transport of aroma compounds to the gas phase. *In vitro* studies using dynamic headspace analysis can show differences for aqueous and viscous solutions (Rankin and Bodyfelt 1996; Bylaite *et al.* 2005). Bylaite *et al.* (2005) suggest that xanthan concentration and the nature and physicochemical properties of aroma compound, rather than viscosity, play a major role affecting aroma release *in vitro*. However, the situation *in vivo* is more complex. Using four different hydrocolloids, Cook *et al.* (2003b) found the well-documented decrease in sensory perception but did not find a corresponding decrease in aroma release measured *in vivo*. Weel *et al.* (2002) reported similar results for sensory perception of whey protein gels. These discrepancies between *in vitro* and *in vivo* results suggest that different processes are influencing the release of aroma compounds with concentrations of thickener and nature of aroma compounds being more relevant than the actual changes in viscosity of the system.

1.6.2 Boiled Sweets

Boiled sweets (or hard candies) are one of the most definitive solid model systems. The simplicity of the matrix combined with the well established mode of breakdown makes it a useful model. Hills and Harrison (1995) based their stagnant layer theory of release on the effects observed for the release of a food dye during the sample degradation. In this situation, aroma release occurs as the sugars and volatiles of the sweet gradually dissolve into the surrounding saliva phase. The aroma compounds diffuse to the gas-saliva interface and partition into

the headspace providing a rate of release dependent on the dissolution of the sugar-glass matrix.

Recently other authors have investigated the behaviour of aroma compounds in boiled sweets. De Roos (2003) showed that if aroma was added in vegetable oil, which formed a biphasic system with the sugar mixture, then lower retention was observed due to volatilisation from the oil phase. Following on from this, Schober and Peterson (2004b; 2004a) investigated the effects of compound-compound and compound-solvent interactions from boiled sweets. They reported that incompatibilities between aroma compounds and the matrix may form "pockets" where the aroma compounds will reside rather than dispersion throughout the matrix.

1.6.3 Gelatine-Sucrose Gels

Gelatine is a high molecular weight polypeptide, produced by the thermal degradation of collagen, and obtained from the treatment of animal bones and skin. It comes in two forms depending on the type of treatment; an acidic treatment (Type A) or an alkaline treatment Type B. The alkaline treatment, converts asparagine and glutamine residues to their respective acids and results in a higher viscosity.

Gelatine consists of a large number of glycine, proline and 4-hydroxyproline residues. In brief, the protein is made up of peptide triplets, glycine - X - Y, where X and Y can be any one of the amino acids but proline has a preference for the X position and hydroxyproline the Y position (Bailey and Light 1989). Gelatine is primarily used as a gelling agent forming transparent, elastic, thermoreversible gels on cooling below about 35°C (depending on the bloom strength), which dissolve at low temperature to give 'melt in the mouth' products with useful flavour release. For further information on gelatine the reader is referred to the following review (Poppe 1992).

It has been suggested that aroma release from gelatine-sucrose gels occurs via dissolution of the matrix and subsequent partition of the aroma compounds into the saliva. The driving force for flavour release from gelatine-sucrose gels is dependent on the melting temperature of the gels and the sucrose concentrations which will affect the rate of melting and release. Increased sucrose concentrations will increase the melting temperature of the gel such that if the sucrose content exceeds 40% the melting temperature will be higher than that of the mouth. Aroma release will therefore depend on the rate of diffusion of sucrose out of the gel sample. This will lower the gel melting point of the gel and allow aroma release to occur (Harrison and Hills 1996). These authors based their model on the penetration theory of interfacial mass transfer mentioned previously. Oakenfull and Scott (2003) found that 50% sucrose increased the melting temperature of a gelatine-sucrose gel by 3.9°C.

Several studies have used gelatine-sucrose gels to study aroma release. Baek *et al.*, (1999) showed that the different rates of release observed for different gelatine concentrations were not due to binding of volatile to protein in the gel, nor to mucous membranes, but were due to different rates of gel breakdown in-mouth. Linforth *et al.* (2000) studied release of 27 different aroma compounds from gelatine-sucrose gels. They then used QSPR to identify the physicochemical parameters of aroma compounds that governed the release from gelatine-sucrose gels. Hydrophobicity, volatility and Hartree Energy were the key factors. Hollowood *et al.* (2000) showed that quantity of volatile delivered to the nose from gelatine gels (6%) was directly proportional to the concentration in the sample. Furthermore, the relationship between perceived intensity and sample concentration was linear for the sensory data.

The melting of the gels was a crucial aspect of the release mechanism for this thesis. In our studies, the gels were made with high sugar content and thus aroma release would be dependent on the dissolution and diffusion of sucrose prior to melting of the gel samples. Another important factor was the ability to produce

layered samples which was key to some of the experiments discussed in Chapter 4.

1.6.4 Chewing Gum

Chewing gum is a two-phase system consisting of a water insoluble gum base and a water soluble sugar phase. Due to the hydrophobic nature of the gum base the aroma compounds will be preferentially soluble in this phase. It is a simple, semi-solid matrix, which can be chewed for a long period of time in a semi-controlled fashion (Ovejero-Lopez *et al.* 2004). This contrasts to the gelatine-sucrose gel matrix which is rapidly broken down during the mastication period. The lack of change in bolus length described by Prinz (2000), means that it provides the ideal comparison, for layering, to the gels, which will have vastly altered surface areas.

Other studies on chewing gum have correlated flavour perception with release of sugar rather than menthone (Davidson *et al.* 1999). However, this effect is disputed by Ovejero-Lopez *et al.* (2004) whose results suggest that there is no dramatic decrease in sweetness perception over the mastication period. These authors however, used the intense sweetener sorbitol rather than sucrose which may have explained the differences observed as sorbitol will have a high sweetness intensity and may show lower levels of depletion over time compared to sucrose. Niederer *et al.* (2003), have suggested that incompatibilities between chewing gum base and flavour molecules can lead to the incorporation of the flavour molecules as droplets. This may have implications for the release patterns observed and is also an important consideration for the section on concentrated aroma regions (Chapter 6).

1.6.5 Yoghurt

Yoghurt consumption has increased in recent times and subsequently research into the aroma release from yoghurts has also increased. Yoghurts have numerous aroma compounds present due to the process of milk fermentation (Ott *et al.* 1999). However, these are only present at low quantities and in many cases extra aroma compounds are added to the plain yoghurt base. Flavoured yoghurts contain flavour preparations and a number of fruit based yoghurts also contain pieces of solid fruit. Mixing of these components can occur in the industrial process, however, a common theme nowadays is to have a separate section included with the plain yoghurt. This will contain additional "flavourings" in the form of fruit compotes, chocolate pieces or other mixtures. Fruit purees have been described as being mixed into yoghurts by static mixer systems (Navratil *et al.* 2004).

Yoghurt is a relatively consistent product with a range of fat levels. Hence it lends itself to these types of experiment as a 'real' foodstuff. Yoghurt consists of a complex emulsion of fats and proteins in water, with other components (sugars, acids, etc.) making up a considerable proportion of the formulation (Brauss *et al.* 1999). The fat in yoghurt will act as a solvent for flavour compounds, and the alteration of the fat content of foods will change the rate and concentration at which food flavour molecules are volatilised during consumption. The other components of yoghurt may affect the binding (proteins, carbohydrates) and partitioning (sugars, acids) of aroma compounds in the yoghurt.

Aroma release and mixing behaviour on yoghurts has been studied previously but a combination of the two has yet to be investigated. Brauss *et al.* (1999) showed that increasing the fat content of yoghurt from 0 - 10% had a similar maximum intensity of release for anethole. However, increasing the fat content increased the time to reach this intensity and increased the levels of persistence observed. Kant *et al.* (2004) investigated the effects of β-cyclodextrin. When β-

cyclodextrin was added to fat free yogurt, the release of a commercial lemon flavouring was modified and produced a level of aroma release similar to that from a regular fat yoghurt in that it allowed a better concentration of flavour to be retained in the yoghurt than a low fat yoghurt. Mei *et al.* (2004) used PTR-MS to study thickener effects on aroma release from yoghurt. They found that sweeteners significantly reduced the aroma release but the other thickeners had no effect. These studies show the applicability of using APcI-MS for measuring aroma release from yoghurts and illustrated the importance of the components of the yoghurt on aroma release.

Decourcelle *et al.* (2004) concluded from studying aroma release from stirred yoghurts, that the nature of ingredients in added fruit preparations explained the effects on the aroma release rather than any differences in rheology. This final reference suggests that in a stirred yoghurt system the changes in rheology produced by stirring would have negligible effects on the release of aroma compounds.

1.6.6 Emulsion Systems

As yoghurt is regarded as an emulsion it is important to include a small section on the behaviour of emulsions. The oil phase itself can have effects on the partitioning of aroma compounds with more hydrophobic compounds showing the largest effects (Carey *et al.* 2002; van Ruth *et al.* 2002b; Relkin *et al.* 2004). In multi-phase systems such as emulsions, the release will be determined by the partitioning of the aroma compounds between the different phases. The oil phase is not the only important component of the yoghurt that may influence aroma release. The emulsifiers present may also have an effect due to specific binding or adsorption at the oil-water interface. Harvey *et al.* (1995), by means of the 'rotative diffusion cell', showed that as the concentration of the emulsifier sodium caseinate increased from 0 to 0.5%, the liquid-liquid partition coefficient of ethyl butanoate decreased by 50%. The reduced diffusion rate between layers

of water and tributyrin caused by the presence of sodium caseinate was explained by an increase in the interfacial resistance due to the presence of an adsorbed layer of protein at the interface. Aroma compounds will not all behave in the same way. Variations in partition will be dependent on the physicochemical properties of the compound.

1.7 Methods of Measuring Aroma Release

Measurement of aroma release is not a simple process due to the nature of food systems and the interactions between components. The inability of machines to successfully reproduce sensitivities close to that of human olfaction is also another stumbling block to the analysis of aromas. This may mean that although aroma compounds can be detected by human panellists the subsequent measurement techniques are not sensitive enough to be able to measure this.

Static equilibrium concentrations have been studied for many years and are based on the principles described in Section 1.2.1. Since the late 1960's (Nawar 1966; Wientjes 1968; Buttery *et al.* 1969), considerable numbers of studies have focussed on the measurement of volatile compounds above solutions (headspace analysis). Originally the technique involved direct injection of a quantity of the gas phase into a GC-MS. Subsequently, a number of other systems were developed to trap aroma compounds from the headspace prior to transfer to the GC-MS. These included Solid Phase Micro-Extraction (SPME) fibres (Marsili 1999; Widder *et al.* 1999) and Tenax trapping (dynamic systems only) (Karahadian and Johnson 1993; Buttery and Ling 1995). These methods however, are time-consuming and are unable to measure aroma release from human subjects in real time.

Quantification of volatile release during consumption presents a different range of problems. Mackay (1978) was one of the first researchers to measure the volatile release from panellists. They collected the expired air and injected it into

a GC-MS. Later investigations used more sophisticated methods for collecting the aroma compounds from the breath including cryo-trapping (Linforth and Taylor 1993), and Tenax trapping (Linforth and Taylor 1993; Delahunty *et al.* 1994; Rabe *et al.* 2002).

Soeting and Heidema (1988) first applied direct mass spectroscopy in an attempt to measure volatile compounds from human breath. Their method involved the exhaled air having to pass through a semi-permeable membrane to remove many of the air and water components but encountered problems with the sensitivity (only ppm range) and selectivity of the technique. This situation was not ideal and the technique was not considered further until development by Linforth and Taylor (1998). They developed a system based on Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APcI-MS), a technique that was first suggested for breath analysis by Benoit *et al.* in 1983. The Linforth and Taylor method involved the development of an interface. This comprised of a heated transferline which could carry gas phase concentrations, from either headspace or directly from panellists, directly into the ionisation region of the mass spectrometer.

1.7.1 APCI-MS for Measuring Aroma Release

The APcI-MS technique developed by Taylor and Linforth allows the rapid measurement of gas phase concentrations with sensitivities of between 10-100 ppbv depending on the volatile compound (Taylor *et al.* 2000). The technique is discussed in detail below.

A deactivated fused silica tube is surrounded by a flow of nitrogen at high flow rates. This produces a venturi effect which serves to draw a continuous gas phase from the outside environment into the ionisation source. A heated transferline (approximately 150°C) surrounds the external section of the silica to prevent condensation of any compounds present in the sampled gas phase (Figure 1.2).

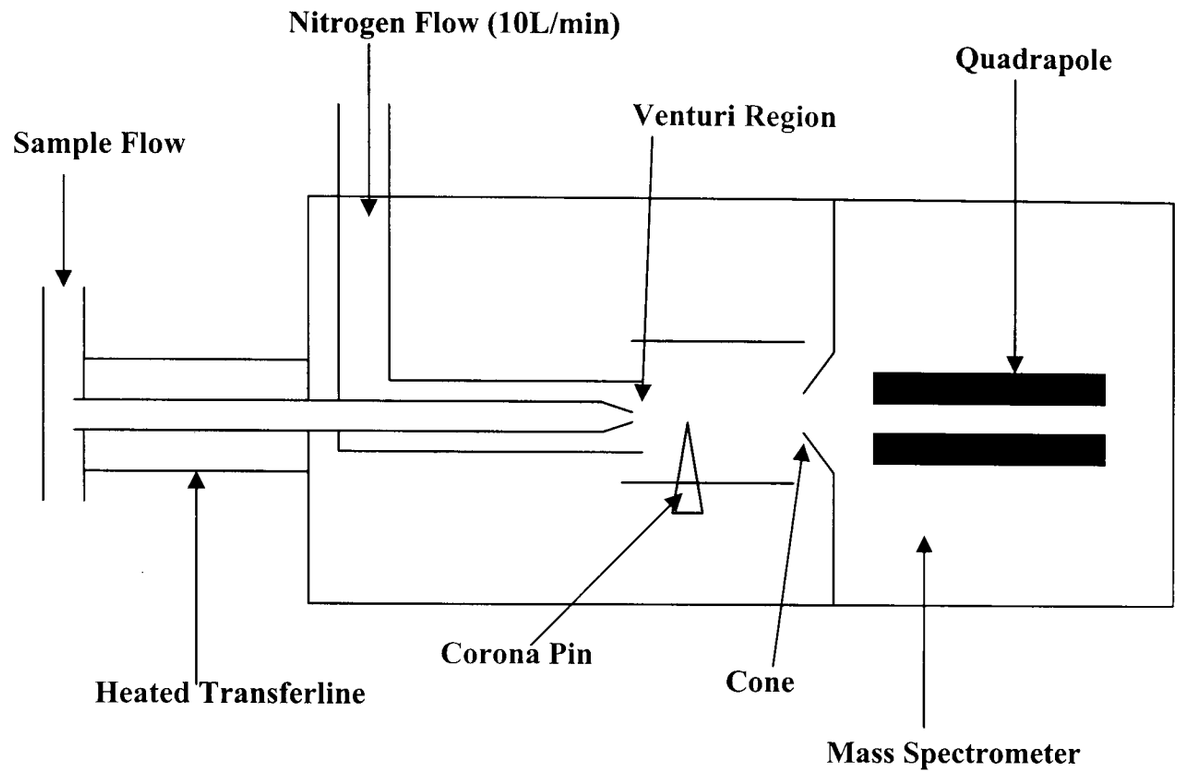


Figure 1.3 Diagrammatic representation of the APcI-MS system used for aroma release studies.

Upon reaching the ionisation source the water present in the sample is subjected to a 4kV corona discharge. This serves to protonate the water molecules and produces the hydronium ion (H_3O^+). Previous methods struggled to function accurately due to the high levels of water present in expired air which interfered with the analysis. In the APcI-MS technique the water vapour present in the expired air is essential for producing the reagent H_3O^+ ion. These ions are produced as opposed to any ions from ambient air due to the higher proton affinity of the H_2O molecule. The ionisation of the aroma compounds is achieved by proton transfer reactions (Figure 1.3). These occur as the aroma compounds generally have higher proton affinities than water and will thus become protonated on collision with the hydronium ions. The positive ions produced are transferred in a vacuum to the quadrupole mass spectrometer region, where they are separated, and detected based on their molecular mass. This is also known as the mass to charge ratio (m/z) as the molecules generally produce protonated ions. This technique, in contrast to some other ionisation processes, is regarded

as 'soft' and therefore very little fragmentation of the aroma molecules is observed. This means that most ions detected will be the original molecule plus a proton (MH^+). In some cases, some fragmentation may occur and this is especially the case in longer chain alcohols which tend to dehydrate and lose a water molecule.

$$M + H_3O^+ \rightarrow MH^+ + H_2O$$

$$H\text{-}M\text{-}OH + H_3O^+ \rightarrow MH^+ + 2H_2O$$

Figure 1.4 Representation of some of the proton transfer reactions that occur in APcI-MS

APCI-MS has allowed significant steps to be made in the studies of aroma release, especially with regard to release from panellists and temporal profiles. However, it is not without its limitations. Although it is capable of monitoring the release of a wide range of compounds simultaneously, the technique of soft ionisation precludes the formation of distinctive fragmentation patterns. This means that compounds producing the same molecular ion cannot be resolved from one another using this analysis. For example, amyl acetate and isoamyl acetate both produce a molecular ion of 131 but analysis by APcI-MS will be unable to resolve the contributions of each. Ion trap APcI-MS or MS/MS may resolve some of these resolution issues but as a consequence of the technique involved some of the time-resolution will be lost which may be important in measurement of samples undergoing rapid changes such as is the case during consumption *in vivo*. Another potential problem arises when samples containing ethanol are monitored. If the ethanol concentration in an aqueous sample exceeds 4% then changes in ionisation or compounds may occur. This is due to the higher proton affinity of ethanol compared to water, causing production of 'ethanolic' ($[C_2H_5OH+H]^+$) reagent ions in preference to H_3O^+ ions. The different proton

transfer characteristics of ethanol have been shown to have different ionisation effects on a range of aroma compounds (Aznar *et al.* 2004).

Another significant limitation of APcI-MS is the potential of competition between ionisation of molecules in the source. This will be especially important for flavour mixtures where a number of aroma compounds will be present simultaneously. What this suggests is that as proton transfer is dependent on the proton affinity of the compounds, some compounds will be preferentially ionised in a system and it may be such that different compounds will affect the ionisation of other compounds in the mixture. For example, it has been demonstrated that ethyl hexanoate in mixtures can increase the ionisation of other molecules present and thus alter the levels detected by the mass spectrometer (Le Quere, Personal Communication). Competition between compounds may therefore adversely affect the quantitative nature of this technique when aroma mixtures are used.

An alternative technique based on proton transfer, Proton Transfer Reaction Mass Spectrometry (PTR-MS) has also been developed and allows measurement of volatile release. It acts on the same principle of chemical ionisation as APcI-MS with H_3O^+ as the ionising reagent but has been shown to be able to measure up to pptv levels (Lindinger *et al.* 1998). If long sampling times are used the key differences between APcI-MS and PTR-MS are that the reagent ions are formed separately and then allowed to react with the analytes in the sample under reduced pressure (Taylor and Linforth 2000). A number of authors have used PTR-MS as an alternative to APcI-MS for real time monitoring of aroma release (Mayr *et al.* 2003; van Ruth and Buhr 2004; Mestres *et al.* 2005).

1.7.2 Flavour Perception

Time-intensity (TI) techniques have been studied intensively for numerous products, from gels (Wilson and Brown 1997) to chewing gums (Davidson *et al.*

1999) and can provide useful information on time courses of release from solid food products and their relation to perceived changes (Overbosch 1986; Piggott and Schaschke 2001). Other measurement techniques to study differences in flavour perception involve paired comparison, difference tests and ranking tests. These tests allow discrimination between certain samples based on a single criterion given to each panellist. Perception studies are important as it is not only the release characteristics that are of interest but the fact that consumers are able to differentiate between any changes. It is all very well maximising the release from a system but this may not reflect the required profile to give the optimum sensory response.

1.8 Aims and Objectives

Hypotheses related to the degree of aroma compound dispersion suggest that different food matrices will behave in different ways. Higher scale mixing requires a number of systems to be developed whereby physical separation of aroma compounds into distinct regions of the matrix can be achieved. Carrier solvents can be used to disperse aroma compounds by increasing their solubilities in the aqueous phase of a matrix. This provides separation on a higher degree of scrutiny. Finally, in some systems, phase separation may occur due to low aroma compound solubility either engendered by low moisture content, or by other phase separation mechanisms

The aim of this work was to study the effects of these different length scales of aroma dispersion on the overall mixing and subsequently to assess the release. Finally some effects of these different dispersion techniques were studied to assess whether these differences had an impact on perception.

The main objective of this work was to:

Assess the effects of different dispersion mechanisms on the dispersion, level of delivery and subsequent release of a range of aroma compounds from differing food matrices.

Several secondary objectives were also considered. These are listed below. To:

Determine suitable methods for analysis for aroma release and suitable parameters for testing of samples.

Investigate the use of different mixing techniques (stirring and static mixing) on the partition and release of a commercial strawberry flavour.

Determine the effects of separating aromas in separate layers and the subsequent effects on release and perception.

Determine the effects of carrier solvent type on aroma release from model confectionery systems, during consumption, for a range of aroma compounds based on their physicochemical properties.

Study the release of aroma droplets from model systems in comparison to solubilised aromas and to attempt to model the behaviour based on physicochemical parameters.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Volatile Aroma Compounds

The volatile aroma compounds used for all experiments are detailed in Table 2.1. Compounds were obtained from Sigma-Aldrich, Gillingham, UK; Acros Organics, Loughborough, UK; Fisher Scientific, Loughborough, UK or Firmenich, S.A., Geneva, Switzerland. All volatiles were at least 97% purity and were used without any further processing. Physicochemical parameters were generated using a group contribution software package EPI Suite, Environmental Protection Agency, USA. Strawberry flavours (502-301T and 504-273A) were provided by Firmenich, S.A.

2.1.2 Carrier Solvents

Carrier solvents used were distilled water (reagent grade), ethanol, glycerol (Fisher Scientific, Loughborough, UK), propylene glycol and triacetin (Aldrich, Gillingham, UK). The chemicals were ACS reagent grade or higher.

2.1.3 Other Non-volatile Compounds

The remaining non-volatile components used are listed in Table 2.3. These include not only the sugars and salts but also the macromolecular food components.

Table 2.1 Volatile Aroma Compounds. *Properties generated using a group contribution software package (EPI Suite, Environmental Protection Agency, USA). Log P represents the logarithm of the octanol-water partition coefficient and Log solubility is the logarithmic value of the aqueous solubility (mg / L) of each compound in water.

Volatile Compound	Molecular Weight	Log P*	Vapour Pressure* (mm HG)	Log Solubility*
2,3-diethyl pyrazine	136.20	2.02	0.78	3.65
2,5-dimethyl pyrazine	108.14	1.03	3.18	4.50
Amyl acetate	130.19	2.34	4.16	3.00
Butan-2-one	72.11	0.26	98.5	4.88
Butanal	72.11	0.82	108	4.38
Carvone	150.22	3.07	0.13	2.56
Citral	152.24	3.45	0.0913	1.93
Cyclohexane	84.16	3.18	93.9	1.63
Diacetyl	86.09	-1.34	70.2	6.00
Ethyl acetate	88.11	0.86	98.3	4.48
Ethyl butyrate	116.16	1.85	14.6	3.44
Ethyl hexanoate	144.21	2.83	1.80	2.49
Ethyl octanoate	172.27	3.81	0.235	1.52
Ethyl pentanoate	130.19	2.34	4.8	2.97
Hexane	86.18	3.29	150	1.24
Hexanal	100.16	1.80	9.57	3.55
Hexan-2-one	100.16	1.24	13.6	3.89
Hexanol	102.18	1.82	0.881	3.84
Limonene	136.24	4.83	1.45	0.66
Linalool	154.25	3.38	0.0832	2.83
Menthofuran	150.22	4.29	0.0805	1.21
Methyl acetate	74.08	0.37	52.7	4.97
Methyl salicylate	152.15	2.60	0.0534	3.27
Octanol	130.23	2.81	0.099	2.91
Octanone	128.22	2.22	1.87	2.95

Table 2.2 Carrier Solvents used. * Properties generated using a software package (EPI Suite, Environmental Protection Agency, USA). Again the solubility is the aqueous solubility (i.e. in solubility of compound in water).

Carrier Solvent	Molecular Weight	Supplier	Grade	Log P*	Solubility* (mg/L)
Ethanol	46.07	Fisher	Reagent	-0.14	7.92E+05
Glycerol	92.10	Fisher	Reagent	-1.65	>1.00E+06
Propylene Glycol	76.10	Sigma	Reagent	-0.78	>1.00E+06
Triacetin	218.21	Aldrich	ACS	0.36	2.15E+04
Water	18.02	n/a	Distilled	-1.38	>1.00E+06

Table 2.3 Other non-volatile products used.

Compound	Supplier	Supplier Address	Type of Product
Citric acid	Aldrich	Gillingham, UK	Ingredient
Electric Blue	Firmenich	Geneva, Switzerland	Colourant
Gelatine	Gelatines Weishardt	Cedex, Belgium	Gelling agent
Glucose Syrup	Cargill Sweeteners	Tilbury, Essex, UK	Ingredient
Green food colourant E102 Tartrazine E142 Green S	Supercook	Leeds, UK	Colourant
Ponceau 4R	Firmenich, SA	Geneva, Switzerland	Colourant
Red food colourant E122 Carmoisine E110 Sunset Yellow	Supercook	Leeds, UK	Colourant
Sucrose	British Sugar	Peterborough, UK	Ingredient
Valencia-T Gum Base	Cafosa	Barcelona, Spain	Gum base
Xanthan Gum	Red Carnation Gums	Laindon, Essex	Thickening agent
Yoghurt	Yeo Valley	Cannington, Somerset, UK	Ingredient
β-carotene	Aldrich	Gillingham, UK	Colourant

2.2 Sample Preparation

2.2.1 Preparation of Solutions

2.2.1.1 Solutions for Static Headspace Analysis

For aqueous solutions, aliquots of pure volatile compound (5-50 mg L^{-1}, volume dependent on compound) were predissolved in 4-30 mL of distilled water prior to addition to an glass Erlenmeyer flask (glass was used to reduce the level of volatile binding) and the solution made up to 200 mL. For addition using co-solvents, the aroma compounds were pre-dissolved in the same volumes (4-30 mL; 2-15 %) of the appropriate solvent prior to being made up with distilled water to a total solution of 200 mL in a glass Erlenmeyer flask. Each solution contained a maximum of two volatile compounds to reduce the probability of volatile-volatile interactions in solution and at the source (competition) as demonstrated in Section 3.7. The aliquots were added at volatile concentrations such that they produced gas phase concentrations which gave similar ion intensities when monitored by APcI-MS. The solutions were shaken for 2 hours at 550 oscillations min^{-1} using an SFT1 Flask Shaker (Stuart Scientific, Redhill, UK) at 20°C, to increase the effectiveness of compound solubilisation. Portions of solution were then added to Schott bottles for analysis.

2.2.1.2 Droplets in Solution

Droplets were initially studied in solutions, however, most aroma compounds, being less dense than water, rapidly progressed to the surface of the solution and volatilized into the headspace. This rapid migration did not allow these systems to be investigated therefore a thickened solution was developed to restrict droplet movement. Xanthan gum (Red Carnation Gums, Laindon, Essex, UK) was

selected for its ability to thicken solutions at relatively low concentrations and to increase stability of emulsions and suspend droplets. These properties suggested that it would provide a suitable medium to restrict volatile compound droplet movement in aqueous solutions (Garcia-Ochoa F *et al.* 2000). Concentrations between 0 and 0.8 % were investigated (Section 3.9) as these are similar to levels used in the food and pharmaceutical industries to increase product stability.

Initially 1000 g of 0.8 % xanthan solution was produced. Approximately 970 g of distilled water was weighed out in a 2 L glass beaker (which had been previously weighed). The water was stirred at 500 rpm using a paddle stirrer (Stuart Scientific Stirrer SS2, paddle diameter 80 mm) to produce a vortex. Xanthan (8 g) was added gradually, ensuring that no aggregations formed and that the xanthan became well dispersed. Mixer speed was altered between 200-600 rpm and stirred for a further 2 hours to ensure complete mixing. After two hours the beaker containing the solution was reweighed, the weight of the beaker subtracted, and the final solution made up to 1000 g by addition of the necessary quantity of distilled water. The solution was then stirred again for a further 10 minutes. Sample dilution used 200 g of the 0.8 % xanthan stock solution and the dilution conditions shown in Table 2.4. Once dilutions were made, the samples were agitated for a further 90 minutes using a roller mixer to make sure that the samples were fully mixed. The samples were refrigerated, at 4°C, after mixing to reduce growth of micro-organisms.

Table 2.4 Dilutions of 0.8% xanthan stock solution to create a range of xanthan concentrations (v/v %)

Xanthan Concentration (v/v %)	Stock Solution (g)	Distilled Water (g)
0	0	200
0.1	25	175
0.2	50	150
0.3	75	125
0.4	100	100
0.5	125	75
0.8	200	0

Droplets of volatile compound (1 μl) labelled with β-carotene (Aldrich) were injected into 15 mL of 0 %-0.8 % xanthan solutions to a depth of 15 mm in a glass testube prior to consumption. Injection of droplets was assessed in a range of xanthan concentrations to develop a system whereby movement was restricted to a minimal extent, i.e. the droplet was retained in solution long enough for *in vivo* analysis to occur.

2.2.2 Preparation of Gelatine-Sucrose Gel Samples

2.2.2.1 Gel Preparation

The gelatine-sucrose gels were prepared by pre-soaking 30 g of gelatine (Gelatines Weishardts, Type A, 225 Bloom) in 100 mL of distilled water for 30 minutes at room temperature. A sugar syrup containing 150 g of sucrose dissolved in 75 mL of water was prepared and then 200 g of glucose syrup (Cargill Sweeteners, 42 DE) was added. The syrup was heated to 112°C and stirred to achieve complete dissolution; it was then cooled to 75°C and the gelatine solution was added and stirred in. Citric acid (5 g) dissolved in 20 mL of water and then added.

To prepare gels with solubilised aroma compounds, aliquots (250 g) of the hot gelatine-sucrose acid (70°C) were weighed out and the appropriate volatile compound was added at this stage and stirred throughout the gel for a period of at least 20 seconds. The hot liquid was then poured into an aluminium tray to a depth of 20 mm and allowed to set for 1 hour at 20°C and then refrigerated overnight at 4°C to allow full gelation to occur. Samples were covered to reduce dehydration. Gel samples were cut into cubes using a knife (20 mm x 20 mm x 20 mm; ~7 g).

2.2.2.2 Preparation of Layered Gel Samples

Gelatine-sucrose gels (6 %) were prepared as per the aforementioned protocol except quantities added were one-quarter of those mentioned above. This allowed production of a layer of gelatine-sucrose gel which had a thickness of 5 mm. Volatile compounds (125-250 mg L^{-1}) dissolved in propylene glycol (2 % in the final gel) were stirred into the hot gel prior to gelation for a period of 20 seconds to ensure complete mixing. Food colourant (200 μL; Supercook, Leeds, UK) was added to the gel to distinguish between the layers and illustrate that no mixing of layers occurred, green for flavoured layers, red for unflavoured layers (colourant was not added for samples to be used for sensory testing to prevent biasing the panellists). Gel samples were allowed to set for no less than 90 minutes at 20°C to ensure gelation. The subsequent layer was then added to the surface of the previously set gel when the mixture had cooled to 45°C to reduce interfacial melting of the set gel. Any volatile compounds or solvents were mixed in prior to the layering of the gel. Samples were produced with combinations of flavoured and unflavoured layers. These are described in Table 2.5, and a graphical representation in Figure 2.1, with 1 denoting a flavoured layer and a 0 denoting an unflavoured layer.

Table 2.5 Description of Layered Gelatine-Sucrose Gel Samples

Sample	Description	Arrangement of Layers	Thickness of each Layer (mm)
1	A gel without any layers. Volatiles homogenously dispersed throughout	One flavoured layer only	20
10	A two-layered sample with flavour in one layer (100 %) and no flavour in the other layer.	One flavoured layer on top of one unflavoured layer	10
1100	A four layered sample with two flavoured layers each containing 50 % of the total gel flavour and two unflavoured layers	Two flavoured layers adjacent to each other on top of two unflavoured layers	5
1010	A four layered sample with two flavoured layers each containing 50 % of the total gel flavour and two unflavoured layers	Two flavoured layers alternately placed with two unflavoured layers	5
1001	A four layered sample with two flavoured layers each containing 50 % of the total gel flavour and two unflavoured layers	Two unflavoured layers adjacent to each other in between two flavoured layers	5
0110	A four layered sample with two flavoured layers each containing 50 % of the total gel flavour and two unflavoured layers	Two flavoured layers adjacent to each other in between two unflavoured layers	5
1111	A four layered sample with four flavoured layers each containing 25 % of the total gel flavour	Four flavoured layers adjacent to each other	5

Alternative gel samples were also produced with 2 and 4 layers for comparisons against the 1 layer sample. These had the same quantity of overall aroma but it was distributed in the following combinations (Figure 2.2).

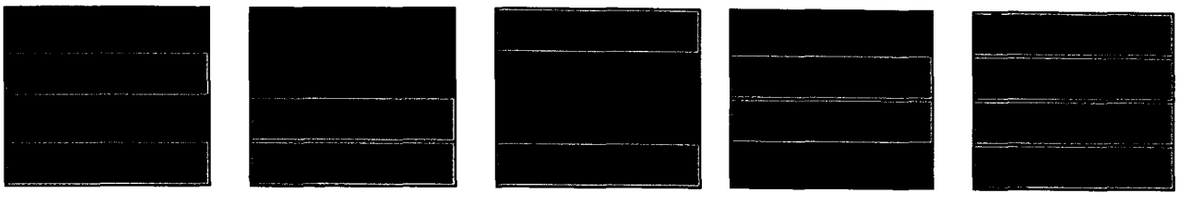


Figure 2.1 Schematic of Different Layered Gelatine-Sucrose Gel Samples. Aroma dispersed throughout green layers, red layers contain no volatile compounds. From left to right samples are 1010, 1100, 1001, 0110 and 1111.

The two and four layer samples were produced in the same manner as described previously with the exception that the thickness of the layers varied. The one layer sample was produced as per the standard gelatine-sucrose gel recipe as stated in Section 2.2.2.1. The 2 layer sample used half the quantities to produce layers of gel of 10 mm thickness. The volatile concentration added was however, 100 % in the flavoured layer. The four layer samples were produced as described above. The layers were again coloured as before, green for flavoured gel and red for unflavoured. Gels were cut into cubes (20 mm x 20 mm x 20 mm).

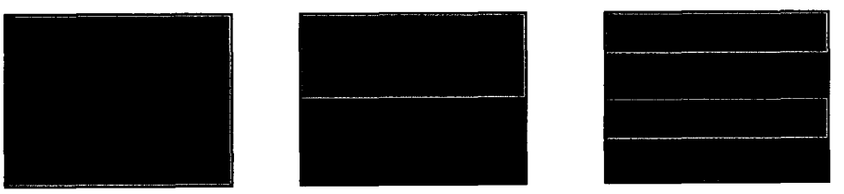


Figure 2.2 Schematic of Different Layered Gelatine-Sucrose Gel Samples. Aroma dispersed throughout green layers, red layers contain no volatile compounds. From left to right samples are 1, 2 and 4 layers.

2.2.2.3 Addition of Volatiles in Solvents

Pure volatiles were dissolved in 5 mL of the appropriate solvent at concentrations ranging from 100-250 mg L^{-1}. Glass apparatus was used as limonene has been shown to absorb to other surfaces (Massaladi and King 1973). The solvents used

were distilled water, glycerol, ethanol and propylene glycol. Gels were prepared as per Section 2.2.2.1. The solvent-volatile mixture was added to the hot gelatine-sucrose solution prior to gelation and was stirred in for a period of 20 seconds. The solvents were present at 2 % within the final gel. Setting and sample portions of the gels was as per section 2.2.2.1.

2.2.2.4 Addition of Volatiles as Droplets

To visualize volatile compounds added as droplets, they were coloured with β-carotene (20 mg) dissolved in pure aroma compound (2 mL). For solubilised samples, propylene glycol was used as a carrier solvent to increase volatile compound solubility (2 % propylene glycol in final gel). Volatile compounds were added to give a concentration in the gel of 125 mg L^{-1}. For gel samples containing droplets, 250 g of hot gelatine-sucrose-acid was weighed out and stirred for 20 seconds to ensure the same treatment as the solubilised sample (including 2 % propylene glycol). The gel was poured out onto an aluminium tray and allowed to cool for 15 minutes before the droplets were injected. Droplets were injected at this stage as this was found to be the optimum point at which droplets were retained at the injection site in the gel (Section 3.10). Figure 2.3 shows a schematic representation of the samples with 1 drop (1 μL), 2 drops (0.5 μL / drop) and 4 drops (0.25 μL / drop). Gels were then also allowed to set for a further 45 minutes at 20°C before being refrigerated overnight at 4°C.

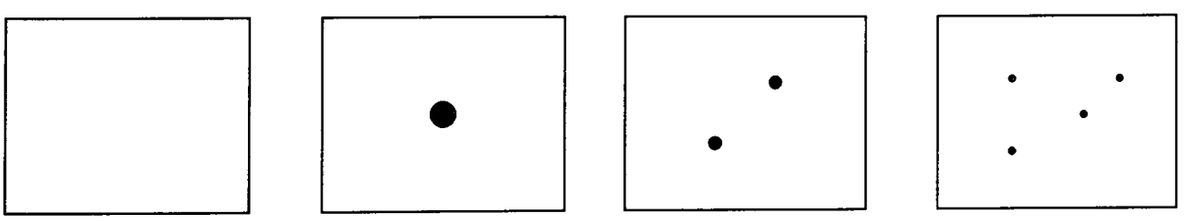


Figure 2.3 Schematic representation of droplets injected into gels (not real sizes). From left to right; dispersed, one droplet, two droplets and four droplets.

2.2.3 Preparation of 'Sugar-mix' Solutions

The sucrose-rich ('sugar mix') solutions were prepared as per the aforementioned gelatine-sucrose gel preparation with the exception that the gelatine phase was not added. This allowed a comparison to the gel samples, without the setting of the system engendered by the presence of the gelatine. Once cooled, the sugar-rich solution was poured into sealed Schott bottles and stored at 4°C. This solution would better mimic the process of partition in the mouth as the aroma release from the gels would be dependent on the dissolution and melting of the samples in mouth. Therefore, unlike the aqueous samples the sugar mixture would give a better indication of partition in mouth without the presence of the gelling agent.

2.2.4 Preparation of Chewing Gum Samples

Chewing gum samples were produced by pre-heating a mixer to 50°C and then adding 200 g gum base (Valencia) which was allowed to melt for 5 minutes. Once the gum base had begun to melt, the mixer was started and the molten gum base mixed for 5 minutes. Glucose syrup (190 g; 38 DE) was added and the heat was removed. Sucrose (295 g) and 5 g of glycerine was added and mixed for a further 2 – 3 minutes followed by another 295 g of sucrose and further mixing for 2 minutes. Finally, flavouring (1 mL), colouring (1 mL), and citric acid (1 g) were added and mixed for further 2 minutes.

Two portions of gum mixture were produced with one set flavoured (strawberry flavour 502-301T) and the second set left unflavoured. Red colouring (Ponceau 4R) was added to the flavoured gum and blue colouring (Electric Blue) to the unflavoured gum such that the flavoured and unflavoured layers could be distinguished. The two layers were rolled together using a pastry sheeter (Rollfix 13-452) to a thickness of 15 mm and a two layered product with one flavoured

layer and one unflavoured layer was generated. Subsequent layered samples were produced by cutting and rolling, to produce 4, 8 and 16 layers. Final samples were cut to 20 mm x 20 mm x 15 mm. In this method the samples with only one layer (all flavour) had double the aroma concentration of the other samples.

2.2.5 Preparation of Yoghurt Samples

2.2.5.1 Stirred Yoghurts

Fruit compote (Apple and Strawberry, Yeo Valley, UK) was strained to remove any pieces of fruit using a sieve (600 μm pore size). Natural yoghurt (50 mL; Yeo Valley, UK – fat content 4.1 %) was added to a plastic pot and 10 mL of the strained compote was added to the surface. Initial sampling of compote on a full scan using the APcI-MS gave no significant ion concentrations. Strawberry aroma (504273A, Firmenich, SA) was dissolved in propylene glycol (5 mL in 100 mL) and a 0.25 mL aliquot added to boost the ion concentration with characteristic ion contributions from esters at 117 and 89. Yoghurt and compote were stirred at 50 rpm (Yellowline, OST Basic) with a specially designed stirring blade, which fitted the dimensions of the pot used. Yoghurt was stirred for 10, 20, 30, 40, 60, 80, 120, 180, 300, 420, 600 and 900 seconds with a cover to reduce volatile loss. Headspace samples were also produced to analyse the partition behaviour of the samples.

2.2.5.2 Static Mixer System

A static mixer system was developed using Kenics mixer blades such that a defined level of mixing could be established. Two stock samples of yoghurt (2 L) were produced. One sample had volatile compounds (limonene and methyl acetate) dissolved in propylene glycol, added to it (Yoghurt A). The volatiles

were added at 250 mg L^{-1} and 25 mg L^{-1} (in final 2 L sample) respectively to a solution containing 20 mL propylene glycol and 80 mL of distilled water. This flavouring mixture was added to the yoghurt (1900 mL) and stirred for 15 minutes with a Kenwood mixer (Chef HM220, Kenwood, Havant, UK). The other yoghurt stock sample had the propylene glycol-water mixture added but the volatile compounds were omitted to produce a non-flavoured stock (Yoghurt B). This sample was also stirred for 15 minutes at 500 W to ensure similar treatments for the two samples. Mixing elements containing 2, 4, 6, 8 and 10 blades were placed into the mixer tube. Yoghurt A and Yoghurt B were then pumped simultaneously at 5 mL min^{-1} using a Cole-Palmer MasterFlex L/S (twin Easy Load II pump head) peristaltic pump into the static mixer test rig, as shown in Figure 2.4. Both yoghurt A and yoghurt B were then blended together to investigate the degree of mixing, before reaching the sampling section where samples of the blended yoghurt were collected. The valves were closed every 20 seconds and the sampling section, which was filled with the blended yoghurt, was removed. Cling film was used to retain the blended yoghurt in the sampling section tube. Yoghurts A and B were blended to assess the efficiency of mixing using the static mixer with colourant to observe the mixing pattern and aroma to measure the effects on release *in vivo*.

The same protocol was carried out at Birmingham University to measure the mixedness of the samples. The only exception was that in Yoghurt A the volatile compounds (in propylene glycol) were substituted with black food colouring (Supercook) and the propylene glycol in Yoghurt B with water. In this experiment, coloured yoghurt and uncoloured yoghurt were blended laminarly through static mixer to produce a sample of yoghurt which contained different level of colouring. The samples were then frozen and sliced before being scanned on a flatbed colour scanner to produce electronic images for image analysis. The whole experiment was carried out in a cold room at 4°C.

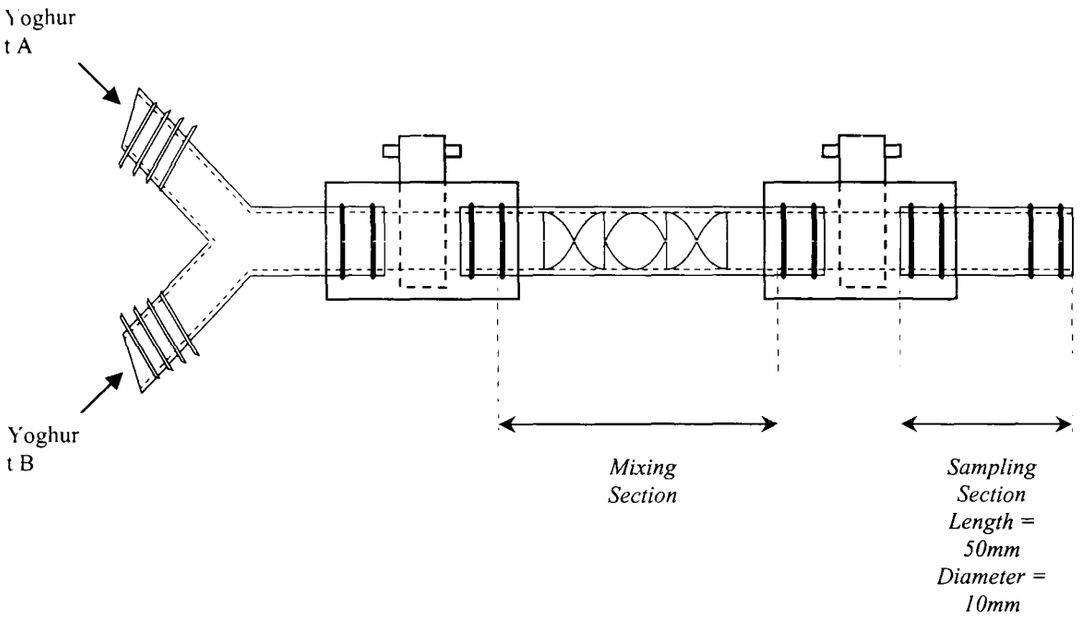


Figure 2.4 Schematic of the Static Mixer Test Rig

A calibration was performed using samples with known quantities of food colouring prepared by mixing known quantities of Yoghurt A and Yoghurt B with the Kenwood Food Mixer (HM220) for 15 minutes at 500 W. The mixed samples were measured into sampling section tubes and frozen at -20°C in the freezer overnight.

2.2.6 Preparation of Boiled Sweet Samples

A sucrose (200 g), glucose syrup, (200 g), water (97.5 g) slurry was produced and heated slowly, whilst stirring to ensure total dissolution. The slurry was heated to 150°C taking care to avoid splashing solution onto the sides of the container. At 150°C the pan was removed from the heat and allowed to cool to 90°C when the citric acid (2.5 g) and flavouring (Section 2.2.6.1) were stirred in. Samples were deposited into moulds and permitted to set at 20°C for 1 hour before being refrigerated at 4°C overnight.

2.2.6.1 Flavouring

Volatile compounds were dissolved in the carrier solvents, water, propylene glycol or ethanol (5 % in total sample) to a final concentration of 125 mg L^{-1}. The compound-solvent mixture was added as the flavouring (see above).

2.2.6.2 Droplets

Volatile compounds were either dissolved in propylene glycol (5 % in total sample) or injected as droplets for a final concentration of 125 mg L^{-1}. In the case of dispersed aroma, 63 μL was dissolved in 25 mL of propylene glycol and dispersed in 500 g of boiled sweet mixture. Droplets of pure volatile compound (1 μL; labelled with β-carotene) were injected once the sample had cooled to 70°C.

2.2.7 Sensory Examination of Intensity of Release from Gelatine-Sucrose Gels

Thirty untrained panellists (aged 22-50) with some previous knowledge in the use of sensory studies were recruited from the Food Sciences Division. In a Paired Comparison Test, two samples were presented to the panellists and they were instructed to consume each sample in its entirety, in the presentation order, and to note the sample with the strongest overall flavour intensity. The samples were coded with 3 digit random codes and were randomized using the Fizz program (Biosystemes, Couternon, France). Panellists were given crackers and water to cleanse their palate between samples.

2.2.7.1 Droplet Samples

A paired comparison test was carried out for two aroma compounds; ethyl butyrate and limonene. The two samples compared were 1 drop against 2 drops of volatiles and the panellists were asked to note the samples with the strongest overall flavour intensity. Volatile droplets were not labelled in this case to prevent influencing the panellists' response.

2.2.7.2 Layered Samples

A paired comparison test was carried out for two aroma compounds; methyl acetate and linalool based on previous comments from panellists involved in breath-by-breath analysis. Sample comparison was made between samples flavoured in all 4 layers and flavour dispersed in only 2 of the layers (1111 and 1010 from Section 2.2.2.2). Samples were not coloured to prevent bias from the sensory panel.

2.3 Analytical Methods

2.3.1 Volatile Analysis

Volatile analysis was performed using an Atmospheric Pressure Chemical Ionisation Mass Spectrometer (APcI-MS). The key feature of this technique is the ability to measure the concentrations of compounds in a gaseous phase based on the mass to charge ratio of the ions over a selected time period (real time monitoring). As chemical ionisation is a 'soft' ionisation technique, few fragments are produced and subsequently most compounds can be measured as their positive ion $(M+H)^+$. Further description on limitations and aspects of

APcI-MS analysis are described in Section 1.7. Volatile compounds in the gas phase were drawn into the source via a heated transferline (160°C) by way of a N_2 venturi flow. At this stage, the volatile compounds in the gas phase were subjected to ionisation by a corona discharge (4 kV) and were then sampled into the high vacuum region of the mass spectrometer. The APcI-MS was setup to monitor in Selective Ion Monitoring Mode (SIM) and to produce MH^+ ions with minimal fragmentation. This mode allowed specific ion intensities to be measured as well as the change in these ions over time. Calibrations were performed in order to convert the data to actual ion concentrations (mg m^{-3}) using a defined calibration method (Taylor *et al.* 2000). Calibration was carried out using a 300 μg L^{-1} concentration of the aroma compound dissolved in hexane (Fisher Scientific UK Ltd., Loughborough, U.K.) directly injected into the APcI-MS at a gas flow rate of 75 mL min^{-1}.

2.3.1.1 Static Equilibrium Headspace Analysis by Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APcI-MS)

Volumes of solution (50 mL) were placed in sealed Schott bottles with adapted lids and allowed to equilibrate for at least 4 hours at 20°C (Section 3.8). The headspace (73 mL) was sampled into the APcI-MS for a period of approximately 40 seconds at a flow rate ranging between 3 and 11 mL min^{-1}, depending on the volatile compound being assessed. The low flow rates associated with this system led to little disturbance of the headspace above the samples and thus retained an equilibrium situation. Indicative of this, the ion traces typically show an almost immediate increase in ion concentration followed by a plateau phase for the duration of the sampling and a rapid decline once the sample is removed. Concentration of volatiles in the headspace was calculated by subtracting the background signal from the detected ion intensity of the plateau.

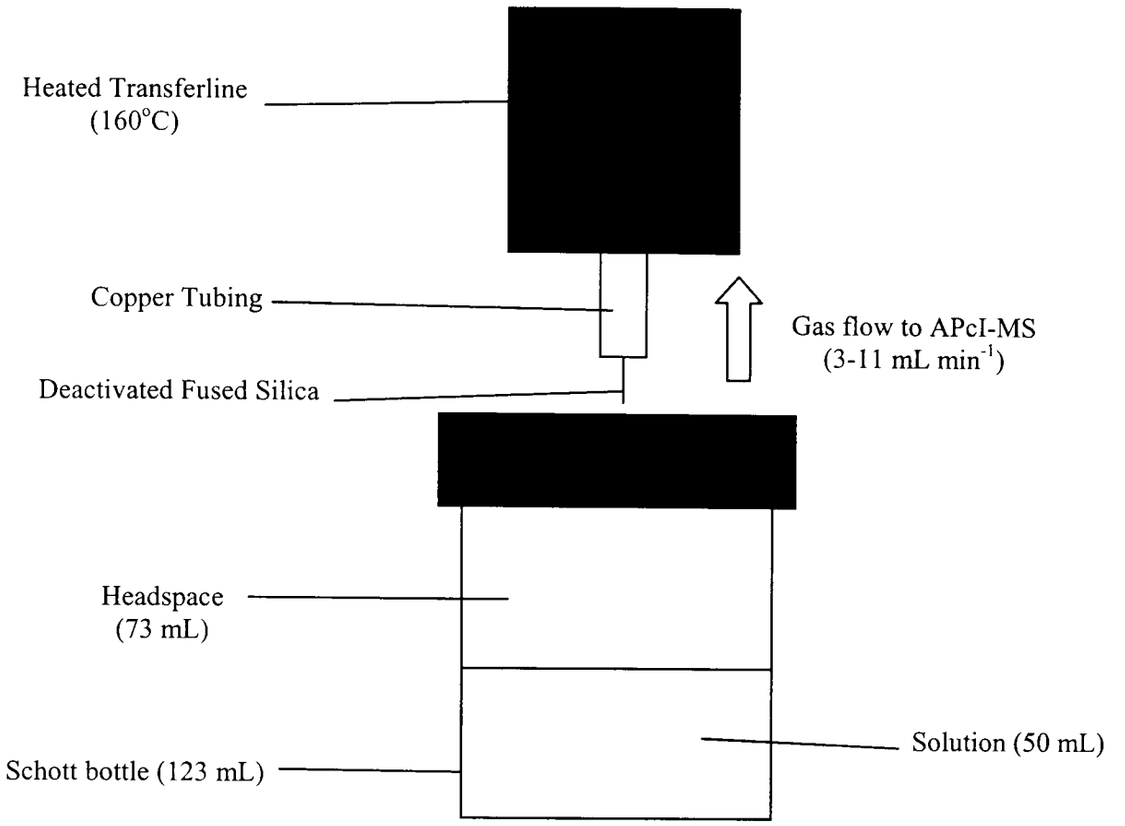


Figure 2.5 Setup used for sampling the gas phase in Static Headspace Analysis

2.3.1.2 Dynamic Headspace Analysis by Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APcI-MS)

In a dynamic situation the equilibrium of the system is not maintained and this better represents the conditions likely to exist *in vivo*. Volumes of solution (100 mL) were placed in sealed glass Schott bottles. A lid adapted for dynamic headspace measurements was fitted and the headspace (23 mL) above the solution was swept with a nitrogen flow of 70 mL min^{-1}. A portion of this flow (3-5 mL min^{-1}) was sampled into the APcI-MS with any excess N_2 passing out through the outlet tube (Figure 2.6).

For gel samples a higher temperature and some agitation were required to increase the rate of dissolution. Water (100 mL) was maintained at 37°C in a water bath prior to addition of the gel portion. A heated, magnetic stirrer (37°C, 50 rpm) was placed below the Schott bottle during the sampling process.

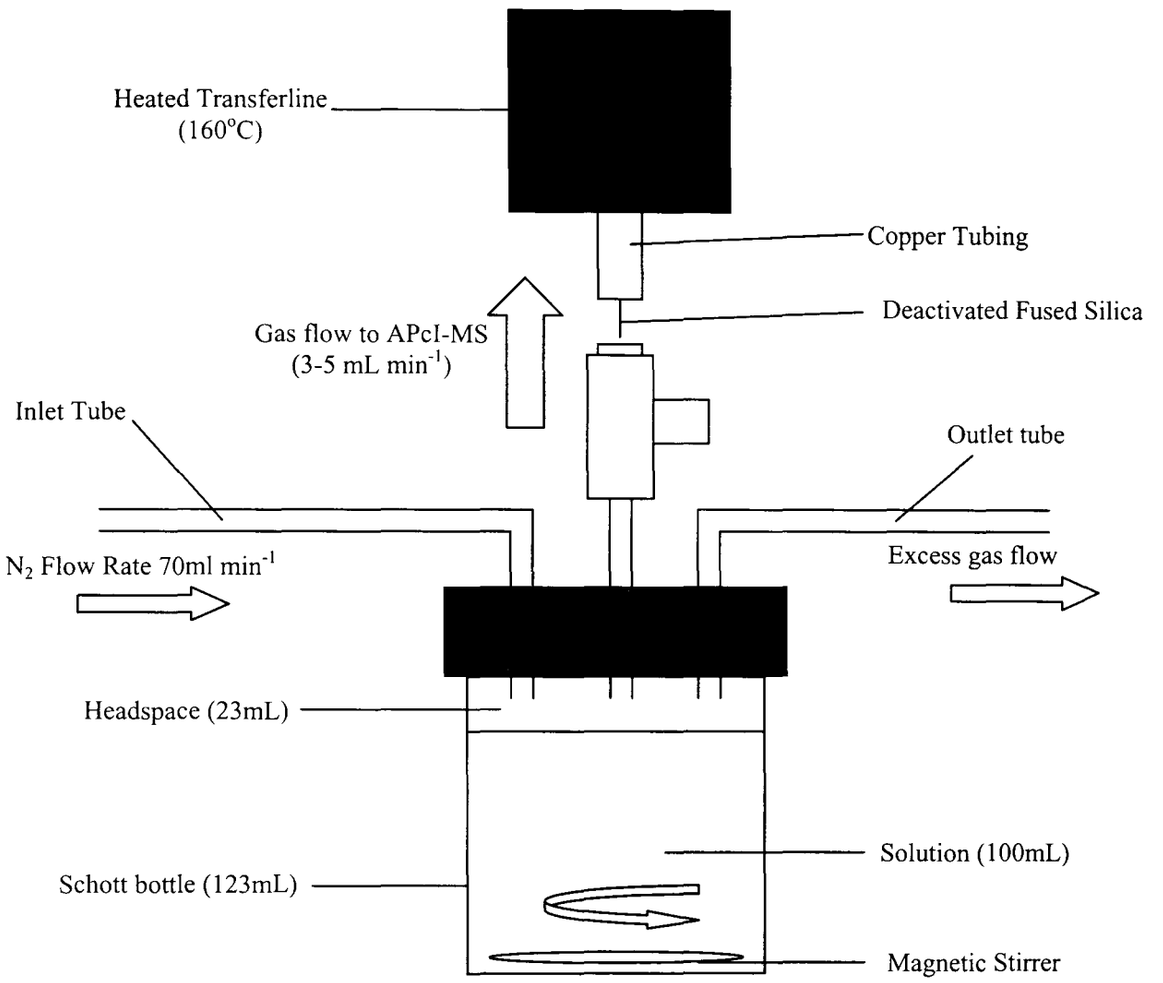


Figure 2.6 Setup used for sampling the gas phase in Dynamic Headspace Analysis

2.3.1.3 Breath-by-Breath Analysis by Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APcI-MS)

For breath-by-breath analysis the APcI-MS with its adapted interface was again used. Flow rates were generally higher (25-30 mL min^{-1}) due to high dilution of the volatile compounds *in vivo*. SIM mode was used to study each individual volatile compound; however, a shorter dwell time was used. This meant that the ion intensity was scanned every 0.02 seconds for each ion as opposed to 0.2 seconds for headspace analyses. This allowed measurement of volatile release in 'real time' without the delays involved when measuring headspace samples.

Acetone (ion 59) was monitored as an indicator of breathing rate and any swallowing events that may occur (Linforth *et al.* 2002). Acetone is present in panellist's exhaled air and is a result of fatty acid metabolism by the liver. The acetone dissolves into the bloodstream where it circulates to the lungs and is present in the expired air. Panellists were instructed to consume the samples whilst exhaling through their nose into a plastic tube, attached to a T-piece, which was connected to the APcI-MS transfer line. The other section of the T-Piece allowed any excess air to escape. A range of products ranging from solutions to boiled sweets were assessed using this technique and hence a range of protocols were required.

Solutions

Solutions were consumed directly from the glass test tubes. The entire volume of solution (15 mL) was taken into the panellist's mouth and swallowed. The first exhalation after the swallow was measured by the panellist placing their nostril onto the tubing connected to the APcI-MS, with a sampling flow of 25 mL min^{-1}. Subsequent breaths were also measured to observe any persistence effects of the samples.

Yoghurts

A spoonful (~5 g) of stirred yoghurt was taken from each sample and taken into the mouth before being swallowed, whilst exhaled air was drawn into the APcI-MS with a flow rate of 25 mL min^{-1}. As previously established, the ions monitored were 117 and 89 (Section 3.1). Samples were consumed by 4 panellists with 3 repetitions of each. Each panellist had one pot for each level of mixing but the replicates were taken from the same pot.

Samples produced by the static mixer system were presented to panellists within the stainless steel tubes (i.d. 10 mm, length 50 mm). Samples were drawn into the mouth from the tubes by the panellists, their nostril placed onto the tubing attached to the APcI-MS, and the sample was then swallowed. The APcI-MS was set up in SIM mode to monitor for ions 137, 75 and 59, at a flow rate of 26 mL

min^{-1}. Three panellists each consumed 3 replicate samples of each of 0, 2, 4, 6, 8 and 10 mixer element samples.

Gels

Four panellists were asked to chew the samples, with their mouths closed, for a period of 45 seconds and then swallow and to continue breathing into the APcI-MS for a further 15 seconds. Each panellist completed three replicates of each of the samples. The volatiles from the exhaled air were sampled at a flow rate of between 5 and 25 mL min^{-1} (depending on the compound) into the APcI-MS source (MicroMass, Manchester, UK).

Gums

Samples were initially consumed using APcI-MS in full scan mode, which measured all the ions detected between a defined m/z range (40-200), to ascertain the important ions released from the chewing gum (Section 3.1). These ions were monitored in subsequent experiments using the APcI-MS as the indicators of the release. Panellists were instructed to chew the gum samples for 5 minutes whilst exhaling into the APcI-MS transferline. Flow rate was 25 mL min^{-1} and dwell time was 0.02 seconds.

Boiled Sweets

Samples were placed into the panellist's mouth and held there for the time taken for the entire sample to dissolve. This time period was approximately 7 minutes as an average for all of the panellists. During the consumption period the panellists were instructed to breathe normally and to swallow whenever they felt was necessary, whilst breathing into the APcI-MS tubing. No mastication of the samples was involved in this instance.

2.3.1.4 'Ethanolic' System

The presence of ethanol in samples has been shown to influence the ionisation of some volatile aroma compounds when analysed by APcI-MS (Aznar *et al.* 2004). Therefore a system was setup to observe the influence of the carrier solvents on compound partition when ethanol was used as the source ionising gas. A solution containing 2 % ethanol (Fisher Scientific, Loughborough, UK) had a flow of N_2 bubbled through it. This produced a carrier gas containing ethanol, which subsequently acted as the charge transfer medium. The flow rate of the nitrogen was altered depending on the concentration of ethanol required at the source which was related to the concentration of ethanol present in the samples being tested.

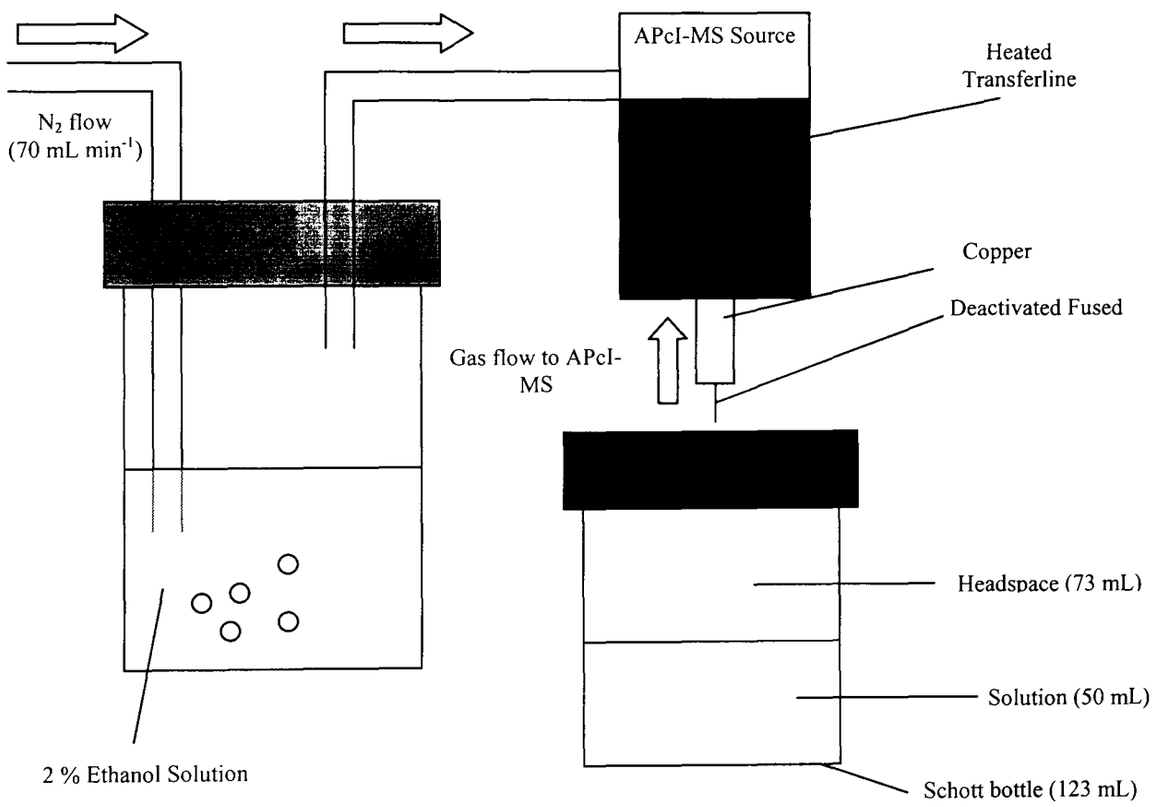


Figure 2.7 Setup used for sampling the gas phase in an ethanolic system

2.3.1.5 Droplet Injection System

A system to monitor volatile release in aqueous solutions was evolved based on the dynamic headspace technique using APcI-MS. A schematic of the altered methodology is included in Figure 2.8. Essentially, the Schott bottle containing 100 mL of solution was inverted and the tubing carrying the gas flows extended to the gas phase. A specially adapted lid was used with the two inlets for the tubing for gas flows but also an injection port with a septum. This allowed a droplet of pure volatile compound to be injected into the solution. Owing to the low density of the compounds they migrated to the solution surface from where the headspace was measured. Volatile compounds, as droplets, showed high levels of release when dynamic headspace flow rates were used (Section 6.1), therefore a reduction in N_2 flow rate to 18.5 ml min^{-1} and a decrease in droplet size to 0.2 μL was used. The reduced droplet size was used to achieve detection by the APcI-MS as most compounds of 1 μl in size exceeded the maximum detection limits of the mass spectrometer.

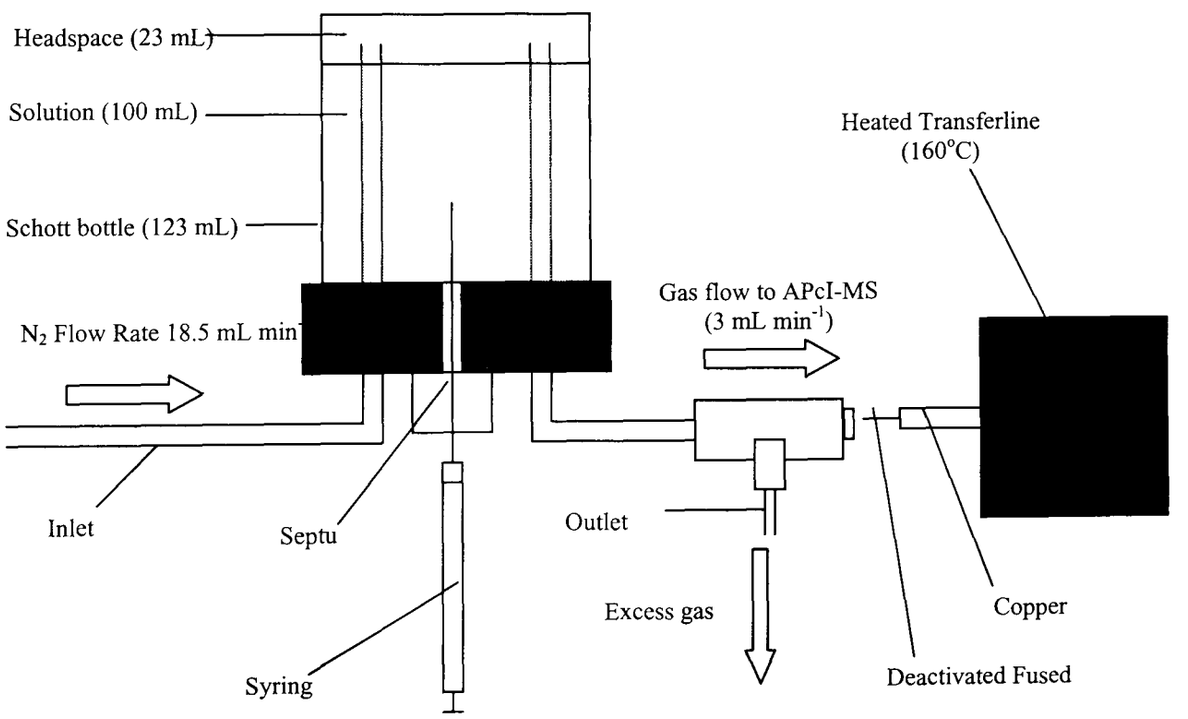


Figure 2.8 Setup used for sampling gas phase from droplet injection system

Dispersed volatile compounds were also assessed using this setup. Volatile compound (0.2 μL) was added to the 100 mL of solution and agitated for 30 minutes to solubilise the compounds.

2.3.2 Other Analyses

2.3.2.1 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a useful methodology to investigate the thermodynamic properties of major food components. DSC is a commonly used technique for studying phase transitions and was thus ideal to analyse the melting point of the gels. DSC experiments were carried out using a Perkin Elmer DSC 7 (Warrington, Cheshire, U.K) using the Pyris software. Gels containing 2 % of different carrier solvents were assessed to compare melting temperatures. Aluminium pans with approximately 16.9 ± 4.6 mg of sample were tested. For scanning DSC, samples were heated within a range from 20 to 70°C at a rate of 5°C min^{-1} with no re-run. Samples were studied in duplicate.

2.3.2.2 Texture Analysis

Gels containing 2 % of different carrier solvents were also assessed to observe any differences in the compressibility and fracture of the gels due to the presence of the solvents. Samples were taken from the fridge 2 hours prior to testing and allowed to equilibrate to room temperature (20°C). Portions of gel were then cut into 20 mm cubes to match the samples used for *in vivo* experiments. A TAXT2 Texture Analyser (Stable Micro Systems, Godalming, UK) with a 5 kg load cell and a 12.8 mm diameter probe was used. Fracture analysis using this probe showed that no fracture occurred in any of the samples studied. Calibration was performed using a 2 kg weight. Samples were tested for compression at a test

speed of 1 mm sec^{-1} for a distance of 16 mm with a force of 0.049N. Three replicates of each sample type were performed and the data was exported to Microsoft Excel for analysis.

2.3.2.3 Image Analysis (work carried out by Vern Lee at Birmingham)

Image analysis was performed to assess the degree of mixing of the yoghurt samples produced by the static mixer. The frozen samples both from section (i) and (ii) were gently pushed out of the sampling tube whilst being sliced (Figure 2.4). Cross sectional images of the sliced samples were then taken using a flatbed scanner and a sample holder.

The images scanned were then processed using Image Pro-Plus 4.5 in greyscale of 8-bit and a pixel concentration of 1200 x 1200. The cross sectional concentration profiles, i.e. the concentration of each pixel, were determined. All concentrations were expressed as percentage of blackness relative to Yoghurt A. (Yoghurt A = 100 % and Yoghurt B = 0 %)

The mean concentration, C_m, was expressed as,

$$C_m = \frac{1}{N}\sum_{i=1} C_i \qquad \textbf{Equation 2.1}$$

where N is the total number of pixels and C_i is the local concentration at each pixel. The variance, s^2, of the measurements which indicated the homogeneity of the mixedness, i.e. the extent to which the concentration in various regions of space differ from the mean concentration, was determined using the expression below.

$$s^2 = \frac{1}{N-1}\sum_{i=1}^{N}(C_i - C_m) \qquad \textbf{Equation 2.2}$$

A small variance implied a homogenous system. Maximum variance, s_o^2, occurred if the sample is completely segregated or unmixed and was calculated as,

$$s_o^2 = C_m(1 - C_m) \qquad \textbf{Equation 2.3}$$

Intensity of the mixedness, *I*, which indicates the goodness of distribution, was then determined by the following expression. An intensity of one indicates segregation while zero intensity indicates homogeneity.

$$I = \frac{s^2}{s_o^2} \qquad \textbf{Equation 2.4}$$

The length scales, S, of the mixing were also determined and using the following expressions.

$$S = \int_0^{\phi} R(r)dr \qquad \textbf{Equation 2.5}$$

$$R(r) = \frac{1}{s^2 N}\sum_{i=1}^{N}[C_i(x) - C_m][C_i(x+r) - C_m] \qquad \textbf{Equation 2.6}$$

where $C_i(x)$ is the concentration at a point and $C_i(x+r)$ is the concentration at a nearby point. $R(r)$ represents the "correlation" of the mixedness of a reference point (arbitrary) to an arbitrary points. r denote the distances between two points. In a system, when the mixedness or concentration of a point is very similar to

another abitrary point, the system is "correlated" and hence denotes homogenous. In turn, if mixedness/concentration of a reference point is very different from another abitrary point in the system, the system is "uncorrelated" and hence denotes segregation.

2.3.3 Sample Characterisation

In order to confirm that volatile concentrations in the gel samples were the same, samples of each preparation type were taken and dissolved in 1L of distilled water at 50°C, in a sealed glass Schott bottle. Samples were then allowed to equilibrate at 20°C for 24 hours before the headspace above each of the samples was measured. The headspace was sampled for approximately 40 seconds at a flow rate of 5 mL min^{-1} into the APcI-MS interface. Cone voltage and ion mass monitored, again depended on the compound being analysed. Calibration of the samples was carried out as previously mentioned. The calibration of the data was calculated by subtracting the blank hexane standard from the known concentration of volatile compound. Hexane was used as the calibrant due to the high solubility of most aroma compounds in hexane but its subsequent lower proton affinity allowed the ionization of the aroma compound ions at the source. This calibration was used to calculate the concentration of volatile compounds (mg aroma per m^3 air) detected for each sample.

2.4 Data Processing

2.4.1 APcI-MS

Data was processed using MassLynx (v3.2) (Micromass Ltd., Manchester, U.K.), cdc2000 (Custom software for ApcI-MS analysis), and Microsoft Excel (Microsoft Corporation, USA). To allow a better comparison between the

different panellists and the different aroma compounds, the data was normalised to remove variation on the Y axis. This is also of importance as the main differences observed are inter- rather than intra-panellist and normalising also allows comparison between different panellists.

2.4.2 Calculation of Physico-chemical Parameters

Physico-chemical parameters of the compounds were calculated using a group contribution software package, EPI Suite (U.S. Environmental Protection Agency & Syracuse Research Corporation (SRC), USA) which based its values on chemical structures as well as containing reference values for some parameters.

2.4.3 Experimental Design

Design Expert v6.06 (StatEase Inc, Minneapolis, USA) was used to design experiments such that the system provided a representative sample and a robust data set.

2.4.4 Statistical Analysis

Statistical analyses were performed using Microsoft Excel, Design Expert v6.06 (StatEase Inc, Minneapolis, USA) and SPSS for Windows v11.0.0 (SPSS Inc., LEAD Technologies Inc., Haddonfield, New Jersey, USA).

3 DEVELOPMENT OF METHODS

Preliminary investigations were necessary to define the parameters for the analytical techniques as well as to develop systems that would be appropriate for comparative measurements. These explorations involved answering a number of questions related to; methods of aroma dispersal, concentrations of volatiles required to produce adequate responses, stability of systems used, and the reproducibility of each system. With many of the analytical techniques, it was important to define beforehand the important parameters that were to be assessed. This was particularly important since the products ranged from solutions to semi-solid gels and gums to solid boiled sweets. These different matrices release aroma compounds via different mechanisms and as such have different effects with regard to restriction of mass transfer, volatile components from matrix components, aroma compound solubility and binding, and breakdown of the matrix. Aroma compounds also have varying properties. These would also affect their release. Adequate aroma concentrations for each product type were determined as well as suitable methodologies for producing consistent samples. Headspace and breath by breath techniques were assessed for their reproducibility and protocols were developed for increasing the consistency of *in vivo* studies. Finally, systems to represent the different levels of aroma dispersion were developed.

3.1 Evaluation of Important Ions in Chewing Gum and Yoghurt Systems Containing Strawberry Flavour

In these systems, a pre-mixed whole 'strawberry' flavour (502-301T) was utilised (Firmenich SA) for breath by breath experiments. The ions detected by the APcI-MS needed to be ascertained prior to investigation. Samples (6 g) of chewing gum containing the strawberry flavour were chewed by a single

panellist, whilst monitoring the ion counts in the exhaled breath via APcI-MS (Section 2.3.1.3). The APcI-MS was set up in full scan mode to monitor the total ion count for a range of ions from 40 to 200 m/z. Figure 3.1 shows the spectrum for the full scan of a single sample and indicated that the ions with the highest ion intensity were 117 and 89. These ions correspond to the protonated molecular ions of ethyl butyrate and ethyl acetate, respectively.

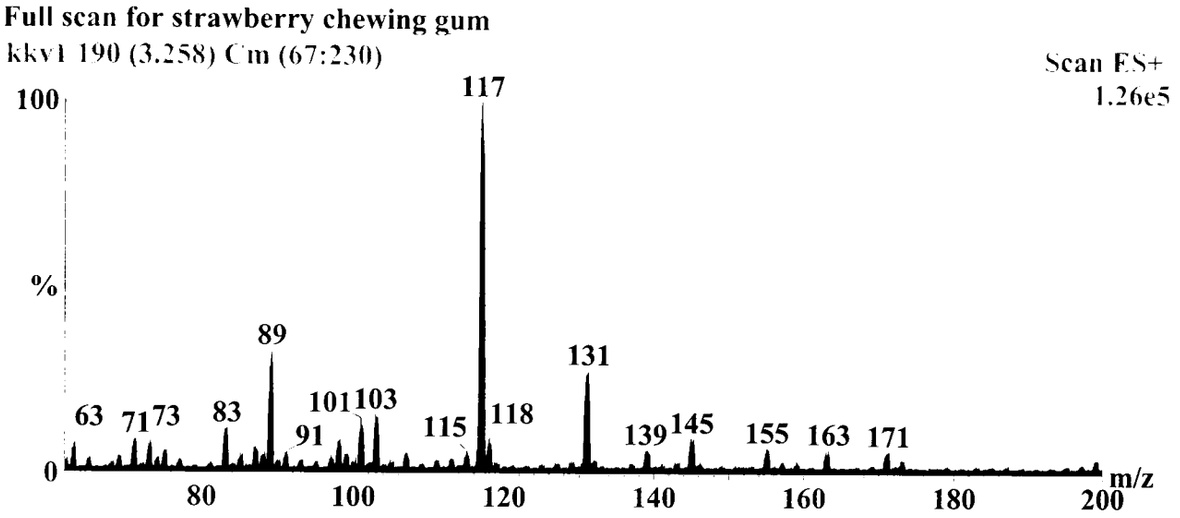


Figure 3.1 Full scan spectrum of strawberry aroma release from chewing gum (m/z 65-200).

In experiments where strawberry aroma was added to yoghurts a different flavour was used (504273A). However, the major ions observed were the same. Plain yoghurt was investigated using APcI-MS. Consumption by a single panellist, was tested to identify if any ions were detected from the unflavoured yoghurt. A spoonful of yoghurt without added flavouring was consumed and the exhaled air monitored in full scan mode (m/z 40-200). The spectrum of ions showed that the most significant ions detected were ions 55 and 59. Therefore, there were no competing ions in plain yoghurt that might interfere with the monitoring of ions 117 and 89 from the strawberry flavour. This suggests that although compounds such as butyric acid and acetoin which are derived from yoghurt itself both give molecular ions of 89 the contribution is solely from the strawberry aroma and thus ethyl acetate is considered to be the main contributor to the levels of ion 89 detected.

3.2 Evaluation of Gels and Solvents with No Added Volatiles when Assessed by APcI-MS

The gels and solvents used as carriers for the aroma compounds were checked to see if they produced any ions that would interfere with APcI-MS analysis of flavour release from gels. It was possible that during the gel production process, volatile compounds may have been created (due to temperatures of greater than 70°C during processing). Several samples, with no added volatile compounds, were consumed using the protocol described in Section 2.3.1.3, in full scan mode. Breathing was also tested for any ions present in the exhaled air of the panellists when no sample was consumed.

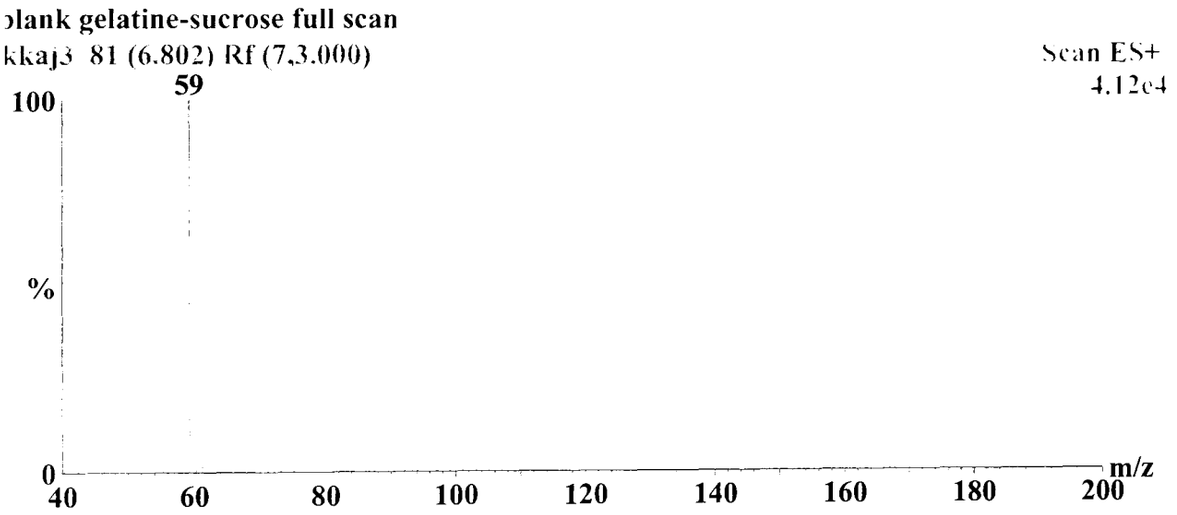


Figure 3.2 Spectrum of ions detected during consumption of a gelatine-sucrose gel without addition of aroma compounds. Peak ions labelled when greater than 5% of the peak ion concentration.

Figure 3.2 shows that the major ion detected from consumption of unflavoured gels was ion 59 which corresponds to acetone present on the breath. All other ions were detected at less than 4.97e2, which is sufficiently low to be disregarded. Ion 59 was also the most abundant during measurement of exhaled air when no sample was consumed.

Solutions containing 2 % solvent solutions were made and their gas phase concentrations monitored in full scan mode. Elmore and Langley (1996) investigated the effects of varying ethanol content on the dynamic headspace concentration using chemical ionisation mass spectrometry and Taylor *et al.* (2001) monitored ethanol release as an aroma compound from gelatine-agarose gels. Ethanol release on APcI-MS can cause instability in the ionisation region if the levels are too high. Up to 4 % ethanol can be analysed using water as the reagent ion, above this concentration, the source needs modification so that ethanol becomes the reagent ion and ensures that ionisation is linear with concentration (Aznar *et al.* 2004).

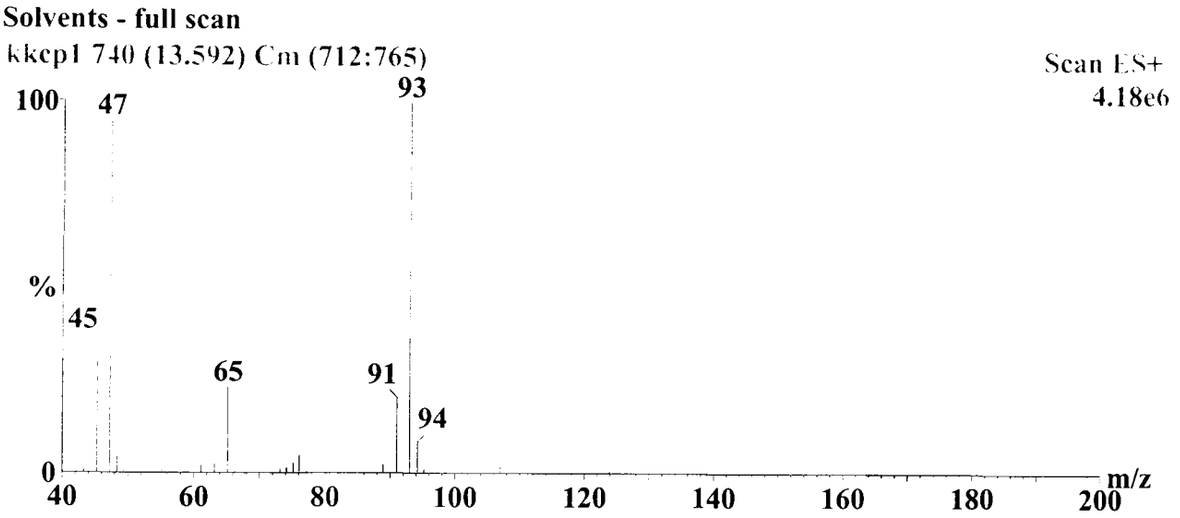


Figure 3.3 Spectrum illustrating ions detected in headspace above 2% ethanol solution.

Figure 3.3 shows the major ions detected in the headspace above a solution containing 2 % ethanol (w/w %). Ions 47 and 93 correspond to the ethanol monomer and dimer respectively and thus other compounds which produce these ions may not be analysed quantitatively when ethanol is present. Other ions which may be affected include ions 45, 65, 91 and 94. Ion 45 is indicative of acetaldehyde which is unsurprising as acetaldehyde is a product of the breakdown of ethanol. Acetaldehyde monitoring should be avoided when ethanol is used as a carrier solvent.

3.3 Dispersion of Volatiles in Gelatine-Sucrose Gels Prior to Gelation

As described in Chapter 2, the addition of volatiles to a liquid gelatine-sucrose-acid mixture was integral to the production of many of the samples assessed for aroma release. Volatile compounds were added to gels using a dispersing agent, which in many cases was propylene glycol although in some experiments other dispersants were used.

Gels were produced as per Section 2.2.2.1 with hexanol (250 mg L^{-1}) and a red food dye (400 mg L^{-1}) added in 5 mL of propylene glycol. Hexanol was chosen, due to its intermediate solubility to act as a marker for dispersion in the system. Thirty seconds of manual stirring with a glass rod stirrer was shown to be a sufficient stirring time to produce a homogenous colour appearance through the gel. Bakker *et al.* (1998) showed that mixing of red dye into gelatine solutions, at 1.67 rps was sufficient to ensure a uniform dispersion. It was assumed that if the colour was uniform then a corresponding dispersion of carrier solvent would be achieved. Depending on the solubility of the volatile compound in the solvent the dispersion of the volatile may vary throughout the solution.

Figure 3.4 shows the release of hexanol over the duration of the consumption event. The curve indicates that hexanol was evenly released into the expired air in the nose. Since mass transfer through the nose is laminar (Hodgson *et al.* 2003b) mixing is unlikely to occur at this stage. The mixing may occur during mastication but, with these gels that fragment and then dissolve, mastication would have a limited role. Because of these factors and in the absence of 'spikes" in the expired air, the conclusion is that the volatile compounds were evenly distributed in the gel during manufacture. It has been commonly shown that lipophilic volatiles, in low fat systems, are subject to initial 'bursts' of aroma followed by a rapid decline in intensity (Malone *et al.* 2000). These 'bursts' are

related to insoluble regions in the matrix corresponding to poor levels of dispersion.

The use of colourants was a useful technique when investigating the layering of the gelatine-sucrose gels. Creation of layered gelatine-sucrose gel samples is described in Section 2.2.2.2. Dispersion of differing colours in subsequent layers allowed the separation to be observed. No mixing of the two colours at the interface indicated that no mixing between the layers had occurred. This is illustrated in Figure 3.5 which shows the clear definitions between the two coloured layers.

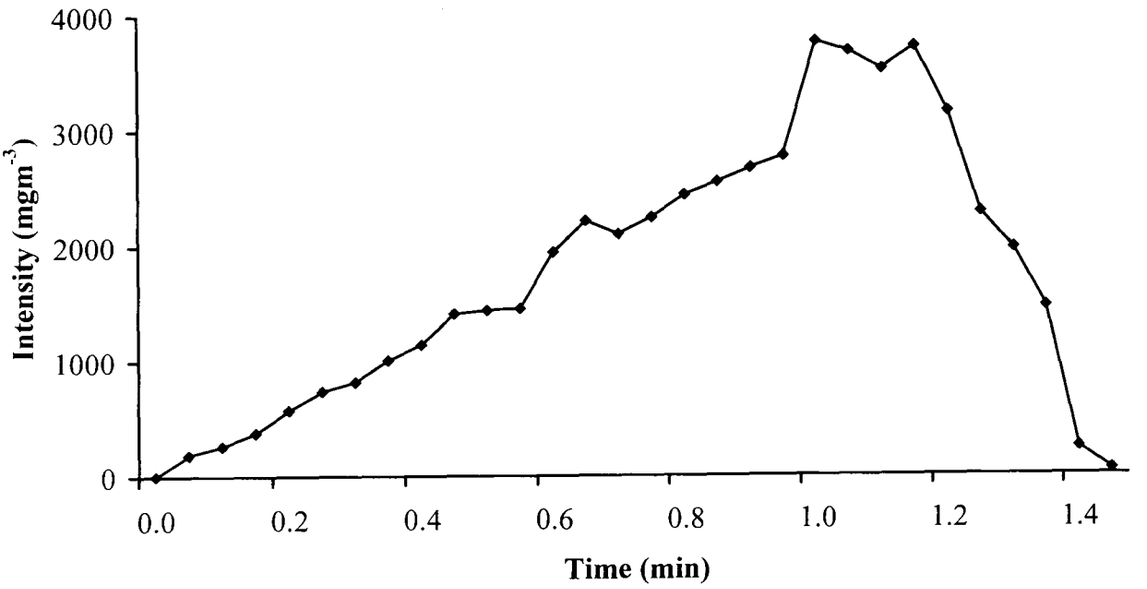


Figure 3.4 Release of hexanol *in vivo* over time during consumption of a gelatine-sucrose gel for 3 panellists with a total of 8 replicates.

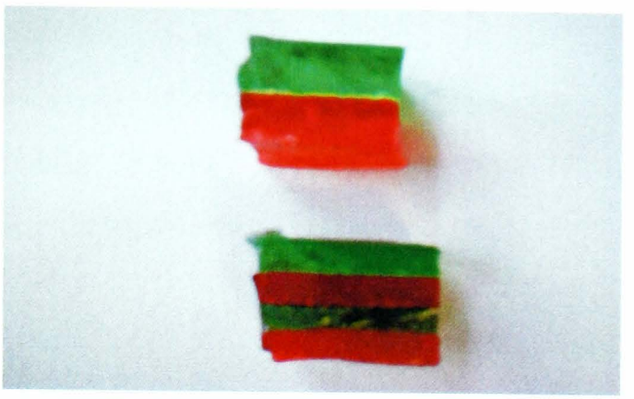


Figure 3.5 Picture illustrating 2 and 4 layer gelatine-sucrose gel samples. Green layers represent layers containing aroma compounds and red layers those without.

3.4 Development of Sampling Protocol for Gelatine-Sucrose Gels

After optimisation of gel sample preparation it was important to ensure that the consumption process of each sample was comparable. A protocol was developed to reduce the levels of variation between samples and also between panellists. It is well documented that differences in aroma release *in vivo* are highly dependent on aspects of physiology between panellists with chewing rates, breathing rates (Harrison and Hills 1997a; Harrison 1998) and swallowing events are of particular importance (Buettner and Schieberle 2000b; Steinhart *et al.* 2000; Buettner *et al.* 2001; Sprunt *et al.* 2002; Hodgson *et al.* 2003b; Pionnier *et al.* 2004). Initial studies demonstrated that a defined gel eating protocol was required to standardise the eating events. These studies showed that if no instruction was given to a panellist, the time taken to consume the whole sample, varied significantly and the release profiles were difficult to compare. Time to maximum intensity (T_{max}) is an important parameter for measuring rates of release especially during the consumption of gelatine-sucrose gels, which are subjected to dissolution and melting (Harrison and Hills 1996).

Gelatine-sucrose gels (6 %) were produced as per Section 2.2.2.1. Hexanol (250 mg L^{-1}) in 2 % propylene glycol was added as a marker compound for aroma release. Samples were cut into cubes of 8 g. A range of protocols were tested ranging in consumption time for each sample from 15-75 seconds, with 15 seconds of exhalation post swallowing. Swallowing occurred after 15, 30, 45, 60 and 75 seconds.

Figure 3.6 clearly shows an increase in both the I_{max} and T_{max} values for hexanol as swallowing is delayed from 15 to 75 seconds. This follows the pattern of aroma release suggested by the model of Hills and Harrison which states that as the gel is broken down and dissolves into the saliva, volatiles are dissolved and subsequently partition into the gas phase (Harrison and Hills 1996). Increasing the time to swallowing will allow a greater period of time for gels to dissolve and release volatiles into the saliva. It is widely acknowledged that the exhalation after swallowing provides a surge of volatiles to the nasal epithelia (Ingham *et al.* 1995a; Harrison 2000; Hodgson *et al.* 2003b; Wright *et al.* 2003). If swallowing is delayed then a greater quantity of volatiles will be present in this exhalation as well as delaying the T_{max}. Another consideration is the comfort of the panellist. As the chewing period was extended, the volume of saliva in mouth increased and subsequently the urge to swallow before the required time increased. Forty five seconds chewing time prior to swallowing was thus chosen as a suitable protocol for future experiments involving gelatine-sucrose gels.

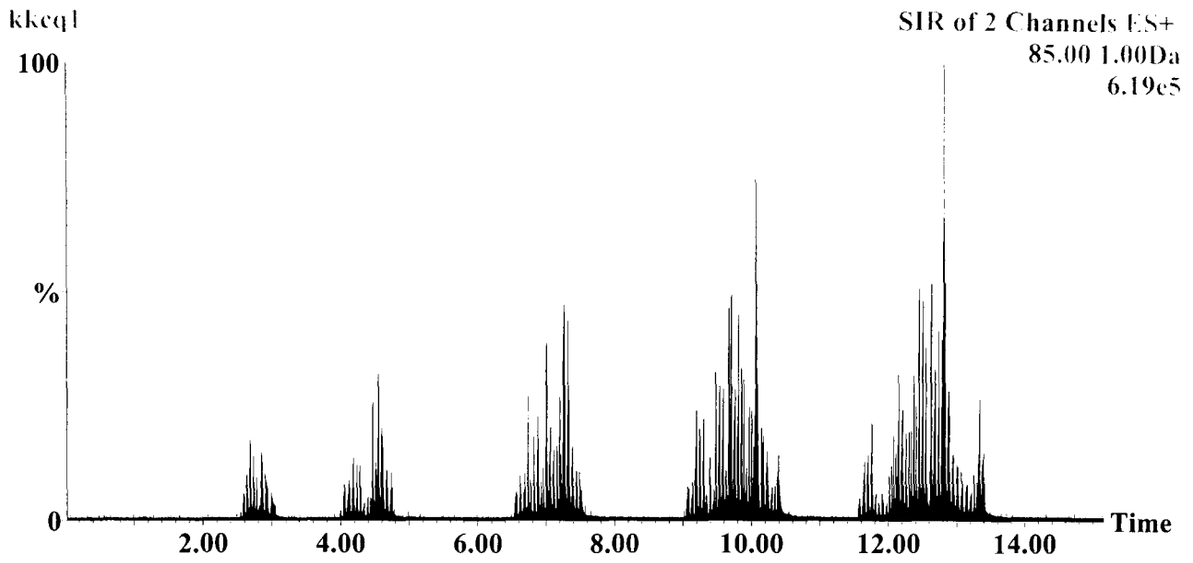


Figure 3.6 Effects of different gel consumption time on *in vivo* release of hexanol. From left to right 15, 30, 45, 60 and 75 seconds before swallowing.

Sample size was also assessed with samples ranging in size from 1 to 8 g using the 45 second consumption protocol mentioned above. Figure 3.7 shows a non-linear increase in the I_{max} of release as the sample size increases until 6 g after which further sample size increase does not significantly increase the aroma release. No differences in T_{max} were observed as swallowing occurred at the same time point for each sample. Prinz and Lucas (1995) showed that swallowing was dependent on the particle size and lubrication of the sample and that chewing events required a minimum particle size to be detected by the oral mucosa. It is unlikely that for the 1 and 2 g samples that sufficient particle sizes remain for the full 45 second period of mastication and these samples are probably inappropriate for use in further studies. The increase in surface area due to the increased size may also play a role in aroma release. As the sample size increased the surface area would increase however, larger sample sizes will be limited by the volume in the oral cavity and saliva coverage. Further increases in the surface area available for release may not occur for larger samples sizes. Samples (6 - 8 g) show similar release patterns and hence these were the sample sizes used in future studies.

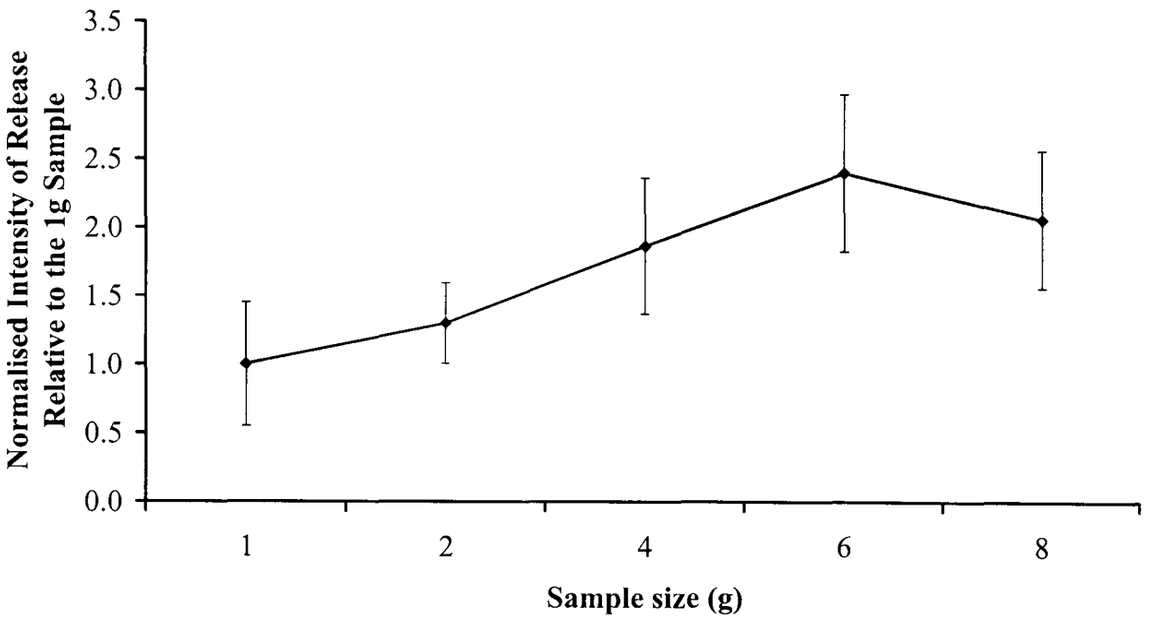


Figure 3.7 Effect of varying sample size on *in vivo* aroma release of hexanol from gelatine-sucrose gels. Normalised data for 3 replicates.

3.5 Definition of Gel Aroma Compound Concentrations and Palatability Study

An integral part of studying volatile compound release from gels was to define a suitable concentration of volatile compounds to be added. The levels chosen were dependent on a number of important considerations. These included: concentrations likely to be present in real confectionery products; levels that would give appropriate ion counts when assessed using APcI-MS; and levels that were able to give sufficient reproducibility. Vuilleumier *et al.* (2002) observed major differences between the equilibrium headspace concentration above the solutions and the retronasal breath volatile concentration. Results for limonene showed a 1000 times lower concentration in retronasal breath than that expected from the results of headspace analysis. This data suggests that the levels of aroma compound loss between the original sample and the final concentration detected at the nasal epithelia varies significantly and that this behaviour is compound dependent. This is summarised by Linforth *et al.* (2002).

It was also important to ensure that the compounds selected had a reasonable palatability. Single volatile compounds, rather than a flavour mixture, may have distinctly unappealing aromas especially at concentrations required to get sufficient ion detection. Since some of the experiments required a number of replicates to be consumed, panellists could potentially consume a large number of samples. If samples were too unpalatable this might adversely affect panellist performance. Volatile compounds were pre-dissolved in 5 mL of propylene glycol and added to hot gelatine-sucrose gel mixture (as per Section 2.2.2.1) at a concentration of 250 mg L^{-1}. This level of aroma is relatively high, however, losses due to heat processing and gelatine binding have been shown to reduce volatile release during consumption by up to 15 % (Burg 1998). Several volatile compounds were tested and the ion counts and palatability (on a scale of 1-5, 1 unpleasant – 5 pleasant) were noted.

Table 3.1 Ion Intensities and palatability ratings for 10 volatile compounds consumed from gels containing 250 mg L^{-1} of aroma compound using APcI-MS as a measuring technique. Palatability ratings 1 = unpleasant, 5 = pleasant.

Compound	Palatability Rating	Peak height Intensity
Acetaldehyde	4	6.39e5
Carvone	3.5	1.38e5
Diethyl succinate	4	5.57e4
Ethyl decanoate	3.5	1.20e5
Ethyl hexanoate	3.5	4.28e7
Ethyl octanoate	3.5	4.31e6
Ethyl pentanoate	3	3.69e7
Linalool	1	1.99e5
octanol	1.5	4.06e5
p-cymene	2.5	1.05e6

Table 3.1 shows that 250 mg L^{-1} was an appropriate concentration level for most of the gel samples tested with ion intensities ranging from 1e5 – 5e7. Thus compounds used were chosen based on their ability to give a good detection signal but without being so concentrated that they became unpalatable. A high

concentration of aroma compounds were used but the potential for losses was not assessed by measuring the final concentrations in the gels. Caution must be taken when interpreting these results as losses between different aroma compounds may not be equal and thus differing final gel concentrations may be the result. Simple methods could be used to test this via a technique other than APcI-MS. These include dissolution of the sample in hexane and extraction of the aroma compounds however, the potential for inaccuaracies may be as high as that for measuring static headspace. An alternative technique involves the direct injection of a SPME-fibre into the solid gel and measurement using GC analysis however (Le Quere, Personal Communication), again this will not give the overall total gel concentration.

3.6 Effective Solubilisation

Volatile organic compounds range in levels of aqueous solubility. A large number of these compounds are non-polar which implies that they have low solubilities in water. Water, is a self associating liquid due to the high levels of hydrogen bonding that it experiences between other water molecules. This means that water has a lattice-like structure that prevents the insertion of non-polar groups and prevents them from becoming solubilised. Addition of two liquids will automatically have an increase in the entropy of the system ensuring that the two liquids will mix to some extent (albeit a very minor one) as a mixture has a level of disorder greater than two pure liquids. Further information on the behaviour of solutions and solvents is discussed in Chapter 1. In order to achieve effective solubilisation of non-polar compounds it is therefore necessary to break up the hydrogen bonding structure of water to form cavities that allow the solutes to become soluble (Yalkowsky 1999a). This can be achieved in a number of ways including, use of a co-solvent or surfactant, changing pH, or thermodynamic methods which involve increasing the energy in the system such as increasing temperature or agitation. Agitation was suggested as the most

appropriate mechanism in this case as it did not involve the addition of any other molecules nor a change in the temperature of the system.

Many of the investigations required volatile compounds to be solubilised in an aqueous phase prior to addition to their final samples. A range of methods were assessed to select a method of agitation which would provide the most efficient and reproducible samples. Flask shaking for 3 hours at 550 oscillations per minute (SRT1 Flask Shaker, Stuart Scientific, Redhill, UK), roller mixing for 3 hours (SRT2 Rollmix, Stuart Scientific, Redhill, UK), and sonication in an ultrasonic bath (FS3006, Decon Laboratories Ltd, Hove, East Sussex) for 3 hours were all compared to a system where no agitation took place. Three volatile compounds ranging in Log P values were investigated (methyl acetate, hexanol, and linalool; with Log P values of 0.37, 1.82, and 3.38 respectively). Aroma compounds were added at relatively high concentrations to show the effectiveness of the solubilisation (linalool and methyl acetate 50 mg L^{-1} and hexanol 250 mg L^{-1}).

Figure 3.8 shows that the method of agitation had no effect on the final headspace concentrations of hexanol. In contrast, linalool was detected at higher concentrations in the final solution when agitated via the SRT1, although this was not significant in all cases due to the high levels of variation. This increased concentration may be due to a decreased solubility in the aqueous phase and hence a higher partition into the gas phase. However, this sample also showed the lowest level of variation, which may suggest that a greater volume was solubilised and hence delivered to the final headspace samples.

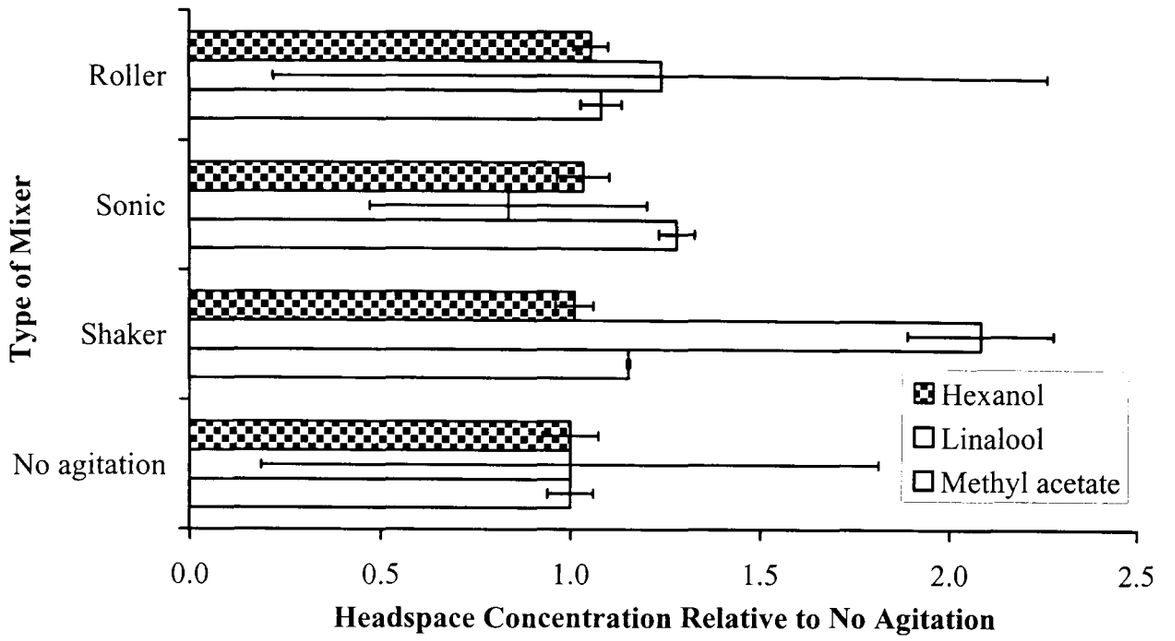


Figure 3.8 Static headspace concentration above solutions with volatiles dispersed using different techniques. Data normalised to sample with no agitation.

Table 3.2 Coefficient of Variation (%) between 3 replicate samples for headspace gas concentrations using different modes of solubilisation.

Compound	No Agitation	Shaker (SRT1)	Sonicator	Roller (SRT2)
Methyl acetate	6.1	0.4	3.7	5.0
Hexanol	7.4	5.0	6.7	4.3
Linalool	81.3	9.3	43.5	82.3

The efficiency of the agitation methods was determined by comparing the variability in static headspace measurements. Replicate measurements were made and the percentage coefficient of variation calculated. Table 3.2 demonstrates that the variation between samples was significantly lower for all of the agitation methods, for the two compounds with lower Log P values. This suggests that agitation is important for solubilising and dispersing volatile compounds even when they have high aqueous solubility. For the hydrophobic compound linalool (Log P = 3.38), the shaking method (SFT1) has the lowest variation of <10 % whereas all of the other treatments show levels of variation of 40-85 %. Agitation is therefore vital for effective solubilisation of hydrophobic

compounds such as linalool. Linalool solubility may also be playing a role in the results observed. Calculated values of solubilities suggest that methyl acetate, hexanol and linalool were all added at well below their solubility limits (Table 3.3).

Table 3.3 Calculated and Experimental Solubility Values for Aroma Compounds in Aqueous Solutions. * Calculated values obtained from EPI Suite (U.S. Environmental Protection Agency). ** value obtained from Hansch *et al.* (1968). * value obtained from Yalkowsky and Dannenfelser (1992). **** value obtained from Stephen and Stephen (1963).**

Compound	Calculated Solubility (mg/L)*	Experimental Solubility (mg/L)
Methyl acetate	93900	243000****
Hexanol	6890	6260**
Linalool	683.7	1590***

In conclusion, although the most time consuming of the methods investigated, use of the shaker (SFT1) as an agitation mechanism produced the most solubilised and subsequently dispersed volatiles. This was the case for both hydrophilic and hydrophobic compounds for which the shaker had the lowest levels of variation.

In a second experiment the repeatability and the variation of solution preparation was measured by static headspace. Reproducibility was evaluated by comparing 5 batches of identical solutions prepared separately with replicates within the samples also measured. Limonene (1 mg L^{-1}) was added in 2 % propylene glycol solutions as per Section 2.2.2.1 and the static headspace concentrations sampled by APcI-MS as per Section 2.3.1.1.

Figure 3.9 shows the repeatability of the headspace sampling technique with no significant differences ($p<0.05$) between the intensity of partition detected for each of the differently prepared solutions. This suggests that at this concentration the limonene would be distributed homogenously throughout the system. The

results indicate that the error between replicates of samples was equivalent to the error observed between the separately produced solutions.

This indicates that the technique for preparation and measurement of solutions is appropriate in this case. The variation in the system may be due to random error which is a result of machine reliability, atmospheric conditions and other external non-controlled factors. Separately prepared solutions may be subjected to a higher potential for random error.

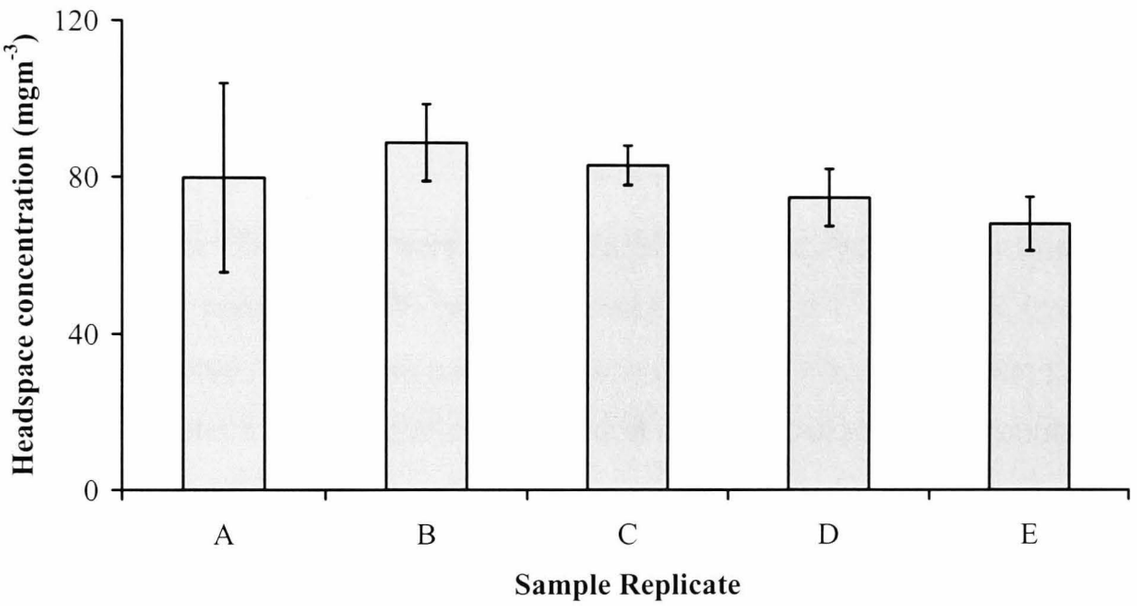


Figure 3.9 Sample replication for 5 different preparations of limonene in 2 % propylene glycol measured by static headspace. Values are the means of 5 replicates. Error bars show ± standard deviation.

3.7 Stage of Addition –Effect of Multiple Volatiles in Solution

Introduction of volatiles into solutions prior to measuring the static headspace can occur at a number of stages throughout the sample preparation process. A number of protocols were developed and compared to assess the system with the lowest variation. Volatiles were added in four protocols:

A. Volatiles (Table 3.4) were pre-dissolved in 4 mL of propylene glycol (Whirly mixer, Heidolph ReAx Top) before addition to 196 mL of water. The solution was agitated for 90 minutes at 500 oscillations min^{-1} on an SFT1 Flask Shaker. Stock solution (5 mL) was added to 45 mL of distilled water and allowed to equilibrate for 24 hours at 20°C.

B. Propylene glycol (4 mL) was pre-dissolved in 196 mL of water. Volatiles (Table 3.4) were added and the sample was agitated for 90 minutes at 500 oscillations min^{-1} on an SFT1 Flask Shaker. Stock solution (5 mL) was added to 45 mL of distilled water and allowed to equilibrate for 24 hours at 20°C.

D. Volatiles (Table 3.4) were pre-dissolved in 200 mL of water by agitating for 240 minutes at 500 oscillations min^{-1} on an SFT1 Flask Shaker. 0.2 mL of propylene glycol was pre-dissolved in 44.8 mL of water and then 5 mL of stock aroma solution was added and allowed to equilibrate for 24 hours at 20°C.

Volatile compounds were added individually or as a mixture to investigate mixture effects. Static headspace concentrations were measured with 4 replicates for each treatment.

Table 3.4 Volumes of volatile addition for different treatments

Compound	Volume of volatile added. Treatments A, B & D
Ethyl butyrate	50 μL
Hexanol	50 μL
Menthofuran	50 μL
Methyl acetate	10 μL
Linalool	80 μL
Mixture	50/50/50/10/80

The different mixing protocols described show no significant differences ($p>0.10$) for each of the methods of preparation (Treatments A-D mentioned above) (Figure 3.10).

Complete flavours generally involve combinations of multiple volatile compounds however; it has been shown that these volatile compounds can influence the behaviour of others in solution. Chaintreau *et al.* (1995) reported that varying the concentration of a single volatile from a mixture of volatiles did not affect the profile of the other compounds in the gas phase. However, other authors (Bohnenstengel 1993, Friel 1999) have suggested that small alterations in volatile mixture makeup can change the partitioning behaviour in certain systems. As it was of interest to assess the effects of different compound parameters on partition and release, inter-compound interactions needed to be minimised.

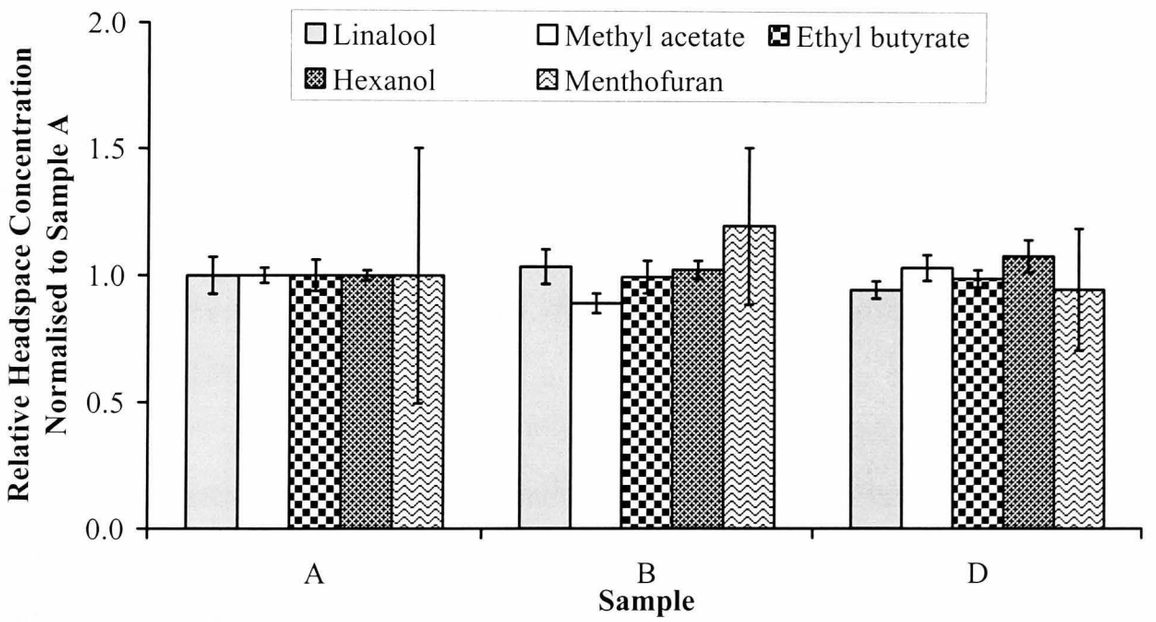


Figure 3.10 Effect of different methods of solution preparation on measured headspace concentrations. Data normalised to preparation method A. Mean values for 3 replicates.

Investigating the effects of partition of a range of volatiles in water when added singly or as a mixture was studied. Five volatile compounds (methyl acetate, ethyl butyrate, hexanol, linalool, and menthofuran) and a mixture of all 5 compounds were investigated for each of the mixing protocols mentioned previously. Results show definite variations for all of the compounds investigated. Data from Table 3.5 shows a reduction in headspace concentration for ethyl butyrate and menthofuran ($P<0.05$). A lower headspace concentration suggested that the volatile compounds may be interacting with one another, possibly by hydrophobic interaction. Hydrophobic interaction between organic molecules in aqueous solution is an attractive interaction between two non-polar molecules which exclude water coming in between the non-polar molecules. This interaction in water involves cavity formation which is then filled by the non-polar molecules and results in restricted motion of the solute (Grant and Higuchi 1990). Linalool and methyl acetate show a slight increase in partition coefficient although this not significant ($p>0.05$). Hexanol shows a significant increase in partition coefficient when added as mixtures and this is difficult to explain. Hexanol showed a 10 fold increase 1.5e5 to 1.5e6) in the detection of ion 85 in the mixture sample when compared to the individual sample which suggests that other compounds in the mixture may affect the level of detection. Other studies have shown the that aroma compound-compound interactions need to be considered when measuring partition (Bylaite *et al.* 2005) and release (Schober and Peterson 2004a).

Competition between aroma compounds for the reagent ion in the source region of the APcI-MS may also contribute to changes in signal intensity. Aroma compounds differ in their proton affinities and the charge transfer pattern may be altered as a result. Competition at the source is in fact the most likely factor for the differences observed between the aroma compounds when added as a mixture or singly. It is likely that as the compound are at infinite dilution their propensity to interact with one another in solution will be negligible (Chaintreau *et al.* 1995). Any differences observed in headspace concentration may be an artefact of the system used to measure the headspace such as competition in ionising

process. To confirm any interactions between compounds experiments using an alternative technique such as GC-MS should also be considered to assess the effect of competition at the source.

Table 3.5 K_{aw} comparison between single aroma compounds and mixtures for Treatment A. Mean and standard deviation values for 4 replicates.

Compound	Solitary K_{aw} (10^4)	Mixture K_{aw} (10^4)
Ethyl butyrate	113 ± 5.91	76.4 ± 3.22
Hexanol	10.7 ± 0.215	89.4 ± 1.12
Linalool	3.89 ± 0.282	5.59 ± 1.13
Menthofuran	414 ± 209	271 ± 160
Methyl acetate	34.3 ± 1.03	44.9 ± 9.23

Figure 3.11 shows that the coefficient of variation in the mixture samples was considerably higher than observed for the individual volatile compounds. Interactions occurring between the volatile compounds will introduce a higher level of variation into the data.

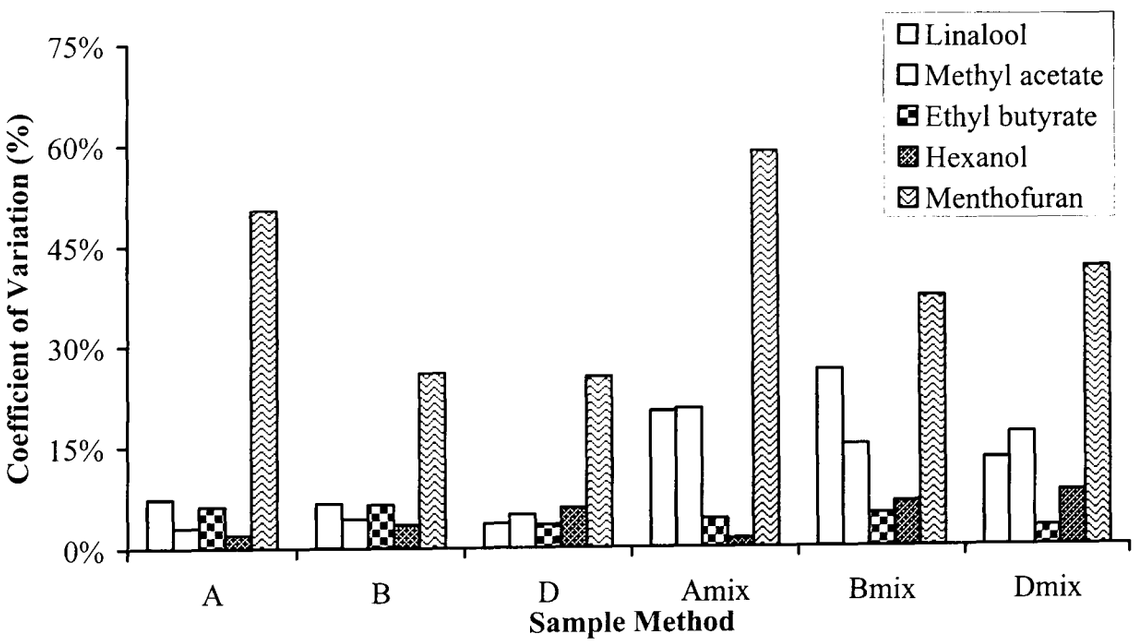


Figure 3.11 Coefficients of variation (%) of headspace concentrations for differing sample preparations for compounds added singly or as mixtures. Mean of 3 replicates.

3.8 Determination of Equilibration Time

In static headspace systems it is important that the system is at equilibrium before the headspace is sampled. If it is not at equilibrium, the true partition may not be measured. Volatile compound partition is dependent on numerous physicochemical properties including Log P, solubility in the aqueous phase, vapour pressure, molecular size, diffusivity, mass transfer, agitation and boiling point. If these factors affect the partitioning of volatile compounds then they may also influence the time required for equilibrium to be reached. A range of volatile compounds, varying in physicochemical properties, were assessed at 1, 2 and 4 hours equilibration time.

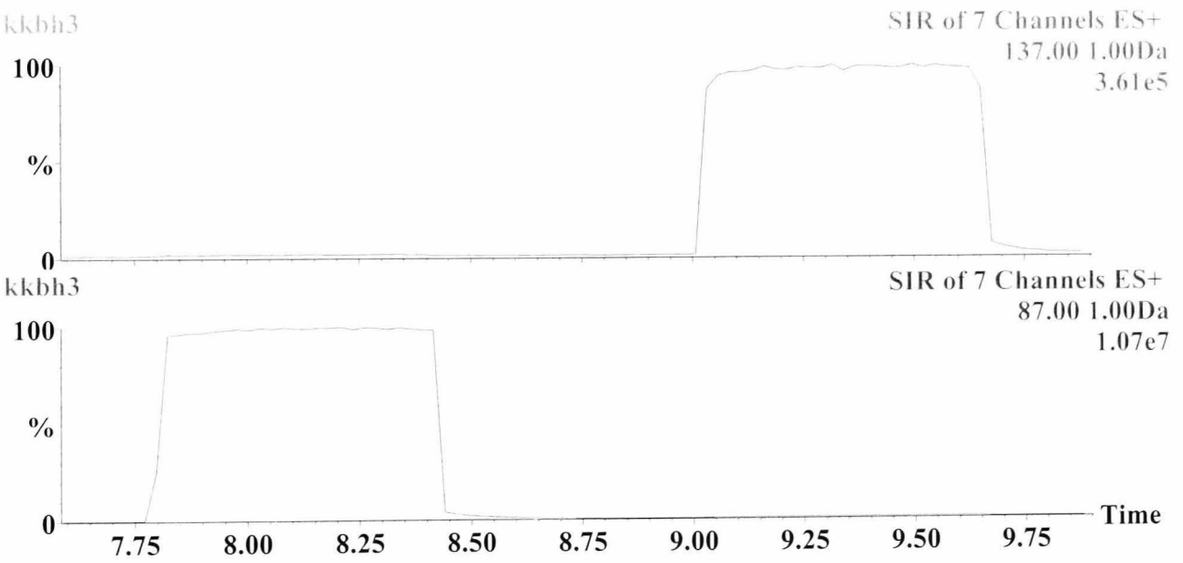


Figure 3.12 Headspace concentrations after 4 hours equilibration. Top – limonene, Below – diacetyl.

Figure 3.12 demonstrates the rapid increase in APcI-MS signal followed by the plateau phase which is an indicator that the system is at equilibrium. In a non-equilibrium situation, the rapid increase in intensity would be followed by a slow increase in the plateau phase. It is clear that both the hydrophilic compound diacetyl and the hydrophobic compound limonene have both reached

equilibrium. This implies that, irrespective of the compounds properties, equilibrium was reached after 4 hours. Table 3.6 shows no change in headspace concentration between 1 and 4 hours equilibration time. This suggests that most of the compounds tested reached equilibrium after 1 hour. All the compounds tested reached a plateau phase after 4 hours. This correlates well with some real applications. An example is the assessment of the headspace for testing blood-ethanol concentrations which uses an equilibration time of only 15 minutes (Penton 2005). Equilibration time of esters, aldehydes and alcohols was shown to be achieved after only 45 minutes although this was at 90°C from Arabica coffee (Sanz *et al.* 2001). These data suggest that the equilibration of molecules is dependent on the properties of the aroma compounds themselves and as such systems should be measured for appropriate equilibration times for all the different volatiles. Essentially though it appears that most volatiles will reach equilibrium within aqueous systems within 4 hours as is suggested by our results.

The results also show a distinct effect of the hydrophobicity of the compound on the time taken to reach equilibrium with hydrophilic compounds not varying between 1 and 4 hours (diacetyl, dimethyl pyrazine) but the more hydrophobic compounds (linalool and limonene) showing a slower equilibration time. A potential explanation for this is that the lower solubility in water may effect the time taken for the compounds to solubilise in the aqueous phase and thus increase the time to reach equilibrium. It may also be related to the fact that more hydrophobic compounds tend to have lower vapour pressures and will thus have a reduced speed at which they become available in the gas phase.

Table 3.6 Amounts of volatiles in the headspace (measured as ion counts) after different equilibration times.

Compound	1 hour equilibration	4 hour equilibration
Diacetyl	1.04e7	1.07e7
Dimethylpyrazine	5.91e5	6.17e5
Ethyl butyrate	9.82e7	1.04e8
Hexanol	3.60e6	3.64e6
Methyl acetate	3.00e6	2.76e6
Limonene	1.98e5	3.61e5
Linalool	1.95e6	2.17e6

3.9 Development of Droplet Analysis Systems

Development of a suitable system for studying the dynamic release of volatile droplets provided a range of challenges:

1. Injection of a suitable droplet size such that it was visible, independent and was released into the gas phase at concentrations suitable for detection using APcI-MS techniques.

2. That the droplet was not subject to migration or disruption during the sample production process.

3. That the system developed was capable of being adapted to measure changes in ion concentrations and that suitable flow rates of sampling could be achieved.

4. It was important that the parameters could be altered to analyse a range of compounds using this system

Initial experimentation showed that all of the volatile compounds assessed could be injected into an aqueous solution as an independent, single droplet. However, the droplets were subject to migration (very rapid <1 second) to the air-solution interface. Aqueous solutions were therefore not a suitable matrix for this system. As an alternative, thickened solutions were investigated. The thickener used in this case was xanthan gum due to its ability to produce thickened solutions at low concentrations. A range of concentrations were investigated from 0 % (water) – 0.8 % xanthan and the migration of the droplet was determined by the time it took to reach the surface from a set injection point (depth in tube 25 mm). However, increasing xanthan concentration may have detrimental effects on the release of the droplet from the solution especially when the samples are consumed by panellists. Yven *et al.* (1998) showed that 0.1 % xanthan reduced the release of aroma compounds and reported reversible hydrogen bonding between xanthan and 1- octen-3-ol. Ten minutes was chosen as a suitable period of time after which if no movement of the droplet occurred it was considered appropriate for measurement.

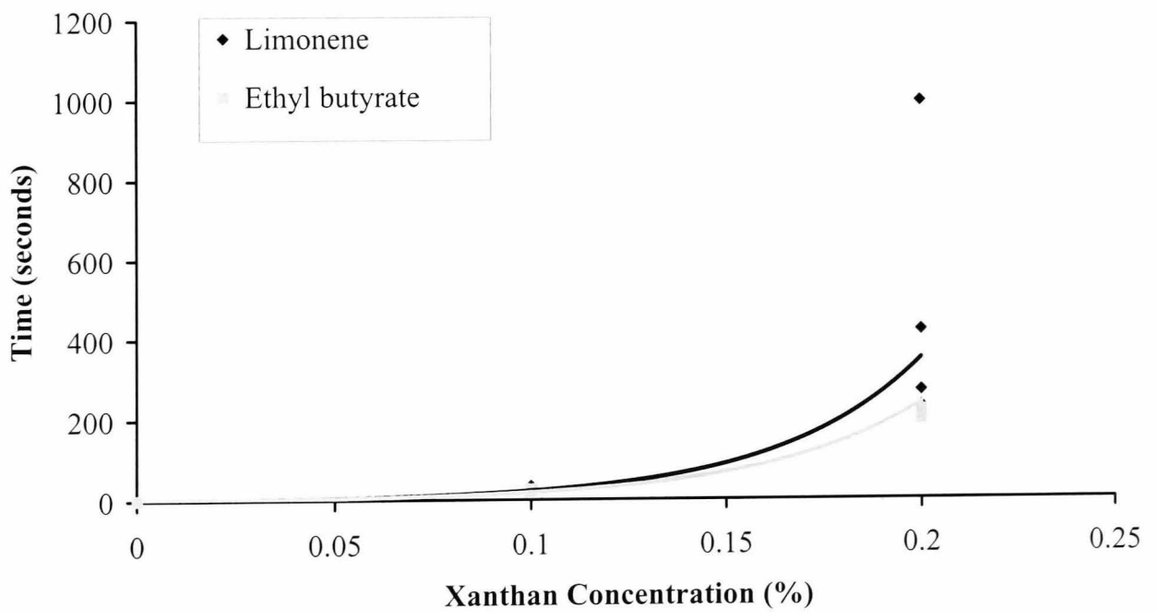


Figure 3.13 Effect of xanthan concentration on time to reach the surface for 2 µl volatile droplets. Points represent replicates.

Figure 3.13 demonstrates an exponential increase in retention of the volatile droplet with increased xanthan concentration. At 0.3 % xanthan the droplet was retained for periods of greater than 600 seconds for all replicates. This suggested that steric hindrance provided by the xanthan coils may be restricting the droplet migration. Differences existed between the two different volatile compounds investigated and this may be due to differences in physico-chemical properties. Ethyl butyrate showed a more rapid progression to the interface than limonene. However, this was unlikely to be due to the specific gravity of the compounds, as limonene has a lower value (0.848 g / mL) than ethyl butyrate (0.878 g / mL). Other factors such as polarity or hydrophobicity may be more important. For instance, limonene may interact with the xanthan coils to a higher degree than ethyl butyrate. Recently, Bylaite *et al.* (2005) have shown that increasing xanthan concentration (up to 0.8 %) significantly reduced the partition coefficient and release rate of ethyl butyrate and limonene but the effect on limonene was more pronounced due to increased binding with xanthan molecules.

Limonene, due to it's oil-like nature, may also be acting more like an oil-in-water emulsion stabilised by the presence of the xanthan which may explain the slower migration times observed. An important point to note at this stage is that dissolution of the droplet into solution has not been measured and this may be important to consider especially for more hydrophilic compounds which may dissolve during the migration period. If this is the case the size of the droplet may become reduced causing more rapid migration through the solution.

3.10 Determination of Gel Injection Time

Migration of droplets also occurred in the hot gelatine-sucrose acid mixture. Preliminary studies assessed the potential of injecting the droplets after gelation of the gel samples. Results showed that a single droplet was not created but that the introduction of the needle formed a 'channel' in the gel into which the aroma compound spread once injected. These gel samples showed very variable release

from droplets, which may be due to the inefficiency of the sealing procedure to ensure retention in the gel. Subsequently, volatile compounds were injected before the gel had fully set. A range of time points during this setting process were assessed, from 2-30 minutes after the hot gel was placed in the sample trays.

Table 3.7 Effects of time during gelation on the movement of pure volatile droplets

Time after gelation (min)	Droplet Retained	Comments
2	n	Migrated rapidly to the surface
5	n	Migrated to the surface
10	n	Migrated to the surface
15	y	Droplet showed no migration
20	y	Droplet showed no migration
25	y	Droplet showed no migration but volatiles became difficult to inject due to gel consistency
30	n	Injection caused cavities in the gel and streams of aroma rather than droplets were produced

The results described in Table 3.7 show that, at the beginning of the gelation period, the volatile droplet behaved as it did in aqueous solution and showed migration to the solution surface. As the gel gained a more rigid structure the droplet was retained at the site of injection and the syringe could be extracted with no adverse effects on the gel structure. At longer time points the gel became increasingly viscous and the syringe served to produce a stream of volatile rather than a droplet. In conclusion, the optimum time point for injection of volatile droplets into the gel was at 15-20 minutes into the gelation period.

3.11 Droplet Injection System

Volatile compound droplets have been shown to migrate to the surface of aqueous solutions (Section 3.10) due to their low solubility and lower density

(compared to water). A system was developed that allowed investigation of droplet release in an aqueous solution. A dynamic headspace apparatus was set up in which the droplets could be injected into the base of the system (Section 2.3.1.5). The droplets migrated to the surface of the solution where they came into contact with the gas phase and the release could be measured by allowing a flow of N_2 to purge the headspace and deliver the volatile compounds to the APcI-MS. The dynamic headspace technique allowed the measurement of changes in headspace concentration as the gas phase above the sample was depleted. Numerous researchers have utilised this approach to measure aroma release in real time (Marin *et al.* 1999; Marin *et al.* 2000; Parker and Marin 2000). They suggest that this represents more closely the situation experienced during consumption *in vivo*.

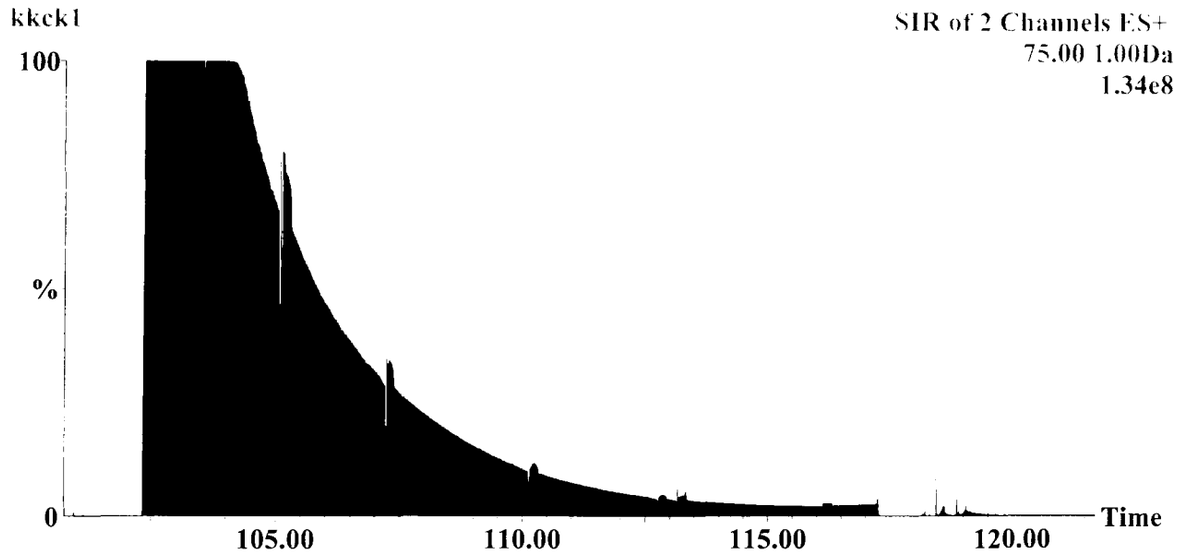


Figure 3.14 Change in headspace concentration for the release of a 1 µl droplet of methyl acetate release from aqueous solution. The droplet was injected at 102 min and the intensity exceeded the dynamic range of the instrument (hence the plateau region) before decreasing as the headspace was flushed constantly with nitrogen.

Initially droplets (1 µL) were injected, as per the aforementioned method. However, as shown in Figure 3.14, the rapid depletion of the gas phase concentration produced levels of aroma which exceed the maximum detection capacity of the mass spectrometer. Reduction of droplet volume to 0.2 µL

decreased the gas phase concentrations detected and ensured that a larger proportion of compounds could be investigated. Importantly, ethyl butyrate and ethyl hexanoate at 0.2 μL still exceeded the detection limit of the mass spectrometer and therefore could not be assessed reliably using this technique.

3.12 Confirmation of Sample Concentrations for Droplets and Solubilised Samples in Gelatine-Sucrose Gels

The levels of aroma compound in the different types of samples (droplets and solubilised) were monitored by dissolving gels in water and measuring the equilibrium headspace. Figure 3.15 shows a typical result for ethyl butyrate where there was no significant difference in the equilibrium headspace (and therefore aroma content) despite the solubilised aroma compound being added when the gel was hotter compared to the droplet injection at lower temperatures. All the aroma compounds studied showed the same behavior.

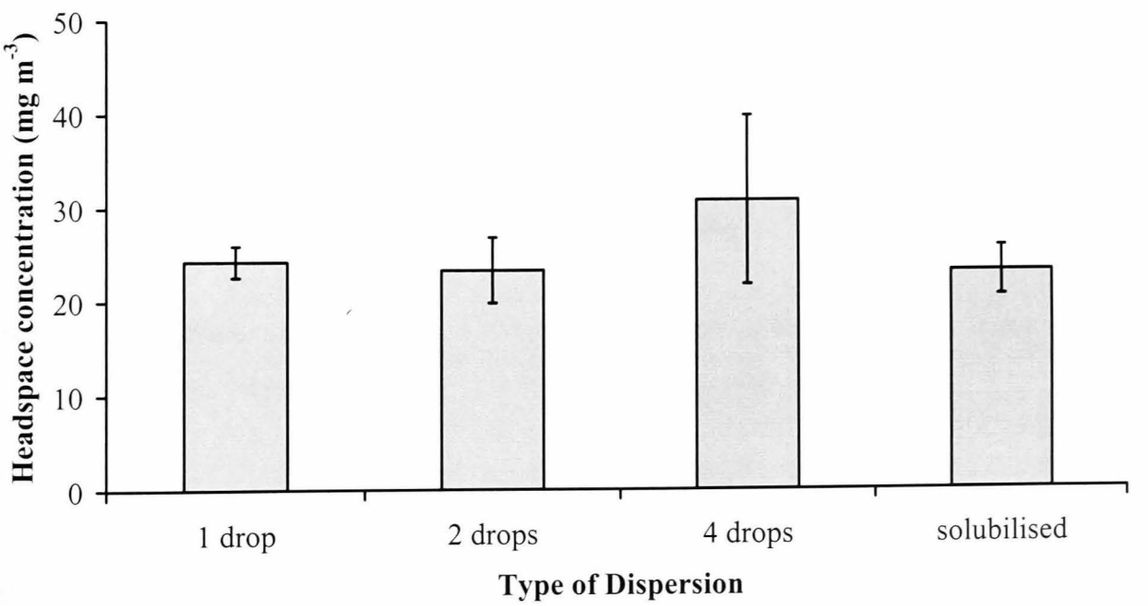


Figure 3.15 Aroma content of different gel samples (containing 1, 2, 4 drops or solubilised ethyl butyrate) measured by dissolution in water and equilibrium headspace measurement. Three replicates were measured, error bars represent the standard deviation.

3.13 Initial Investigations on Stirred Yoghurt

Agitation was shown to be important for dispersing volatile compounds in solution and for dispersion of volatile compounds in hot gelatine-sucrose mixture. Yoghurt is an alternative matrix in which levels of mixing were investigated. A simple test was developed whereby, a yoghurt sample (50 mL) had red food dye (200 mg L^{-1}) (Supercook, Leeds, UK) and linalool (125 mg L^{-1}) added and stirred for differing amounts ranging from 0-30 stirs. The colour mixing and aroma release of linalool *in vivo* were assessed for the different levels of manual stirring with a spoon.

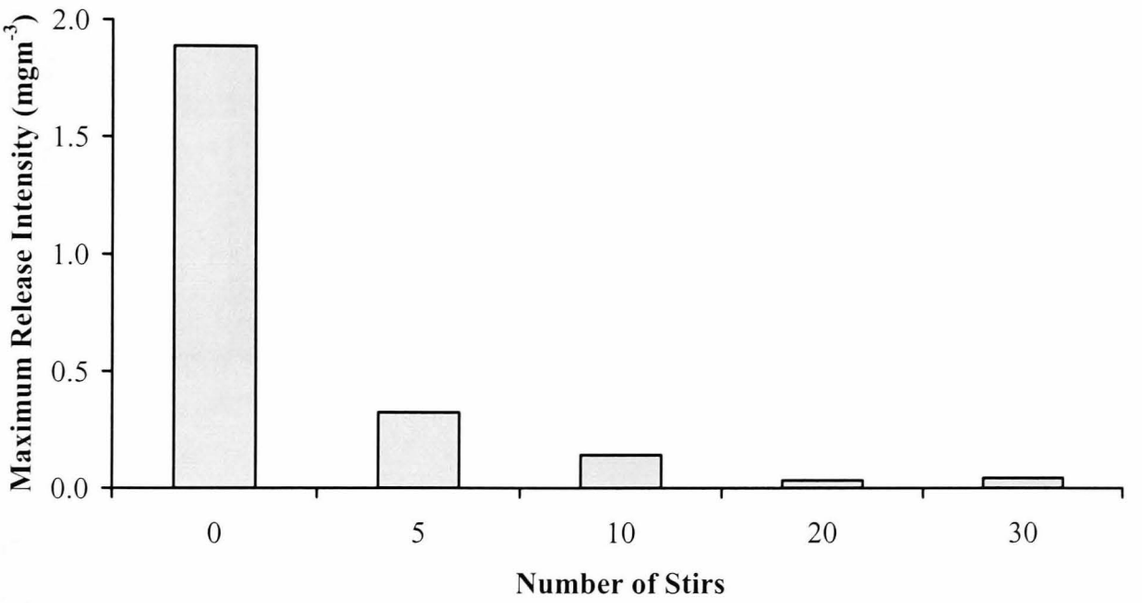


Figure 3.16 Effect of increased stirring on linalool release *in vivo* from stirred yoghurt samples. Mean of 3 replicates for a single panellist.

Figure 3.16 indicates that increasing the number of stirs caused a significant decrease in the intensity of volatile release. Increased dispersion of the volatile compounds would increase the solubility within the yoghurt phase. This reduced the quantities available for partition when consumed in mouth. At low levels of dispersion (i.e. short mixing times) the volatiles would be concentrated in phase separated regions, which would lead to an increase in release when these were

consumed. At higher levels of dispersion, the volatiles would be distributed more homogenously throughout the yoghurt and thus the areas phase separated aroma would be reduced. This was suggested by the significant reduction in intensity observed at higher mixing times. This suggestion is partially supported by Brauss *et al.* (1999). They reported that decreasing the fat content of the yoghurt served to increase the intensity of lipophilic compounds released due to a reduction in dispersion of these compounds in the yoghurt. In this case the reduction in compound dispersion was a consequence of the reduction in stirring time.

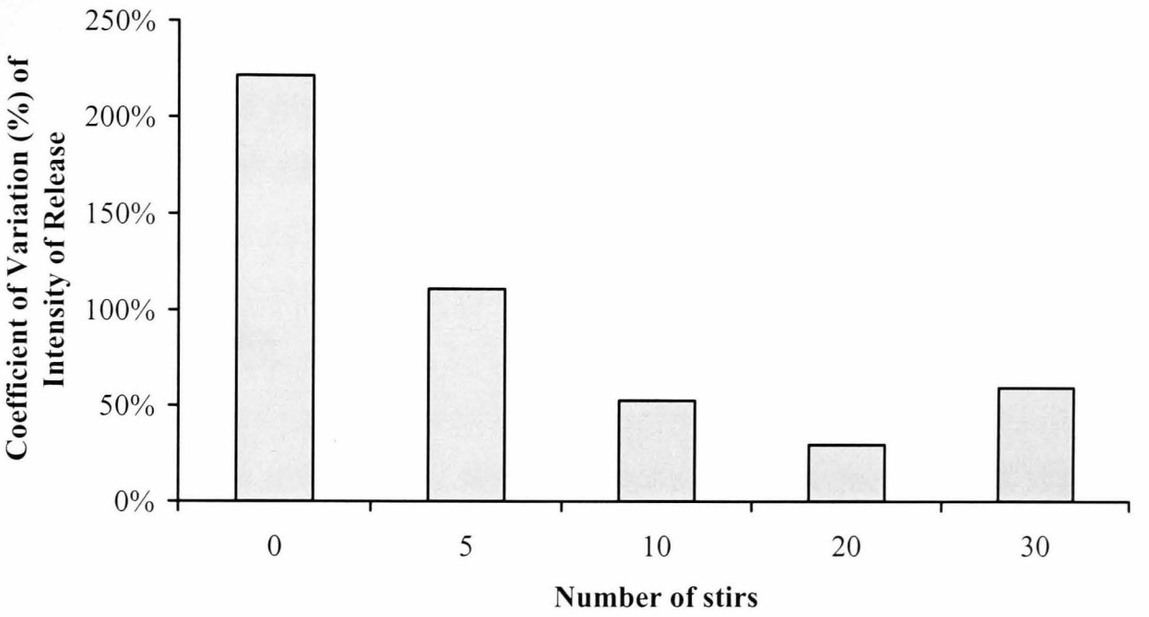


Figure 3.17 Effect of varying stirring levels on the coefficient of variation (%) for intensities of linalool release *in vivo* from stirred yoghurt samples. Mean of 3 replicates.

The previous conclusion is reinforced by Figure 3.17. This illustrates the reduction in percentage coefficient of variation with an increased number of stirs. A more homogenous system will display a more consistent intensity of release and therefore the level of variation will be reduced.

Figure 3.18 shows a visual representation of how stirring affects the distribution. The images indicate that after five stirs the sample was still fairly heterogeneous

with distinct white regions remaining within the coloured regions. Ten stirs shows a higher degree of homogeneity but still with some white regions. By contrast, after twenty stirs, the sample appears to be uniform in colour suggesting homogeneity of the sample. This data correlates well with the decrease in intensity and variation observed in Figures 3.16 and 3.17.

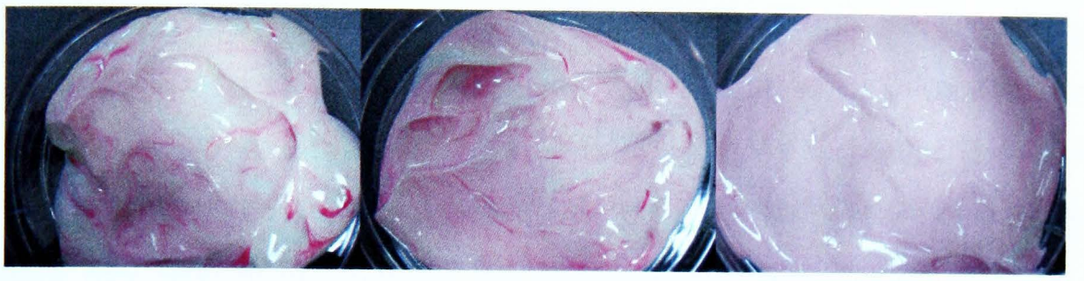


Figure 3.18 Level of stirring on dispersion of red food dye in yoghurt. From left to right (5 stirs, 10 stirs and 20 stirs).

3.14 Conclusions

This Chapter illustrates some of the preliminary experiments that were carried out in order to standardise the methodology and to assess suitable concentrations for further studies. The earlier sections demonstrate the importance of the dispersion methodology as well as assessing the contributions to ion intensities of the different matrices investigated. Sections 3.6 - 3.8 are important in defining conditions and likely sources of error for sample preparation for headspace analysis. Sections 3.9 - 3.12 assess the development of systems to measure volatile release as droplets. Finally, Section 3.13 looks at how these different levels of dispersion can be assessed in yoghurt.

4 HIGHER SCALE AROMA DISPERSION – EFFECTS OF LAYERING AND STIRRING ON RELEASE

In industry, many food products are subjected to a wide range of dispersion techniques in order to produce homogenous systems or to ensure complete mixing of flavours and colourings. This is especially important in the desserts and confectionery industry where foods are continually developed to produce new and innovative products for an ever expanding market. Flavour is an important aspect in these products and the manner in which it is added and dispersed may affect its final perception by the consumer. Layered confectionery and unflavoured yoghurts with additional 'flavour-containing' segments are currently two areas in which there is much interest.

The dispersion of volatile compounds throughout a food matrix can be seen to occur on a number of scales depending on the so called 'Scale of Scrutiny'. This implies that the degree of mixing that occurs depends on the scale at which you observe a product (Chapter 1). This section investigates the dispersion of volatile compounds on this 'higher scale' of mixing, firstly in a semi-solid yoghurt product where mastication is likely to have reduced importance, and subsequently in two confectionery systems where in-mouth mixing may play a larger role in the consumption process.

Numerous studies have been carried out on aroma release from both yoghurt and confectionery systems; however, there is very little published data on the effect of dispersing volatile compounds in the product and their subsequent effects on release. In the case of yoghurts, most data is related to sensory characteristics due to the addition of sweeteners or thickeners (Brennan *et al.* 2002; Kora *et al.* 2003; Kora *et al.* 2004) and varying fat levels (Tuorila *et al.* 1995) on release rather than the method of addition. Lubbers *et al.* (2004) investigated the effects

of stirring on release during storage. Brauss and co-workers (1999) showed that reduced levels of fat (0.2%) increased the intensity and reduced the persistence of lipophilic aroma compound release. Kant *et al.* (2004) and Mei *et al.* (2004) both utilized in-nose techiques to assess aroma release during consumption from flavoured yoghurts by altering the components added to the yoghurt. Although these studies show a wide range of interest in aroma release from yoghurt, the principles of dispersing flavouring agents and their subsequent effects on release have yet to be established. A system was set up whereby the level of volatile compound dispersion could be controlled and assessed and its subsequent release could be followed. APcI-MS was chosen to investigate aroma release and standard yoghurt was used throughout the experiments to avoid confounding factors such as effects of fat content or sweeteners.

Preliminary experiments showed that stirring volatile compounds into the yoghurt using different regimes could affect aroma release by producing areas of high and low concentration throughout the sample (Section 3.13). Following on from this two methods for dispersing aroma compounds into yoghurt were investigated. These were the use of a standard overhead stirrer (OST Basic, Yellowline, Staufen, Germany) and a static mixer system developed by Birmingham University. The stirrer consisted of a moving blade within the sampling vessel to disperse an aroma phase into the bulk yoghurt phase. The static mixer system as described in Chapter 2, was comprised of a number of static mixing blades (Kenics) through which two flows of yoghurt were pumped to increase the level of mixing.

4.1 Effect of Stirring of Yoghurt

The methodology for carrying out static equilibrium headspace and breath-by-breath analysis of yoghurt samples is described in Chapter 2 and the important ions to be assessed are described in Section 3.1. The yoghurt used was Yeo Valley Natural Bio Organic Yoghurt (Cannington, Somerset, UK) and had an

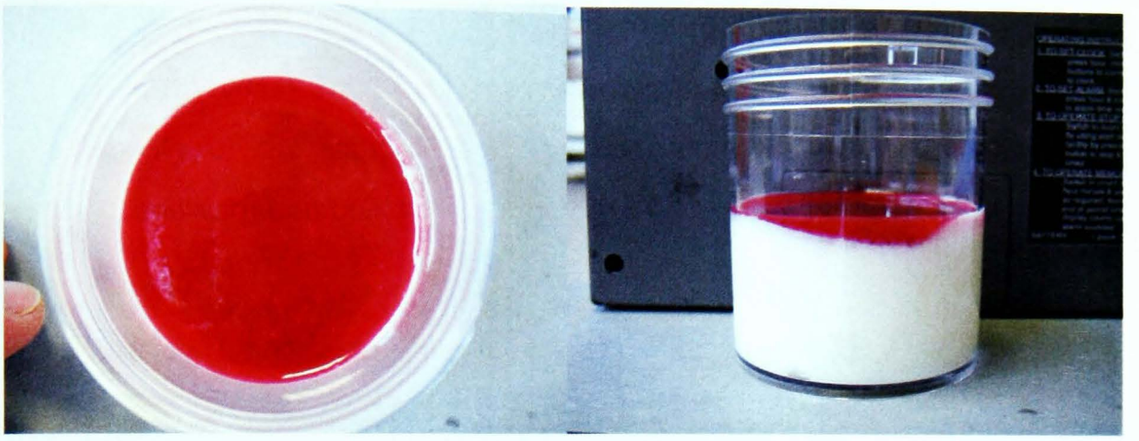
overall fat content of 4.1 %. Sucrose (5 %) was premixed into the yoghurt prior to stirring to improve sample palatability. The flavour added was a commercial strawberry flavour which had been pre-dissolved in a propylene glycol-water mixture (20:80) and added to strained compote (Yeo Valley, Cannington, Somerset, UK) at a final concentration of 25 mg L^{-1}. Compote (10 mL) was added to the surface of 50 mL of yoghurt and stirred for periods of time ranging from 10-900 seconds.

Figures 4.1 and 4.2 illustrate the mixing patterns observed for a range of mixing times. They show that at short mixing time periods (<60 seconds) the compote-flavour mixture was concentrated towards the surface of the samples, whereas increasing the mixing time up to 900 seconds the yoghurt and compote phases became more homogenous. This increase in homogeneity means that the aroma compounds would be better dispersed throughout the matrix. Homogeneity in this instance is related to the appearance of the system with a greater level of mixing achieved between the yoghurt and compote phases with increased mixing time. This is indicated by the increases in colour dispersion observed in figures 4.1 and 4.2. It is important to note at this stage that although the samples will appear to be more mixed the increased mixing may only serve disperse the compote phase throughout the yoghurt phase producing a biphasic system from which a range of molecules (not only aroma compounds) will diffuse. This may have implications for aroma release but the level of dispersion will still be increased by increasing the stirring time.

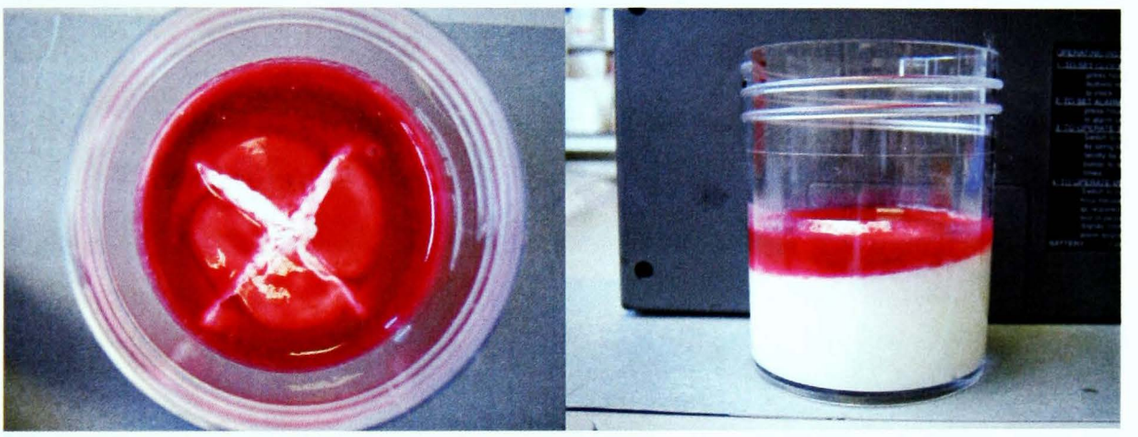
Volatile release was measured in two ways, first by static equilibrium headspace above stirred yoghurt and second by monitoring release *in vivo* during eating. Two marker ions (m/z 117 and 89; Section 3.1) were monitored to follow release of ethyl butyrate and ethyl acetate respectively. Release data was normalised to the most dispersed (mixed) sample.

It was hypothesised that mixing would reduce headspace concentrations as the volatile compounds were mixed from the surface into the bulk of the yoghurt;

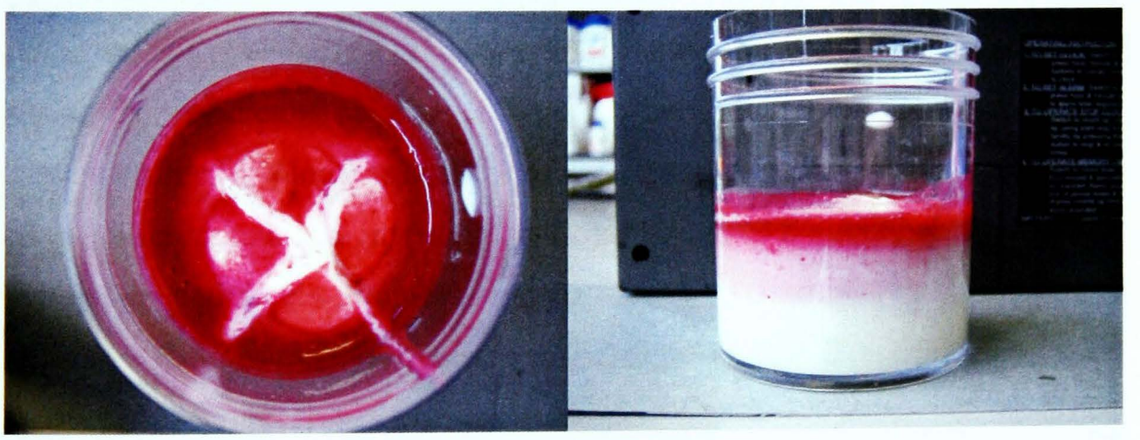
mixing would also affect release *in vivo* by decreasing the intensity and increasing the time to maximum intensity.



No mixing (surface) No mixing (side)

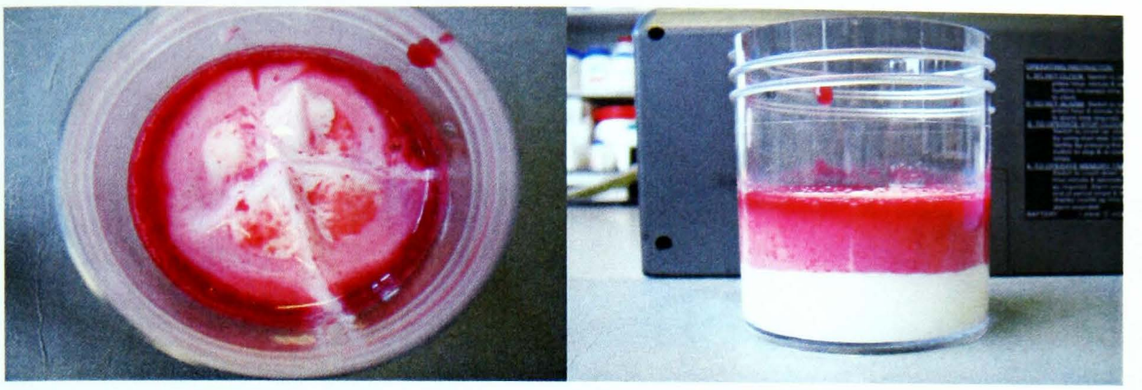


10 seconds mixing (surface) 10 seconds mixing (side)

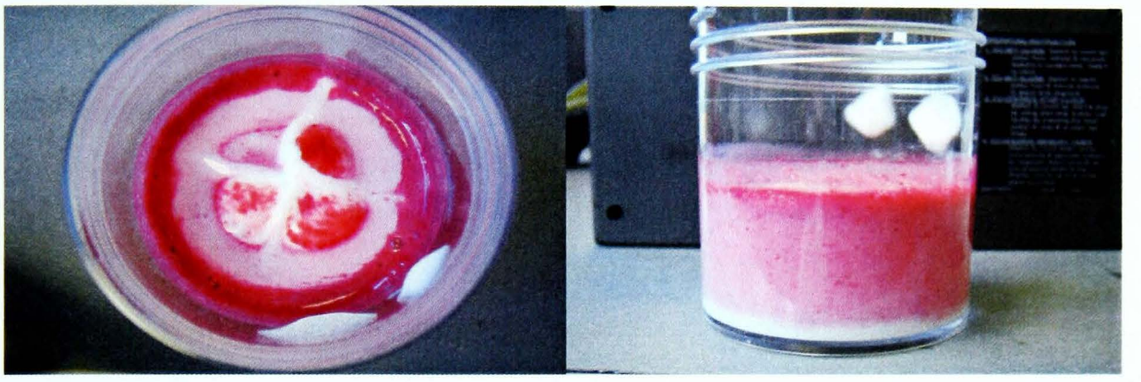


30 seconds mixing (surface) 30 seconds mixing (side)

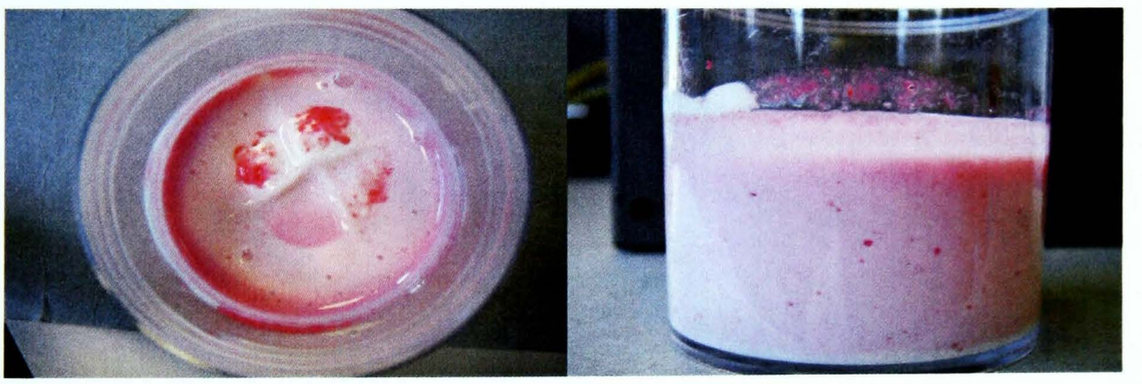
Figure 4.1 Digital images of stirred yoghurts ranging in stirring time from 0-30 seconds. A surface and side view are both included.



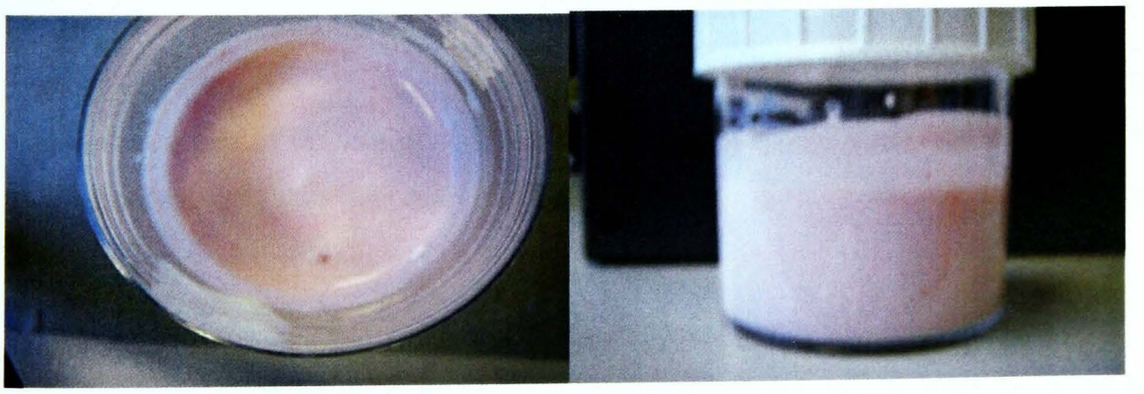
60 seconds mixing (surface) 60 seconds mixing (side)



180 seconds mixing (surface) 180 seconds mixing (side)



300 seconds mixing (surface) 300 seconds mixing (side)



900 seconds mixing (surface) 900 seconds mixing (side)

Figure 4.2 Digital images of stirred yoghurts ranging in stirring time from 60-900 seconds. A surface and side view are both included.

4.1.1 Static Equilibrium Headspace

Figure 4.3 shows the static headspace concentration above the samples after equilibration at 4°C for 1 and 16 hours. The results indicate that increased mixing time reduced the levels of volatile compound that were able to partition into the gas phase during the equilibration period. Low levels of mixing (10-40 seconds) showed significantly higher headspace levels than the homogenous samples (180-900 seconds). This demonstrates that increased mixing time influences the dispersion of the volatile compounds and their ability to partition into the gas phase.

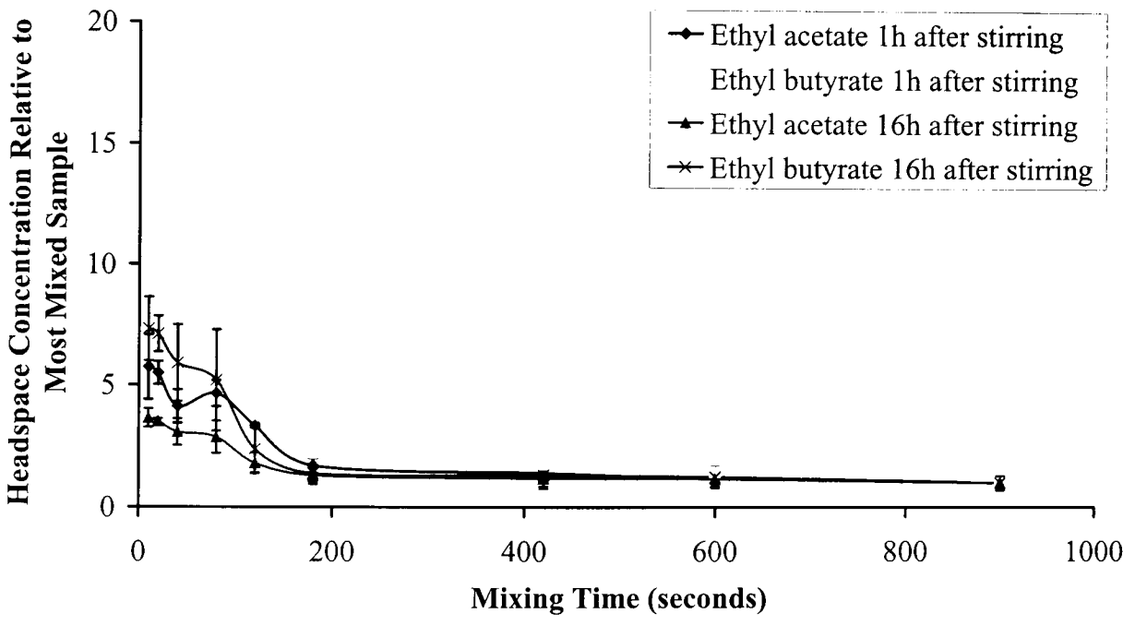


Figure 4.3 Effect of stirring time on the static headspace concentration above yoghurt samples after storage (4°C) for 1 hour or 16 hours. Data normalised to 900 second sample. Mean of three replicates. Error bars represent ± 1 standard deviation.

The headspace decreased with storage time despite being held in a sealed container. This suggests a slow equilibration of the volatile compound between the air, water and fat phases in the yoghurt. Samples mixed for longer time periods show similar levels of aroma partition into the headspace and most of the

differences occur for the shorter mixing times. This is likely to be due to the increased dispersion of the aroma compounds. The data appears to show that the samples become more similar (i.e. a reduction in the differences between the most and least mixed samples) with increased equilibration time and this suggests that eventually the samples may reach the same concentrations in the headspace for all the samples. This may however, require a much longer equilibration time than was observed in this case and this may have implications for storage of these types of products. In a "real" situation where a consumer would add their compote directly to the yoghurt prior to consumption then this storage would not be relevant however, from a production point of view, i.e. addition at factory level where time between mixing and consumer consumption is measured in days, these reductions may influence the final product experienced by the consumer.

Differences were also noted between ethyl acetate and ethyl butyrate. Initially these might be due to differences in partition coefficient but the fact that the two compounds reach a common, constant level with stirring time suggests a slow solubilisation with stirring as the compounds dispersed in the yoghurt phases. This is indicated by the fact that increasing the mixing time reduces the concentration in the headspace closer to that of the most mixed sample (900 seconds). If this is the mechanism, then the more hydrophilic ethyl acetate disperses in the biphasic system faster than the more hydrophobic ethyl butyrate. This is logical as the ethyl butyrate will have to transfer through water into fat and the rate-limiting factor is likely to be matrix solubility. Release of aroma compounds from low fat yoghurts has been shown to increase the intensity and decrease the time taken to reach this maximum intensity for hydrophobic compounds (Brauss *et al.* 1999). Measurements of water activity would have allowed a better idea of the degree of water movement within the system and may have given a better indication of the likely dispersion of the different aroma compounds at different mixing times.

Since yoghurt is a complex matrix, the effect of other components on partitioning should be considered. Sugar was added to yoghurts at 5 % loading but the concentration in the aqueous phase will be higher after taking into account (fat 4.1 %) and solids content (10.1 %). Thus the sugar content is the aqueous phase is about (10.7 %). There are many reports of solutes like sugar affecting partition of volatile compounds but significant effects are usually seen when the sugar content is greater than 20 %. In this case, the headspace concentration is significantly higher ($p < 0.05$) for ethyl butyrate at shorter mixing times than for ethyl acetate although this is not the case for longer mixing times ($p > 0.2$). This may be due to the effects of sucrose on the different compounds with sucrose increasing the partition of ethyl butyrate to a larger extent than ethyl acetate. This is supported slightly by the work of Nahon *et al.* (2000). They showed less effect of sucrose concentrations on ethyl acetate increase, with 10% sucrose solution showing only 10 % increase in gas-solution partition coefficient for ethyl acetate but a 34 % increase for ethyl butyrate. This reduction in salting out behaviour may partially explain why ethyl butyrate has a higher relative concentration than ethyl acetate at shorter mixing times. This may be one explanation however, the presence of the fat phase should lead to a higher retention of ethyl butyrate than was observed from these results. A potential reason behind the higher relative level of salting out is that the aroma compounds were added having been pre-dissolved in propylene glycol and this may have increased their solubility in the compote phase reducing the effects of the fat levels on their ability to retain ethyl butyrate.

4.1.2 *In vivo* Release from Stirred Yoghurt

Having studied the headspace above yoghurts, attention now turned to the effects of stirring and aroma distribution on release *in vivo*. Figure 4.4 shows the I_{max} values from yoghurts stirred for 10-900 seconds for 4 different panellists. Each panellist value was an average of 3 spoonfuls from the same sample pot so each point represents the average of 12 samples. Panellist data was normalised to the

most mixed sample (likely to have the lowest level of variation) to reduce the effects of inter panellist variation. Normalised variation was based on the coefficient of variation multiplied by the normal values. The mean panellist data produced a coefficient of variance (cv %) for each sample and this was multiplied by the normalised values to produce a normalised variation for each sample. Similar results were observed in the trends for individual panellists but the data was normalised due to the high variations in aroma release intensity between panellists rather than between samples.

The results show that overall there was no significant difference ($p<0.05$) in the intensity of release for either of the compounds. This indicates that although the level of mixing varied significantly this had little effect on the release of the volatile compounds during consumption. This is most likely because rapid mixing of the sample in mouth during consumption eliminated any effects of the pre-consumption mixing on the release of aroma *in vivo*.

Samples were mixed as previously described for Section 5.1, in the cold room at 4°C, and the samples were consumed immediately after production such that little time for aroma diffusion or heating could occur.

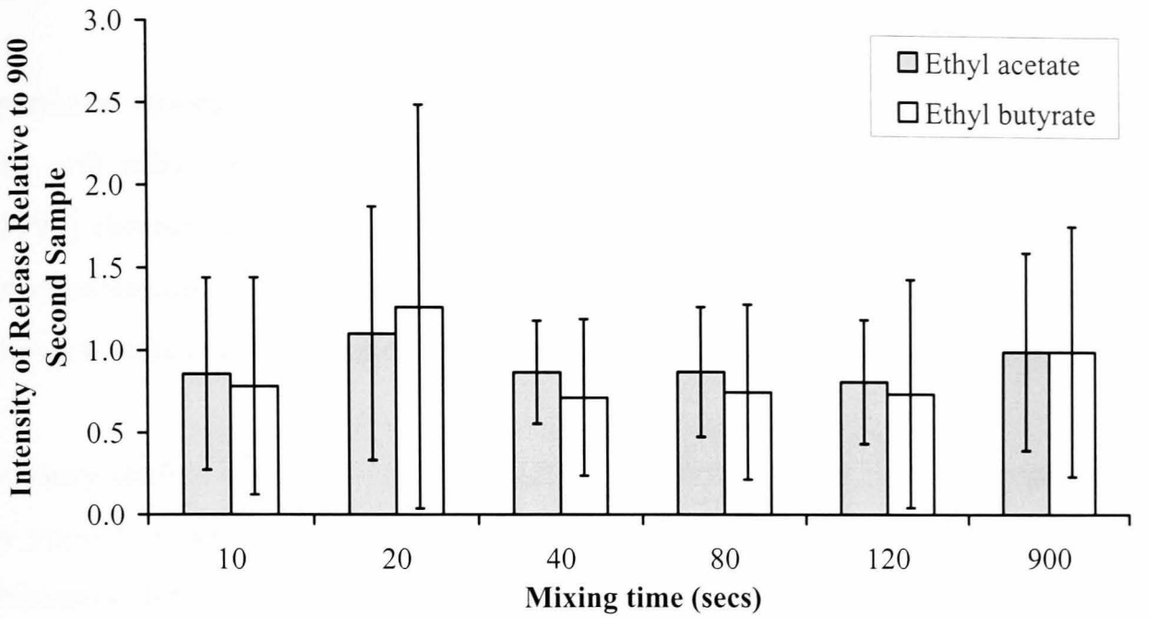


Figure 4.4 Effect of short term stirring times ranging from 10-900 seconds on the I_{max} of aroma release *in vivo* from yoghurts. Data normalised to 900 second sample for each panellist. Four panellists each consuming 3 replicates.

The sample stirred for 900 seconds shows a higher level of aroma release than would be expected if the trend towards a lower intensity of release with increased mixing time is the case. In fact, the data shows that overall there is very little influence on the length of the stirring time on the overall intensity of aroma release even after 900 seconds. Yoghurt is known to shear-thin but viscosity normally recovers with time. It is possible that in this case that there is an effect of yoghurt structure on the amount of aroma released as some of the panellists noted that the 900 second sample not only appeared to be less viscous but also that the flavour was more intense. The main change in the samples due to increased mixing time will be the reduction in viscosity however, Cook *et al.* (Cook *et al.* 2003b) demonstrated that aroma release intensity *in vivo* when measured by APcI-MS was unaffected by the viscosity induced by thickeners but a subsequent was observed in the perception. This may explain why the samples appeared to be more intense in this experiment due to the reduction in viscosity, but the aroma concentrations remained at similar levels. However, with no

measurements of the viscosity the measurement was on a purely panellist opinion basis and may not represent the actual effects on release.

Increased mixing time increases the shear-thinning behaviour of the yoghurt and this will affect its structure and release properties. Benezech and Maingonnat (1993) showed that stirring of commercial yoghurts showed viscosity decline to an equilibrium at about 600 seconds. This corresponds well to our data which shows a reduction in headspace concentration as the mixing time increased.

Sensory studies have shown that increased yoghurt thickness reduced apple notes in yoghurt (Kora *et al.* 2003) yet Brennan *et al.* (2002) showed that yoghurt thickness did not affect flavour intensity experienced by children from 'real' flavours. Cook *et al.* (2003a) demonstrated that although aroma release was unaffected by viscosity of solutions, the perception was related to the oral shear stress of the solution. This suggests that although the aroma release data shows no differences in release, changes in the oral shear stress of the yoghurt may have a significant effect on the intensity of perception.

To investigate the potential shear-thinning effect further, yoghurt was stirred for times between 120 and 900 seconds shown in Figure 4.4. Figure 4.5 shows the effects of intermediate stirring times (180-900 seconds) on the intensity of volatile release. Data is expressed relative to the 900 second sample which acts a marker between the two experiments. No significant difference in the intensity of release was observed. Initial predictions suggested that these longer time periods would show no effects on release because at 180 seconds (the shortest time period tested), the sample appears to have homogenous colour distribution (hence flavour) and limited reductions in viscosity at longer time periods.

A caveat to mention at this point is that although the flavour is assumed to be homogenously distributed in this system the term homogenous is a relative term related to the extent of mixing and colour dispersion achieved. The yoghurt-compote mixture may well form a mixed biphasic system from which aroma and

other molecules will be subject to partition into each phase. However, for the purposes of this study where the extent of mixing is the important aspect, the increase in homogeneity is represented by the samples mixed for longer periods of time.

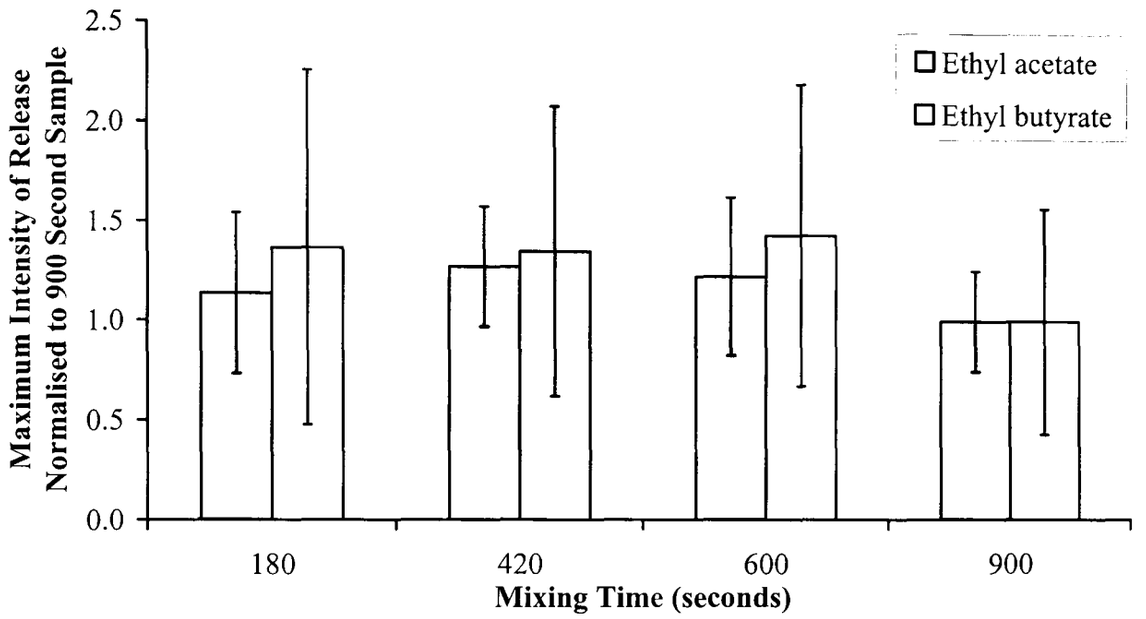


Figure 4.5 Effect of longer stirring times on I_{max} of release from yoghurt. Data Normalised to 900 second sample. Four panellists consuming three replicates each.

The time to maximum intensity (T_{max}) values all occur at values <0.05 minutes and show no difference between samples. The rapid process of yoghurt consumption, whereby the sample is taken into the mouth, moved around and rapidly swallowed, could account for this.

Overall no significant differences in the intensity of release, *in vivo*, were observed for either of the two marker compounds, for any of the mixing times investigated. An important consideration in this experiment was the panellist selection of the sample. If regions from the surface are selected then it would be expected that a higher concentration of aroma would be present compared to more mixed samples. However, as the values represented in this section are mean

values it is possible that the average of the 3 spoonfuls for each sample is masking the differences in mixing per spoonful.

4.1.3 Sample Variation

Variation between samples (Table 4.1) was expected to show that as aroma distribution became more homogenous, samples would show a higher consistency of release and thus less variation between replicates. The results confirm this hypothesis. If the system is more homogenous then the concentration of aroma compounds per spoonful is likely to be more consistent and the difference between the total volatile concentrations of each spoonful will be reduced. Comparing this finding to the headspace concentrations indicates that ethyl acetate potentially has a better solubility in the yoghurt phase than ethyl butyrate. The coefficients of variance demonstrate this by showing a lower percentage variation for ethyl acetate compared to ethyl butyrate. This suggests that the aroma is better dispersed throughout the yoghurt and that each spoonful has a more consistent level of aroma. The lower variation is surprising as it was expected that ethyl butyrate would become more soluble in the yoghurt phase with increased mixing but the effects on ethyl acetate would be minimal due to its lower fat solubility (Le Quere, Personal Communication). Le Quere et al. showed that the percentage retention of ethyl acetate, ethyl butyrate and ethyl hexanoate showed significant increases for ethyl butyrate and ethyl hexanoate in 5% fat yoghurt compared to 0% fat. Ethyl acetate retention showed virtually no change.

However, the results in this case suggest that ethyl acetate has a better dispersion in the yoghurt-compote mixed system which suggests that the level of mixing time is important for ensuring a better distribution of aroma compounds throughout the biphasic system. Increased mixing time also reduces the variation between samples for ethyl butyrate again suggesting a better dispersion. An

influence of the fat phase may also be playing a role as a higher amount of ethyl butyrate will be able to partition into the fat phase in a more mixed system.

Table 4.1 Table of average coefficients of variance for *in vivo* aroma release at stirring times 10 - 900 seconds for two different aroma compounds. Data are mean values from 4 panellists each consuming 3 replicates.

	Mixing Time (seconds)								
Aroma Compound	10	20	40	80	120	180	420	600	900
Ethyl acetate	38%	28%	23%	32%	23%	30%	12%	12%	12%
Ethyl butyrate	69%	45%	40%	63%	63%	61%	49%	38%	28%

4.1.4 Effect of the First Spoonful

The decline in variation with increased mixing time suggested that the more homogenous samples had more consistent release. However, the average variation measured was based on the variation between replicate samples. As the mixing patterns were vastly different, it is likely that the most important indicator of the differences between the samples would be the differences in release between the first sampling event, or in this case the first spoonful. It was thought that this would give an indication of the mixed release without additional mixing from the spoon.

Figure 4.6 shows that there was a higher intensity of release for less mixed systems with a trend towards declining intensity to 120 seconds of stirring. However, this is not a significant difference and is merely a trend in the data set. This was due to the probability that the spoonful of yoghurt was taken from the surface. The surface was the region where the aroma was most concentrated, for the samples with shorter mixing times, and thus the concentration of aroma delivered by this spoonful is likely to be greater than for more mixed samples. The probability of this first spoonful containing a higher concentration of aroma

was higher and it has been shown that increasing aroma concentration in a sample caused a linear increase in the intensity of the aroma delivered to the nasal epithelia, as long as the matrix itself was consistent. It is therefore, important to note in non-homogenous systems that the point of sampling is crucial to the level of aroma release. 900 seconds of stirring again showed an increase in the intensity of release suggesting that the viscosity of the matrix may be more important for increasing aroma release.

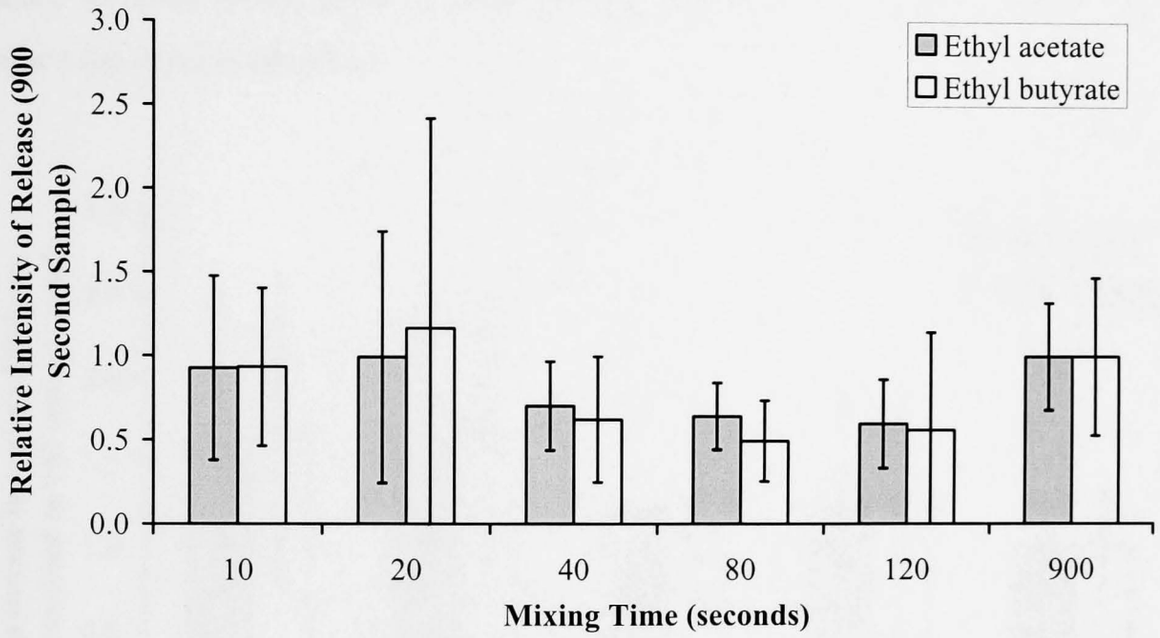


Figure 4.6 Effect of 1st spoonful on the concentration of aroma released *in vivo*. Data normalised to 900 second sample. Data from four panellists consuming one replicate. Error bars represent ± normalised standard deviation.

4.1.5 Effect of Compote on Release

These initial studies involved the use of compote as a vehicle for the dissolved aroma. This compote phase contained a range of other molecules including sugars, pectins and acids which would influence the viscosity, and the release of aroma compounds. In order to eliminate the effect of the compote, the aroma compounds were pre-dissolved in the same volume (10 mL) of water, instead of

compote, prior to stirring of the samples. This significantly reduced the thickness of the yoghurt samples which may play a part in the retention of the strawberry flavour.

Figure 4.7 shows that there was a greater trend towards a decrease in intensity of aroma release with increased mixing time, in the absence of the compote. This may be due to the reduced viscosity, due to the dilution with water, which would therefore reduce the effects of viscosity on mass transfer and release. The more mixed samples would have a lower relative reduction in viscosity which may reduce the release observed.

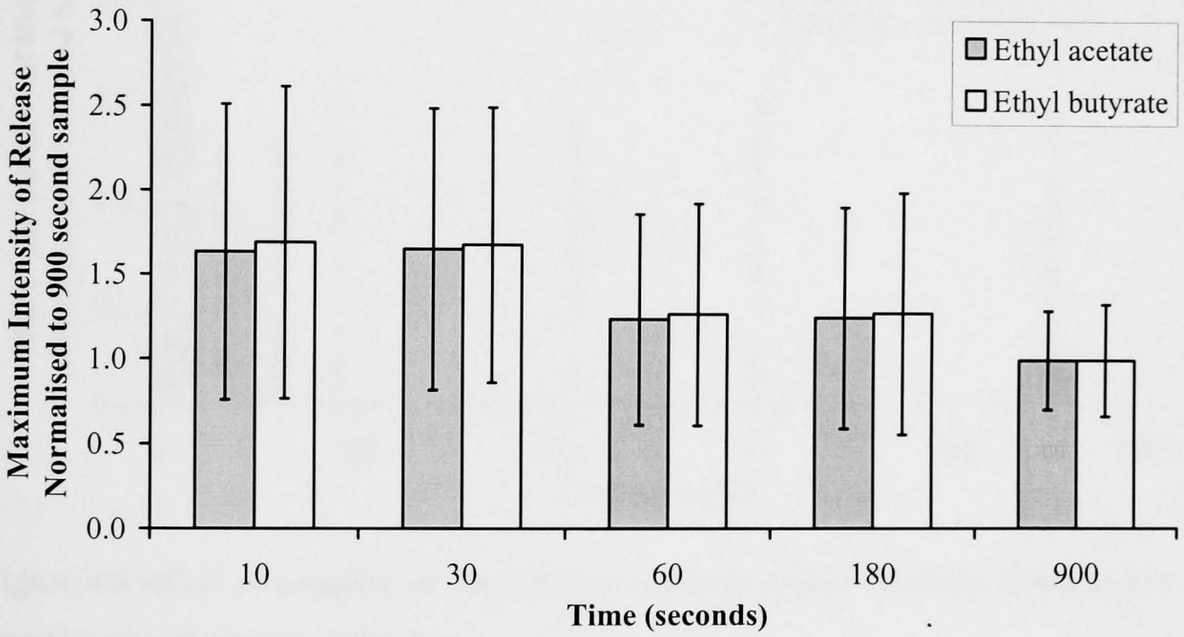


Figure 4.7 Effect of replacing compote with water, on aroma release *in vivo* from differently stirred yoghurts. Means of 3 replicates for 4 panellists.

Figure 4.8 shows that the presence of the compote showed a slight reduction in the overall concentrations of aroma compounds detected in nose however, the high variation due to panellist variability between the samples, means that these levels may not be significant. Lubbers *et al.* (2004) showed that the presence of pectin reduced the headspace concentration of some aroma compounds however, Mei *et al.* (2004) showed that the effects of a range of thickeners did not

significantly effect the release of strawberry flavour compounds when studied *in vivo* using PTR-MS. The results from our experiments suggest that compote may be only slightly reducing the aroma concentration detected at the nasal epithelia, this is unlikely to be significant enough to influence the perception. Any differences in perception would therefore more likely be due to other sensory properties such as thickness or initial sweetness of the sample. Sweetness may be altered by the sugars being better dispersed throughout the system and thus alter the flavour perceived, especially when only the first spoonful is considered.

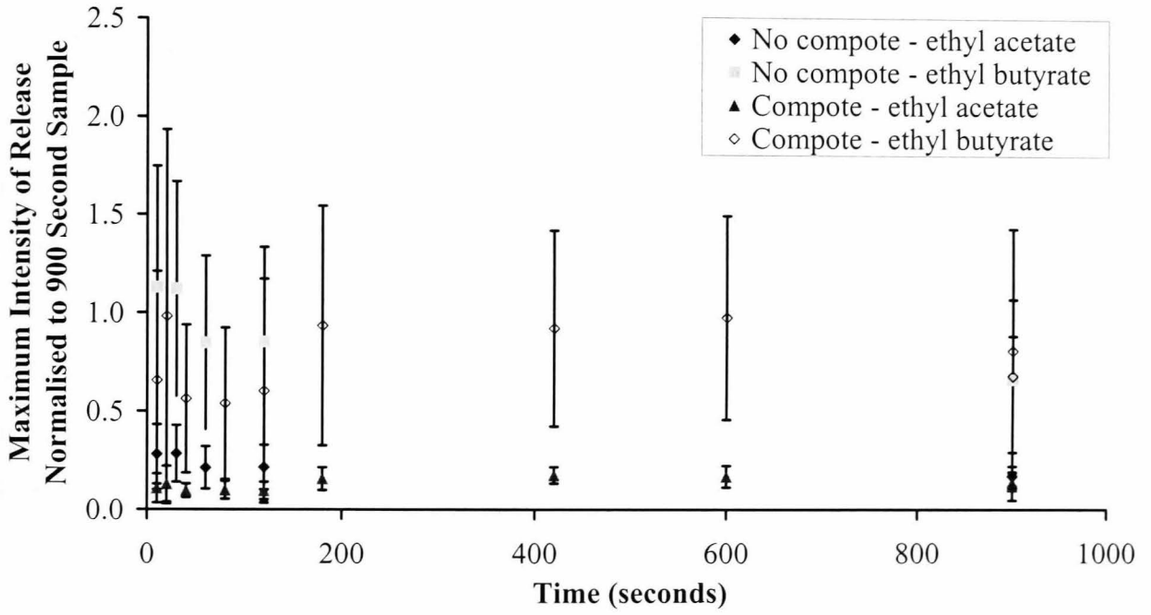


Figure 4.8 Effect of compote on the maximum aroma release intensity compared to samples containing no compote.

4.1.6 Conclusion

Results from stirred yoghurts showed no overall influence of mixing time on *in vivo* aroma release for either of the marker compounds investigated for a strawberry flavour. Headspace analysis indicated that equilibration in the system may take longer than one hour and that increasing mixing time reduced the amount of compound detected in the headspace. Investigation of variation and

the data for the first spoonful showed the importance of sample selection in this system and data for removal of the compote illustrated the importance of the presence of sugars.

Future experiments using a static mixer system will aim to eliminate this panellist choice option by providing mixed samples entirely in a single spoonful and reduced the level of shear by having a static system.

4.2 Effect of Static Mixer System on Mixing of Aroma into Yoghurt

A static mixer system was developed, in collaboration with Birmingham Chemical Engineering department, wherein a range of Kenics mixing elements were incorporated into the system. This allowed a controlled level of mixing to occur, whereby the levels could be quantified by image analysis, and the level of mixing altered. Static mixer systems consist of a number of fixed mixing elements within a pipe or tube. The elements do not move but enable mixing of fluids by complex motion of the fluids induced by the shape of the elements. In industrial processes, the static mixer systems are generally used to produce highly homogenous systems with minimal "dead" volume. They are therefore commonly used in wastewater treatment plants where it is essential that the disinfecting fluids are completely mixed with the bulk fluids (for further information on static mixers see Section 1.4.3). The use of the static mixer system, in this case, was almost in complete contrast to this, whereby the number of elements was selected so that a range of inhomogenously mixed, but consistent samples could be produced. This system would however, allow the measurement of the degree of homogeneity and hence the degree of mixing that had taken place. These measurements could be related to the aroma release data to assess how a defined level of mixing would affect the aroma release *in vivo*.

4.2.1 Effect of Element Number on *in vivo* Aroma Release

Yoghurt was again used as the food matrix because it had a semi-solid nature which would reduce the level of diffusion (thus retaining the mixed pattern). It also possessed suitable flow properties to pass through the mixing elements, and did not require a setting process. Samples were prepared as per Section 2.2.5.2 and were consumed whilst monitoring the breath volatile concentration via APcI-MS. The aroma compounds used were limonene and methyl acetate.

Figure 4.9 shows the results of increasing the number of mixing elements on the maximum intensity of *in vivo* aroma release. It demonstrates that there is no significant difference between the number of elements and the intensity of aroma compound release for either the lipophilic limonene, or the lipophobic methyl acetate. Analysis of Variance (ANOVA) indicates no significant effects on the I_{max} for either limonene or methyl acetate ($P>0.10$) but does indicate a significant difference between the responses for each panellist ($P<0.05$). Therefore normalisation of the data set eliminated the effects of panellist variation and allowed comparisons between the different numbers of elements.

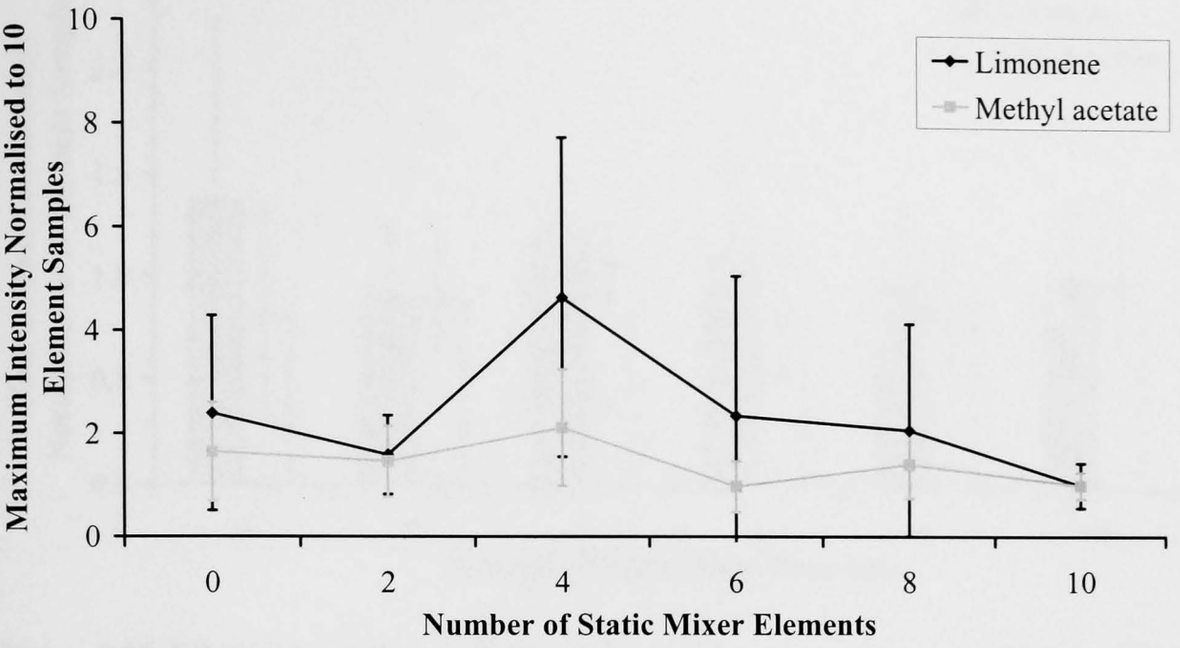


Figure 4.9 Effect of number of static mixer elements on aroma release from 3 panellists. Data is normalised to the most homogenous sample (10 elements) for each panellist. Data is the mean of 3 replicates for each panellist.

It was predicted that, if the sample was less homogenous, then the rate of aroma release would be increased leading to an earlier occurrence of T_{max}. However, the data does not support this hypothesis. Figure 4.10 shows that there is no significant difference between the number of mixer elements and the T_{max} of release ($P>0.10$). The data was again normalised because panellists showed significant differences in intensities between each other ($P<0.001$). These differences have been observed previously for panellist variation. Roberts *et al.* (2003) and Mei *et al.* (2004) both observed inter-person variation, in maximum intensities, of 41-48 % for ethyl butanoate release from milk and yoghurt samples respectively, when the same samples were monitored by PTR-MS.

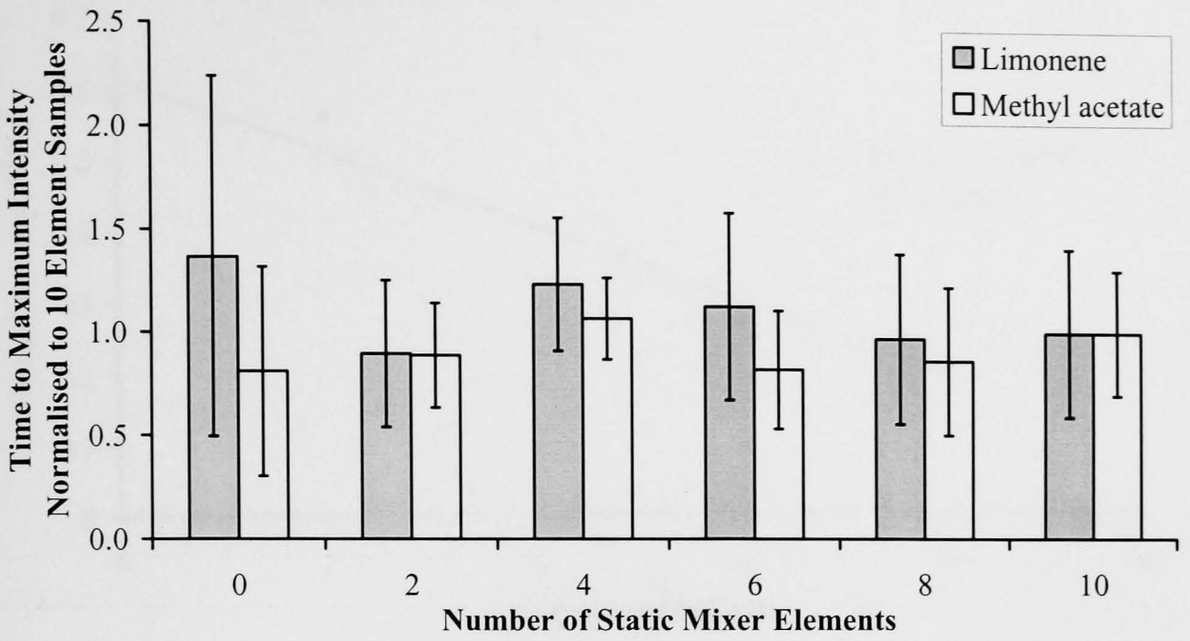


Figure 4.10 Effect of number of static mixer elements on T_{max} of aroma release from 3 panellists. Data is normalised to the most homogenous sample (10 elements). Three panellists with 3 replicates for each panellist.

4.2.2 Measurements of Mixing Intensity

Mixing intensities measurements were carried out by Vern Lee at Birmingham University, using a technique based on freezing the samples in liquid nitrogen and analysing the degree of dye mixing via image analysis (Section 2.3.2.3). Digital images were taken and the degree of mixing intensity was calculated. Figure 4.11 confirms that increasing the number of elements proportionally increases the degree of mixing (indicated by a decrease in mixing intensity). This was expected as, by increasing the level of mixing essentially two-fold for every set of elements, the sample will become more homogenous.

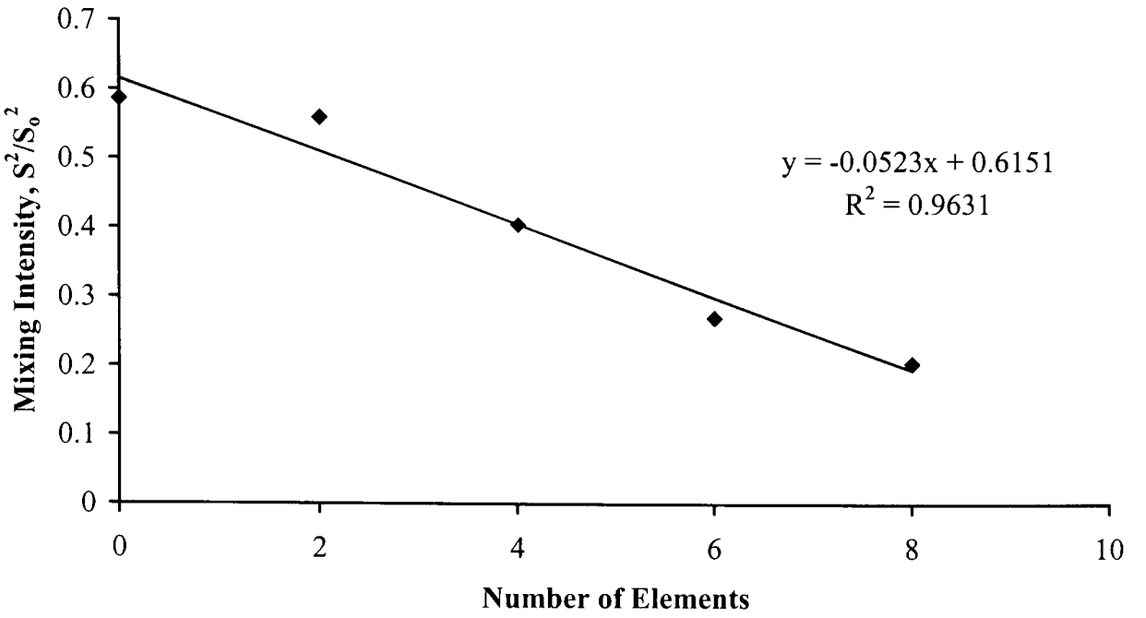


Figure 4.11 Mixing intensity (0<i<1) showing the colour intensity of dye when mixed in via the static mixer system for an increasing number of mixing elements (0-8). Data are proportions of the maximum mixing intensity and are based on approximately 6500 scans.

Development of the model using Design Expert produced the following equation for predicting the mixing intensity with different numbers of static mixing elements. This result showed an excellent correlation between increasing element number and decrease in mixing intensity with an R^2 value of 0.96 (Equation 4.1).

Equation 4.1 Mixing Intensity = +0.61509 - 0.05233* Number of elements

Comparing this result to the *in vivo* release data showed that, although the homogeneity of the yoghurt samples (one containing aroma, one without) was increased, the overall effect on aroma release detected was negligible. This suggests that the rigorous "in mouth" mixing of the sample overcomes any effects of pre-mixing of aroma compounds. These experiments were all conducted and stored at 4°C so that no change of temperature was involved during yoghurt mixing and storage.

4.3 Effect of Layering on Aroma Release from a Semi-solid Gel Matrix

The results presented in the previous section showed that the aroma distribution in yoghurt had no significant effect on aroma release. The short, in mouth retention time and the rapid mixing of the aroma and non-aroma regions showed no significant effects for delivery.

A matrix, which required more mastication, and hence a longer time for volatile delivery, was investigated. Gelatine-sucrose gels were chosen because of their ability to breakdown during mastication to release volatile compounds via dissolution and partition (Harrison and Hills 1996). Gelatine-sucrose gels were a suitable matrix to produce separately layered samples with no mixing between the subsequent independent layers (Section 3.3).

4.3.1 Aroma Release *in vivo*

It was predicted that by separation of volatile compounds into different layers the surface areas available for their release would be altered and thus their ability to partition and be released. Any changes observed would be shown by alterations in the I_{max} and/or T_{max} of the samples. Volatile compounds were pre-dissolved in propylene glycol before being added to the hot gelatine-sucrose mixture and a range of samples were produced as per Section 2.2.2.2. Two aroma compounds were compared (linalool and methyl acetate) with varying physicochemical properties. Final concentrations were normalised for each panellist to reduce any inter-panellist variations and allow the data to be comparable. Gels were prepared with layers of aromatised gel (denoted 1) and layers of unaromatised gel (denoted 0). Therefore the sample denoted 0110 contained layers of unaromatised gel on the outside and two layers of aromatised gel on the inside.

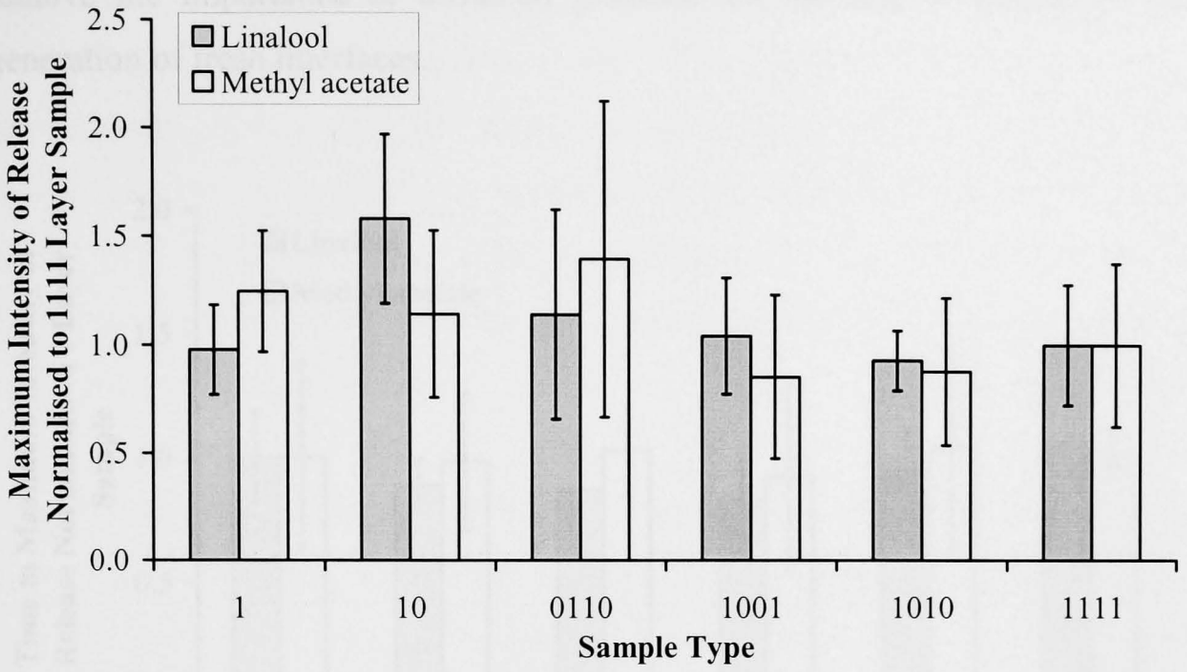


Figure 4.12 I_{max} data for aroma release from layered gels when consumed by 4 panellists with three replicates each. Data normalised to 1111 sample.

Figure 4.12 shows the maximum intensities of release from a range of layered gels. There were no significant differences ($P>0.05$) in volatile release from the samples for either linalool or methyl acetate. This result suggests that during consumption the volatile compounds were being released by the same mechanism, with dissolution and melting of the samples controlling the release of the volatiles as suggested by the model of Harrison and Hills (1996). The different layering patterns appear to have no effect on the intensity of this release which may be attributed to the mastication and dissolution of the samples. As the sugar concentration in the gels is greater than 40 % the melting temperature will be above the mouth temperature (Section 5.3) and therefore the rate-limiting step for volatile release is the diffusion of sucrose from the surface layers (Harrison and Hills 1996). As the sample is masticated, the layers will become disrupted and mass transfer will occur from a range of gel fragments. This rapid increase in surface area will reduce the time taken for gels to dissolve and probably explains why no differences in release intensity were observed. This coincides with the theoretical model by Harrison (2000), which predicts that mastication will

remove the importance of diffusion gradients on the rate of release by the generation of fresh interfaces.

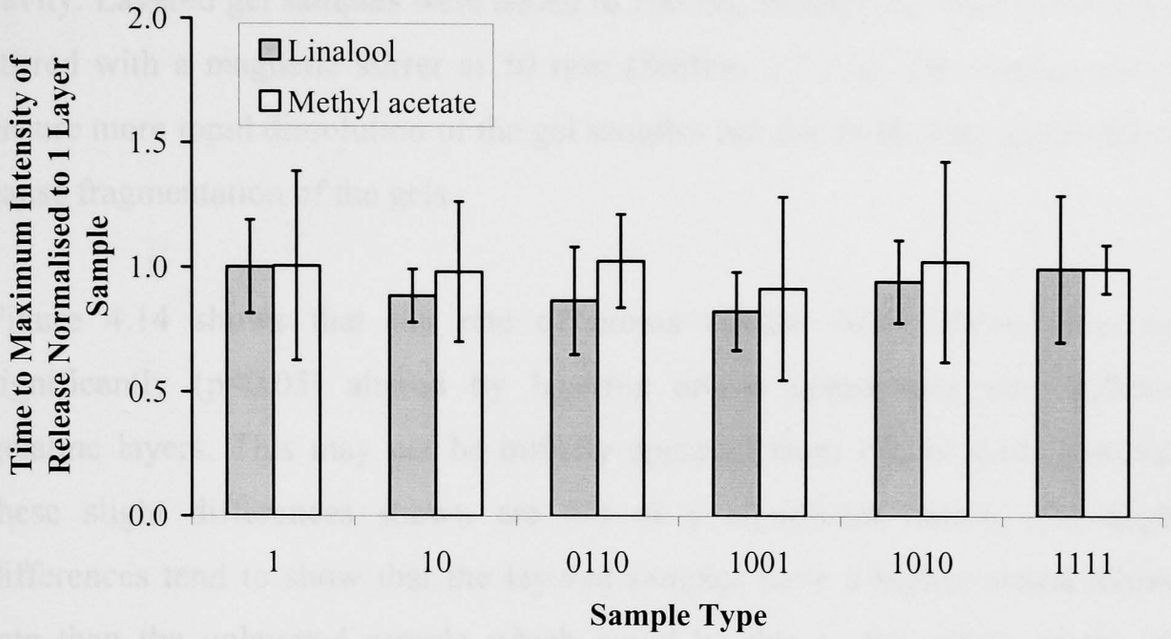


Figure 4.13 T_{max} data for aroma release from layered gels when consumed by 4 panellists with three replicates each. Data normalised to 1111 sample.

The T_{max} value (Figure 4.13) again shows that there was no significant difference ($P>0.05$) between the different samples for either volatile compound. This data implies that the rates of release of the volatiles were similar and that no significant effects were conferred by the different layering patterns. Again the rate-limiting step for aroma release will be dissolution and diffusion of sugars, and the rapid mixing of the samples via mastication will eliminate any differences in release rate observed for the samples.

4.3.2 Dynamic Headspace

Mastication was shown to be an important factor in aroma release from gelatine-sucrose gels and eliminated any impact of layering of volatile compounds on their intensity or timing of release. The dynamic headspace was measured to

assess the release of volatiles in the absence of the chewing and swallowing events associated with *in vivo* consumption. These events are important *in vivo* for increasing surface area for release and pumping of volatiles into the nasal cavity. Layered gel samples were added to 100 mL of distilled water at 37°C and stirred with a magnetic stirrer at 50 rpm (Section 2.3.1.2). The stirring was to ensure more rapid dissolution of the gel samples but due to the low speed did not cause fragmentation of the gels.

Figure 4.14 shows that the rate of aroma release from linalool was not significantly ($p<0.05$) altered by layering aroma compounds into different gelatine layers. This may not be initially apparent from Figure 4.14, however, these slight differences shown are not of a significant nature. The slight differences tend to show that the layered samples have a higher aroma release rate than the unlayered sample which could be due to the nature of the gel layering process. However, no particular trend is observed for release rate between the layered samples and the location of the aroma appears to have a minimal influence. The same result was observed for methyl acetate (data not shown) suggesting that the effects of layering of the compounds was independent of the properties of the aroma compound. Baek *et al.* (1999) showed, by dynamic headspace measurements, that the rate of release of furfuryl acetate from gels was dependent on the gelatine concentration but the overall maximum release concentrations were the same. In this situation, this was confirmed by all of the gel samples having similar overall maximum concentrations, but, unlike in Baek's case, it suggested that the breakdown of the gels was constant as the rates of release were unaffected.

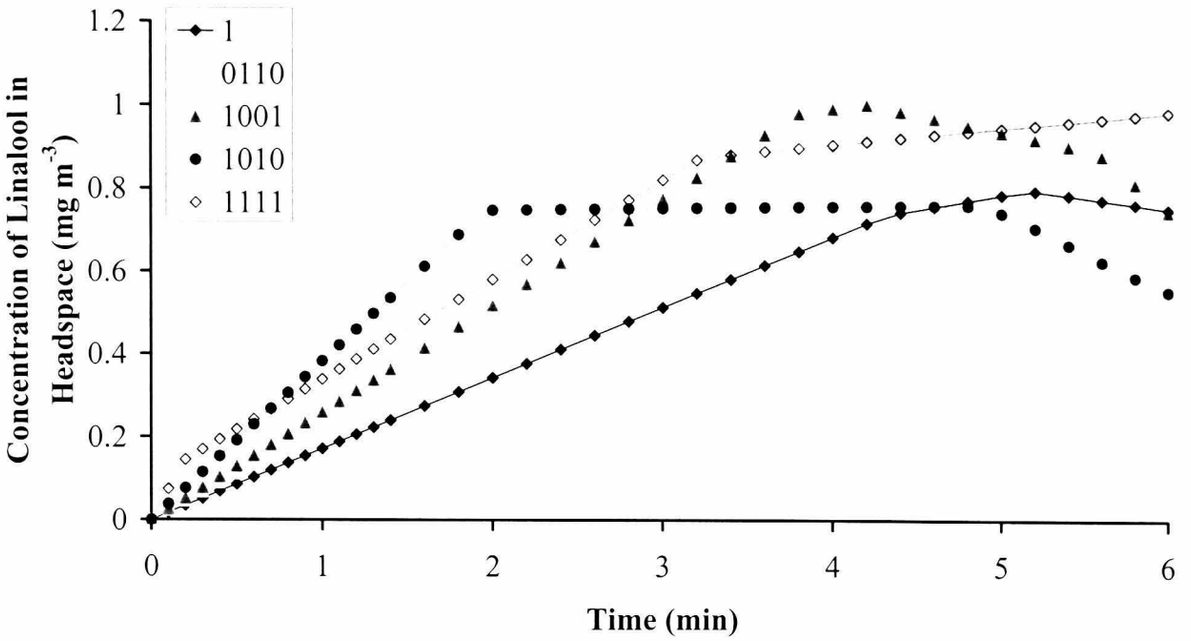


Figure 4.14 Dynamic headspace concentration of linalool during dissolution of multi-layered gels. Mean values of three replicates.

As the gel samples were cut through the layers, each sample would have an aroma-containing region which would be in contact with the liquid phase. This would be the region from which aroma compounds would be dissolving from and partitioning into the solution. The stirring of the system, will serve to deplete the concentrations at the gel-liquid interface and will promote mass transfer of aroma compounds into solution. The partition of volatiles from the solution into the headspace will be related to the concentration in solution (Marin *et al.* 1999). Layering in this system did not affect the release of aroma compounds into the headspace and thus no differences were observed.

4.3.3 Different Layering Patterns

Differences between the actual numbers of layers rather than the location of the volatile compounds appeared to be more important for *in vivo* aroma release. Gels were produced with aroma compounds dispersed throughout the sample (1 layer); dispersed in one layer and an unflavoured layer (2 layer); or dispersed in

two layers, interspersed by two unflavoured layers (1010) (4 layer). Samples were consumed *in vivo* by four panellists as per Section 2.3.1.3.

Release from gels containing aroma in 1, 2 and 4 layers showed no significant difference ($P>0.05$) in the intensity of release between any of the samples for either the limonene or hexanol (Figure 4.15). This suggests that aroma release was dependent on the dissolution and mastication of the sample. This further suggests that these factors were the rate-limiting steps for release and were not dependent on the different layering patterns. Reasons for this behaviour were probably due to rapid mixing of the sample in the mouth by mastication, dissolution and melting (Harrison and Hills 1996). Aroma release is highly dependent on partition, but as the samples have the same volume of aroma the factor determining the extent of release into the gas phase will be the degree of fracture and the interfacial mass transfer (Harrison and Hills 1996). Fracture will be consistent throughout the gel samples and, although not equal, the surface areas for aroma release will be increased during the gel breakdown.

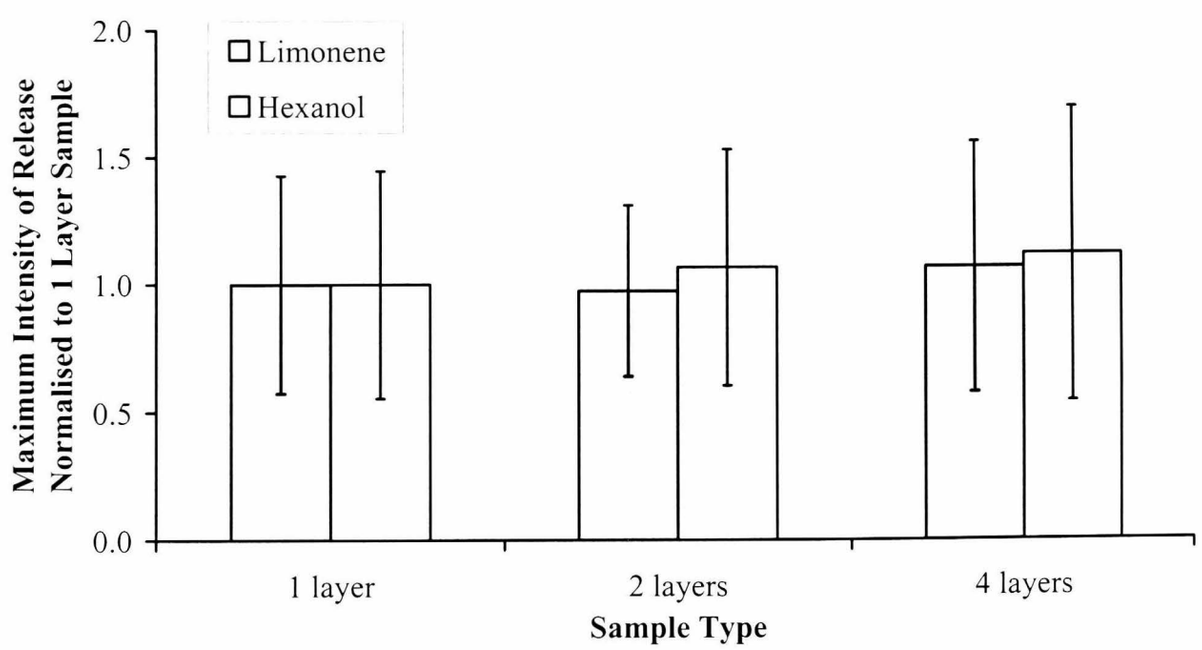


Figure 4.15 Effect of the number of gel layers on I_{max} values of aroma release. Data normalised to 1 layer sample for each panellist. 4 panellists and 3 replicates of each sample.

There was no significant difference in the T_{max} value suggesting similar release rates (Figure 4.16). An interesting finding was that the T_{max} values for limonene appear to be slightly earlier (not significant) than those for hexanol and this may be due to the dispersion of the aroma compounds within the gel layers. This suggested that although the T_{max} between samples did not vary, the properties of the compounds may have been affecting the timings of release. This implies that although supposedly fully solubilised in the gel layers, the aroma compounds may be released differently from each other which will be a consequence of their different properties. This effect was independent of the number of layers implying that the layering was not influencing the timing or rate of aroma release. A similar pattern is observed for linalool and methyl acetate although the effect is less pronounced which may be due to better dispersion of linalool within the gel.

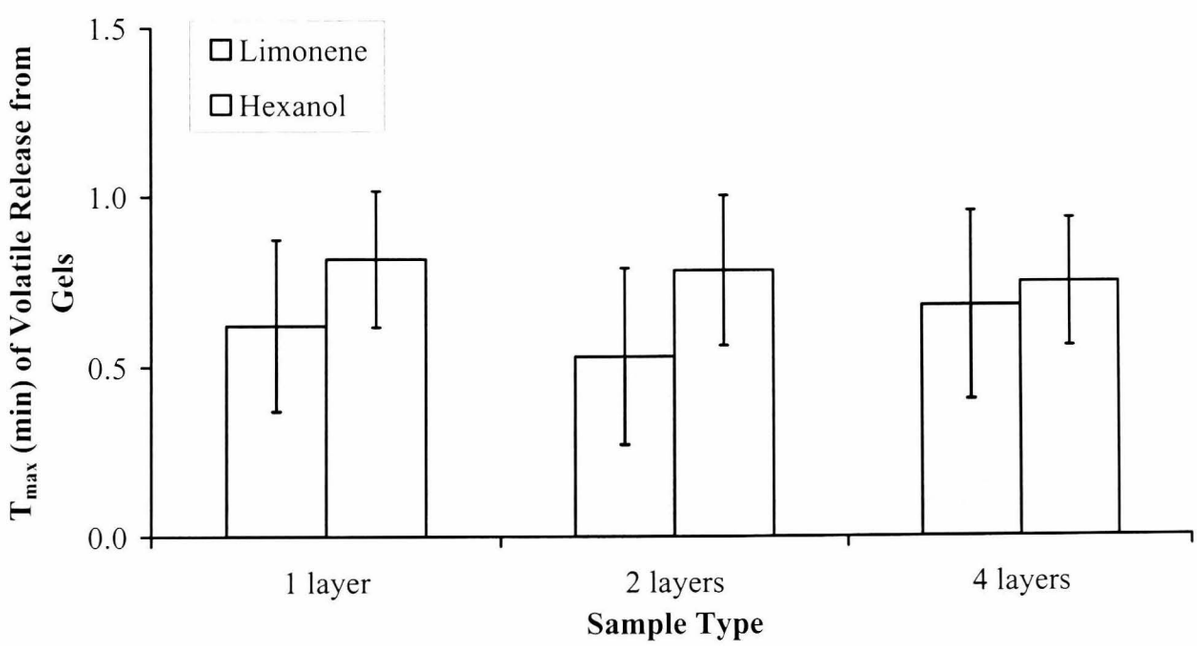


Figure 4.16 Effect of number of gel layers on the T_{max} of aroma release. Data are means of 12 replicates (4 panellists, 3 replicates). Error bars represent ± standard deviation.

A final caveat is that in these studies the maximum number of layers was four. This may be an insufficient level of separation for any mixing differences to be observed. This may be the case as with no significant differences in *in vivo* release observed at long separation, increasing the numbers of layers further will only serve to increase the overall homogeneity of the system. However, due to constraints in sample production, it was not possible to produce reproducible samples with greater than 4 layers.

4.3.4 Sensory Analysis

Sensory perception of aroma release from gels has been shown to be more dependent on the rate of release than the actual maximum intensity of volatile compounds measured at the nasal epithelia (Baek *et al.* 1999). These authors showed that an increase in furfuryl acetate perception was better correlated to the gradient of release over time rather than the I_{max} value. If aroma is being released gradually then the maximum intensity of aroma detected in nose will have a smaller impact on the flavour perception than if the same quantity of aroma is released rapidly.

A Paired Comparison test is an example of a directional difference test where the panellist is directed to identify the strongest (or weakest) sample with specified attribute. In this case the panellists were requested to identify the sample with the strongest flavour intensity. Samples were prepared as per Section 2.2.2.2 and sensory analysis per Section 2.2.7. Linalool and methyl acetate were used as the aroma compounds. The samples tested were 1111 (diffuse aroma) and 1010 (layered aroma).

Results show that 19 of the 30 assessors chose the diffuse (aroma in all the layers) sample as having the highest aroma intensity and 11 the layered sample. No panellists failed to make a selection. Analysis of these results via a two-tailed paired t-test produced a probability of 0.2. Although a higher number of

panellists chose the sample containing aroma in all the layers this was not considered significant. These results reinforce the results obtained from the instrumental measurements and confirm that layering of aroma compounds in gelatine-sucrose gels does not affect the aroma release or perception.

In conclusion, the change in overall sample aroma dispersion in gelatine-sucrose gels via layering has no significant impact on the aroma release concentrations, timing or rates of release. This has been confirmed by studying release, *in vitro*, *in vivo* and finally by sensory analysis.

4.4 Effect of Layering on Release from a Chewing Gum Matrix

Results for higher scale levels of mixing has suggested that layering of aroma into samples which were subjected to breakdown during mastication showed rapid in mouth mixing and a negligible effect of the physical separation of the aroma compounds. Chewing gum was chosen as good model to study flavour release from a matrix that was not subject to breakdown. It permits the chewing of a semi-solid matrix for a prolonged period, releasing volatiles progressively in a semi-controlled manner (Ovejero-Lopez *et al.* 2004). Flavour release is dependent on the partition coefficient and resistance to mass transfer related to the components of the matrix and the interactions of the flavour compounds with these (deRoos and Wolswinkel 1994).

Davidson *et al.* (1999) showed that panellist perception of flavour intensity followed sucrose concentration rather than menthone concentration detected 'in nose'. They showed that menthone release was fairly constant throughout a prolonged chewing period (>5 minutes). Harvey and Barra (2003) reported that release of liquid peppermint oil when concentrated in an encapsulant (Flexarome®, Firmenich) showed a "flavour burst" in the first few minutes from the chewing gum sample. This prevented the flavour volatiles partitioning into the gum base until the encapsulant was broken down and the volatiles released.

When liquid peppermint oil was added directly to chewing gum, the flavour molecules were better dispersed throughout the gum base and lower more consistent release intensity was observed. These observations indicate that aroma release from chewing gum over time for a 5 minute period was not vastly altered unless added as concentrated aroma region (e.g. encapsulant).

In this section the volatile compounds were present in alternate layers throughout the gum samples (Section 2.2.4). This produced layered gums with 1, 2, 4, 8 and 16 layers, half of which had aroma dispersed throughout and half of which had no aroma compound. Because of the method of sample production, in order to use the same batch of gum for all the samples, the 1 layer sample has double the concentration of aroma compounds compared to the other samples. Preliminary experiments using colour showed that the gum could be formed into independent layers. Figure 4.17 shows a digital image of the 2 and 8 layered samples as a comparison and indicates the separation of the layers.

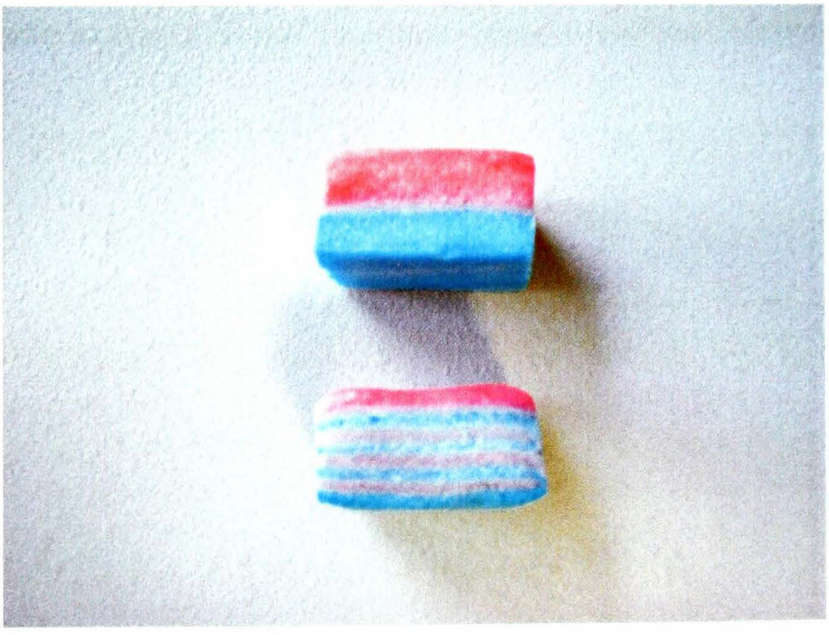


Figure 4.17 Picture illustrating 2 layered and 8 layered chewing gum samples

4.4.1 Effect of Layering on Aroma Release *in vivo*

Samples were consumed by 4 panellists whilst exhaling into the transferline of the APcI-MS for a period of five minutes. This was chosen as an adequate time period for mastication of chewing gum samples. Figure 4.18 shows the volatile release during the first minute of the chewing process. It illustrates that increasing the numbers of layers of gum does not show any definite trend for differences in volatile release detected 'in nose' in the initial release stage. This is the stage at which differences would be expected to occur because after this period of time the sample will be well mixed in mouth and any release will be coming from a homogenous sample. It is unsurprising that over the subsequent four minutes the aroma release patterns are similar as the chewing gum samples will become mixed within the mouth rapidly and any effects of the layering will be lost (Figure 4.19). Even in the case of encapsulants, once they have been released and aroma dissolved into the gum base the pattern of aroma release becomes similar to that of dissolved aroma (Harvey and Barra 2003).

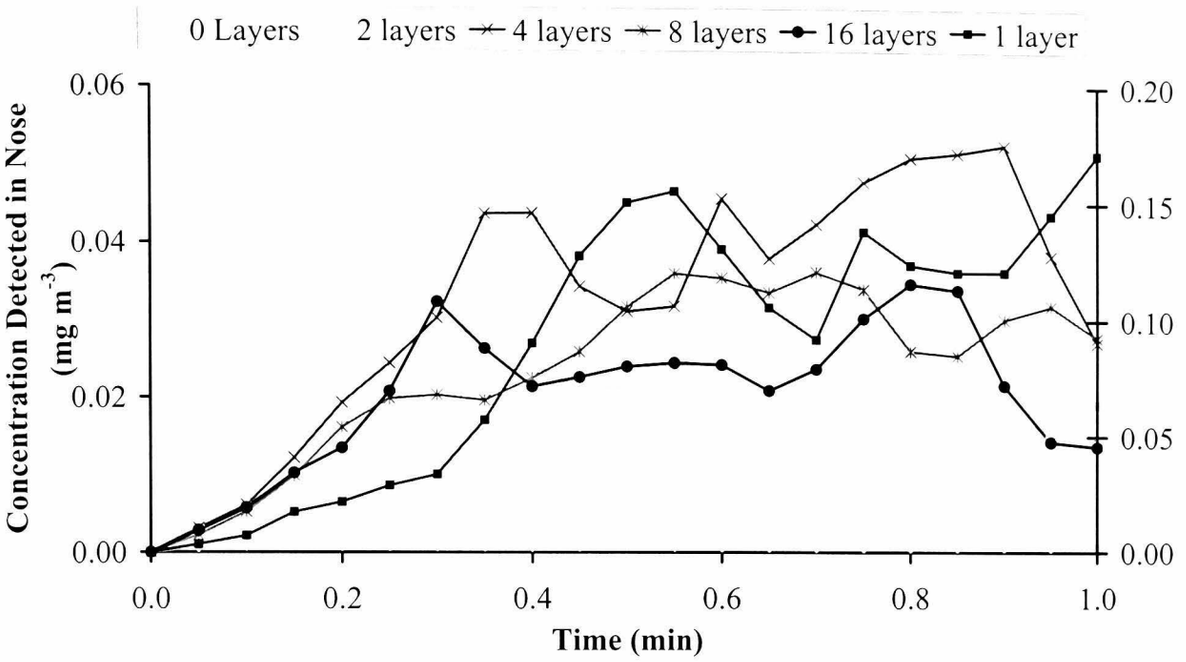


Figure 4.18 Time-release curves of ethyl butyrate for gums containing different numbers of strawberry aroma-containing layers. Data are the mean of 4 replicates. The 1-layer sample has double the aroma concentration and hence utilises the right-hand axis.

Preliminary results showed that 20-30 chews were sufficient to totally mix the colours of the gum and this correlates well with data from other experiments (Prinz 1999). It has also been noted that at sample sizes between 4-18g of chewing gum, the bolus size is not significantly lengthened (Prinz and Heath 2000). The samples involved in this case fell within this remit and so were considered to have overall the same level of bolus lengthening during mastication.

Harvey and Barra (2003) showed that although the encapsulant caused an early burst in the latter stages, when the aroma became dissolved in the gum base, the release profiles for the two samples were similar. Concentrating the volatile compounds into separate layers will not produce the same effect as the encapsulant system. This is evidenced by the similar initial release patterns observed for all of the differently layered samples which is in contrast to the

release profiles from encapsulants shown by Harvey and Barra (2003) and the results demonstrate that there was no significant increase in the intensity of the release when the number of layers was increased from 2-16.

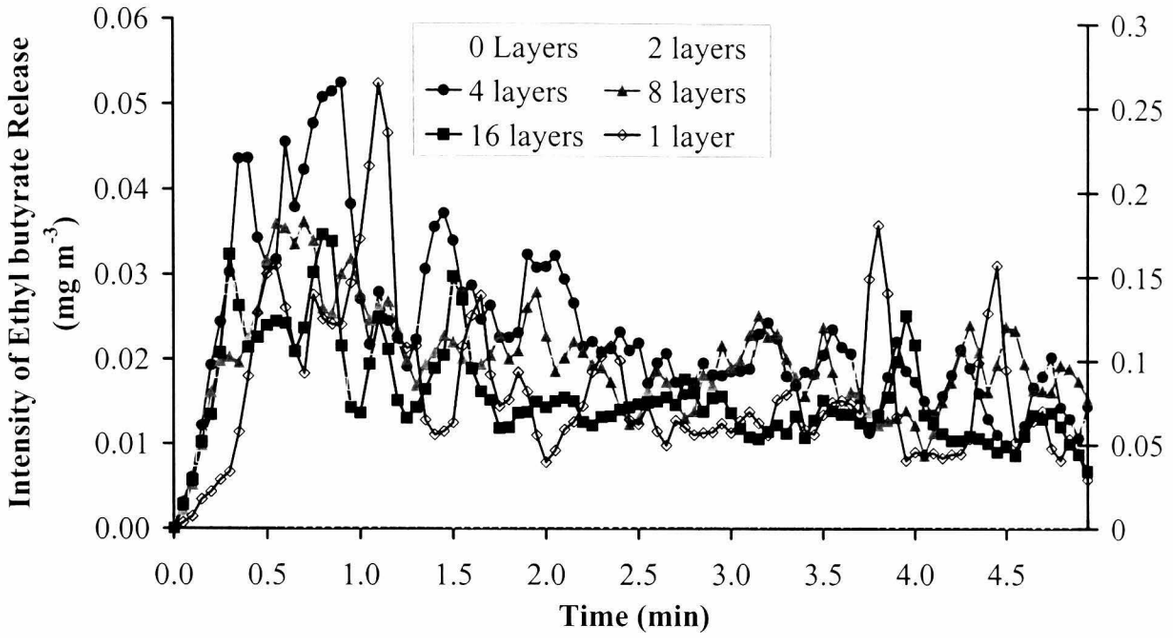


Figure 4.19 Ethyl butyrate release over a 5 minute consumption period from layered chewing gum samples. Data are means of 4 replicates. The 1 layer sample utilises the right hand axis and all the other samples the left hand one.

Figure 4.20 shows the maximum intensities of release for the differently layered samples. The single layer sample has double the volatile concentration of the other samples due to the method of production, however, a four-fold increase in the maximum intensity is observed. A potential explanation for this is that as the entire surface area is subject to aroma release then the potential for release is greater than when areas of unflavoured gum are present. More likely, is the fact that, the 1 layer sample has a lower gum : flavour ratio and hence aroma compounds will be able to partition to a higher extent into the saliva phase

The rate limiting step for flavour release is assumed to be the transport of flavour volatiles across the gum-saliva interface, which can be described by the stagnant-layer theory of mass transfer (Harrison 2000). These authors produced a model for aroma release from chewing gum, predicting that rigorous mouth movements

during mastication will maintain a well mixed saliva phase devoid of any concentration gradients. This means that although the initial surface areas for aroma release may vary, the rapid mixing by mastication coupled to the slow rate of partition between the gum and saliva phases meant that no significant effects of layering aroma in the chewing gum were observed.

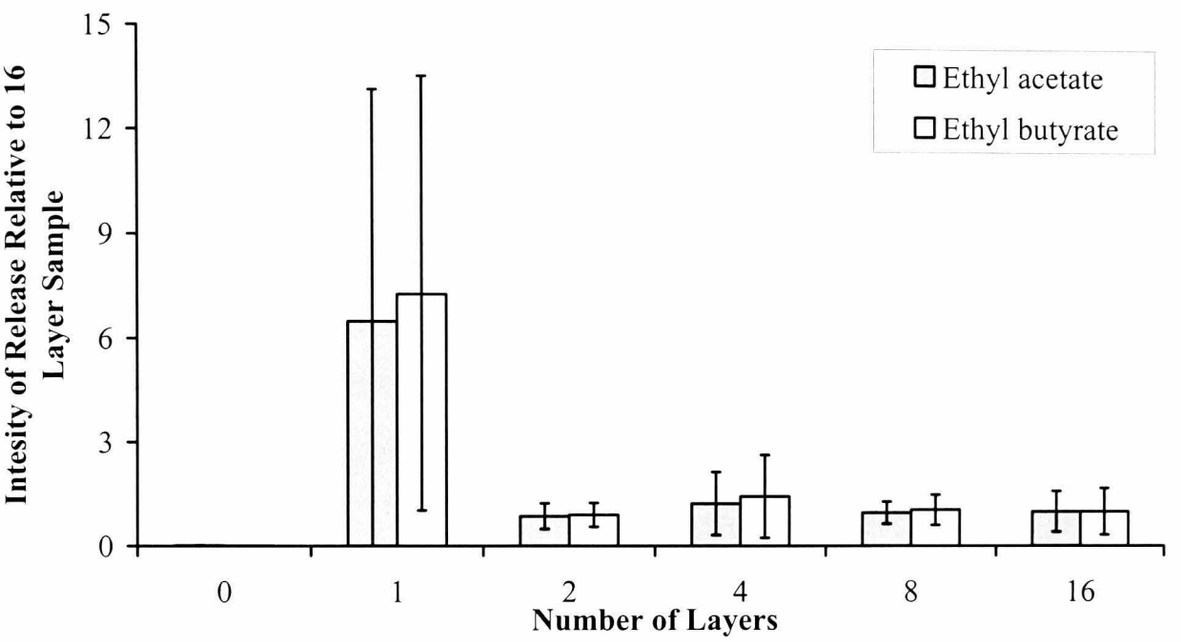


Figure 4.20 Effect of the number of flavoured layers on I_{max} of volatile release from differently layered chewing gums. Data normalised to 16 layer sample. Samples are means of 3 replicates.

4.4.2 Effect of Number of Chewing Actions on Aroma Release *in vivo*

Layering of aroma into separate layers of chewing gums has been shown to have no effects on the intensity or rate of release of the aroma compounds. It was suggested that mastication led to rapid in mouth mixing and that this would eliminate any effects of separation by layers. Samples of chewing gum were analysed *in vivo* using APcI-MS for a differing number of chewing events to assess the effect of the number of chews on aroma release. Chewing number ranged from 5 chews, where the sample would not be completely mixed, up to 50 chews where the sample should be homogenous.

Figures 4.21 and 4.22 show that by increasing the number of chewing actions the intensity and time to reach this intensity were increased. This was probably due to the fact that, increased numbers of chewing actions increased the amount of time for aroma to be released from the gum samples. This indicates that the release of aroma compounds was time dependent and related to a partition style mechanism. Chewing is required to release the volatile compounds from the gum base and deliver them to the nasal epithelia (de Roos 1990). Therefore increasing the number of chewing events will increase the level of aroma that is released. The longer chewing time, indicative of the larger number of chewing actions, explains the later T_{max} values. Increasing the number of chews increases the mixing of the sample with saliva and thus delays the time to reach the maximum intensity. Saliva flow will be an important aspect affecting this rate of release but not the overall quantity or duration (Guinard *et al.* 1997).

Prinz (1999) quantitatively measured colour mixing in gums during mastication using a range of image processing techniques. The author showed that mixing between two different coloured layers of chewing gum was uniform after 30 chews, but varied in mixing patterns up to this point. The techniques they used allowed them to distinguish between the mixing patterns in mouth. Importantly they also assessed the effect of sample size and found that for a 7.5g sample (similar size to sample used in our experiments) not all of the sample was involved in the mixing process but that part was held in the cheek until repositioning of the bolus. These data describe the importance of the number of chews on the in mouth mixing of layered chewing gums and may explain the increases in maximum intensity observed up to 20 chews but a reduction in the increase thereafter. High variation between the different numbers of chewing actions and the intensity of the aroma release make the data difficult to interpret for the different samples. This may be due to the nature of the release from chewing gum which appears as a number of spikes associated with each chewing event. Thus the maximum intensity (I_{max}) may not be a good indicator of the

overall impact of the number of chewing actions on aroma release but the T_{max} may give a better representation (Figure 4.22).

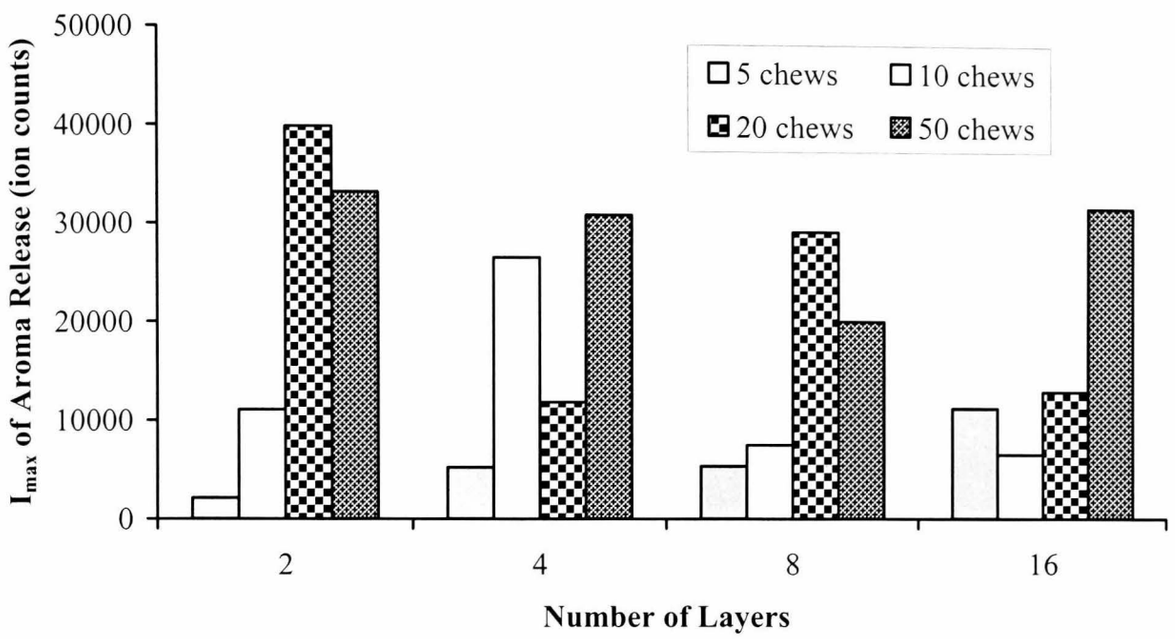


Figure 4.21 Effect of the number of chewing actions on I_{max} of release from differently layered chewing gums. Intensity measured in ion counts detected by the APcI-MS.

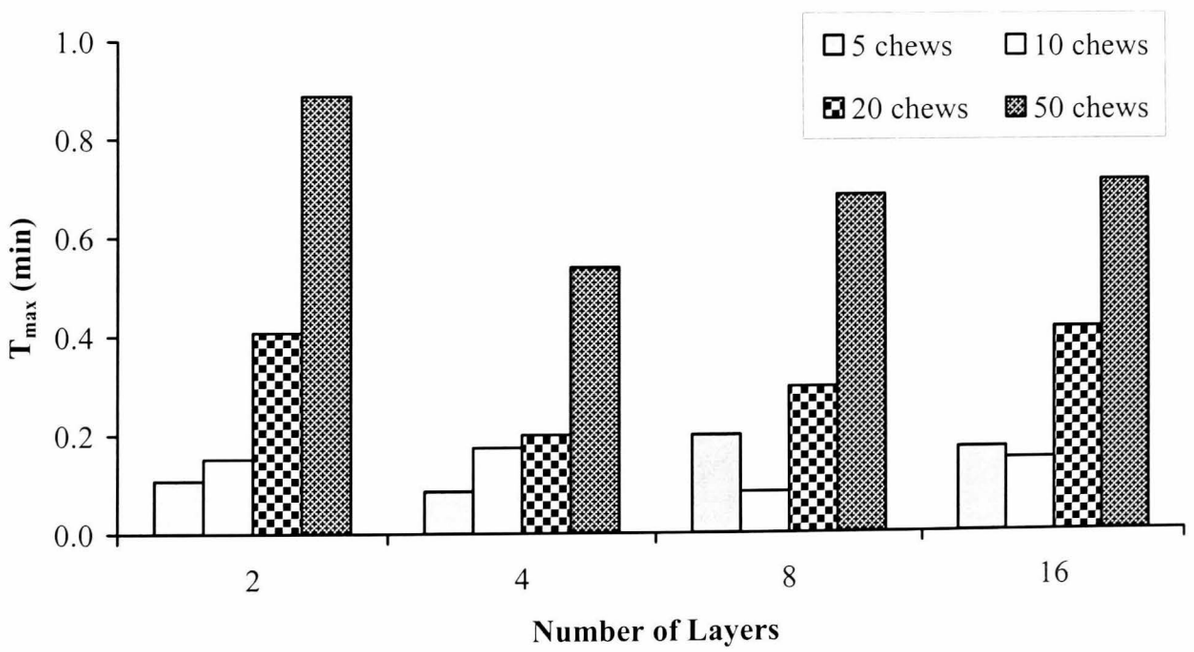


Figure 4.22 Effect of the number of chewing actions on T_{max} of release from differently layered chewing gums.

4.5 Conclusions

Although numerous assumptions with regard to aroma dispersion throughout a matrix have been made in the past it is evident from the data presented in this Chapter that the effects on the aroma release 'in nose' were minimal in the matrices studied. Aroma was dispersed in a range of ways using a variety of techniques and in a number of different matrices. However, despite these different methods of ensuring inhomogeneous aroma dispersion, the overall effects on aroma delivery and release were minimal.

With non-homogenous aroma distribution, the selection of a sample has an influence on the level of aroma delivered and the level of experimental variation is high. Physiological factors such as chewing rate are important when studying release from gels with differing numbers of layers. Mixing with and release from saliva is thought to be an important aspect for aroma release from these systems and methods that can control these aspects may provide better knowledge of the actual influences on aroma release. An example for studying this could be via the use of a model mouth system as developed by Van Ruth and Roozen (2000). They showed that mastication significantly increased aroma release from bell peppers and increased saliva flow significantly decreased aroma release. These findings may give rise to the importance of physiology rather than aroma separation for aroma release in these systems. Maximal aroma delivery may not necessarily require maximum dispersion and rapid 'in mouth' mixing may serve to reduce any differences in mixing method during sample preparation. This may have implications for product design especially in the confectionery industry where development of novel products is a key driving force.

5 EFFECTS OF CARRIER SOLVENTS ON AROMA PARTITIONING AND RELEASE

The previous Chapter showed the impact of differential separation of aroma compounds throughout a range of food matrices and the subsequent effects on aroma release. This Chapter assesses the effects of increasing the levels of dispersion via the use of carrier solvents (co-solvents). Further discussion on solubility and carrier solvents is discussed in Sections 1.4.4 and 1.5.

If the solvent affects the solubility of an aroma compound within a phase, it will also have implications for its partition and dispersion and subsequently its release. A water-co-solvent mixture is a better solvent for many aroma compounds because of their hydrophobicity and the reduced ability to "squeeze out" solutes (Yalkowsky 1999b). The method chosen to assess the effects of the solvents on the solubility and partition of aroma compounds was static headspace analysis. Changes in the headspace concentration were assessed to determine how the presence of carrier solvents affected the partition of the aroma compound in the liquid phase. The hypothesis was that the presence of the carrier solvents would increase the solubility of aroma compounds within the aqueous phases present in foods thereby increasing their dispersion and subsequently having implications such as reductions in the levels of aroma release. A secondary prediction was that the presence of the solvents, when released from the samples, will increase the solubility of the compounds in the saliva phase and will have impacts on their ability to partition into the gas phase above the saliva. Model systems were used to assess the effects of the solvents. In this case the model system was water but a sugar solution was also investigated. Subsequently more complex confectionery products (gelatine-sucrose gels and boiled sweets) were studied to gauge the effects of solvents in a real food system. *In vivo* studies were carried out to assess how solvents affected the release of aroma compounds in nose.

Competition of molecules for ionisation at the source was an important aspect to be considered when using APcI-MS as a measurement technique but results showed that triacetin, glycerol and propylene glycol produced no significant ion concentrations when in solution. Results for ethanol did show affects on ionisation and a modified system was used to account for this.

5.1 Static Headspace Analysis of Gas Phase Concentration Above Aqueous Solutions for Different Solvent Concentrations

The relationship between the concentration of carrier solvents in the liquid phase and the gas phase aroma concentration above a solution was investigated for a range of volatile aroma compounds; diacetyl, ethyl acetate, ethyl butyrate, ethyl hexanoate, hexanal, limonene, linalool, and methyl acetate. The solvents studied were ethanol, propylene glycol, glycerol, triacetin and distilled water, which was used as a standard solvent. Solvent concentrations were studied at 2, 5, 8, 10 and 15 w/w% of the total solutions. Although it is unlikely that some of these solvents will be present at these levels in real food systems, an idea of the effects of similar mole fractions of solvents was assessed. Volatile concentrations were based on predetermined levels to ensure sufficient headspace concentrations for reliable analysis. Compounds were investigated with two compounds in each sample to avoid any compound-compound interactions (Friel *et al.* 2000) as shown in Section 3.7.

The use of different solvents has many effects on the compounds depending on the properties of both the solvent and the compound itself. We predicted that increasing the solvent concentration in the liquid phase would generally increase the solubility of aroma compounds, especially hydrophobic ones, and hence decrease the amount of aroma that partitioned into the headspace. Some

properties related to the solubility of the solvents and aroma compounds are represented in Tables 5.1 and 5.2.

Table 5.1 Some properties of water-miscible solvents (as taken from Yalkowsky (1999b)) * Log P and solubility data as calculated using EPI Suite Group Contribution Software. ** no data from Yalkowsky.

Water-miscible solvent	Surface Tension (dynes/cm)	Solubility Parameter (Hildebrands)	Dielectric Constant (μS/m)	Log K_{ow}	Log P*	Aqueous Solubility (mg/L)*
Water	72.0	23.4	91.0	-4.00	n/a	n/a
Sugars (sorbitol)	73.3	23.4	62.0	-3.37	-3.01	1.00e6
Glycerol	64.9	16.5	42.5	-2.60	-1.65	1.00e6
Ethylene glycol	48.8	14.6	37.7	-1.93	-1.20	1.00e6
Propylene glycol	37.1	12.6	37.7	-1.40	-0.78	8.11e5
Butanediol	37.9	11.6	-	-0.82	-0.29	2.20e5
Ethanol	22.2	12.7	24.3	-0.31	-0.14	7.92e5
Triacetin	**	**	**	**	0.36	2.15e4

Surface tension refers to the cohesive forces between liquid molecules at the surface of the solution measured in dynes/cm, the force in dynes required to break a film of length 1 cm.

Solubility Parameter is the square root of the cohesive energy density as a numerical value indicating the solvency behaviour of a specific solvent as described by Hildebrand. It is calculated using the following equation:

$$D = \sqrt{c} = [\Delta H - RT/ V_m]^{1/2} \qquad \textbf{Equation 5.1}$$

The dielectric constant is the relative permittivity of a dielectric material.

Log K_{ow} is the logarithm of the oil-water partition coefficient.

Table 5.2 Some properties of aroma compounds tested (as defined by EPI Suite).

Compound	Molecular Weight	Aqueous Solubility (mg/L)	Log P	Vapour Pressure (mm Hg)
Diacetyl	86.09	1.00e6	-1.34	70.20
Methyl acetate	74.08	9.39e4	0.37	52.70
Ethyl acetate	88.11	2.99e4	0.86	98.30
Hexanal	100.16	3.53e3	1.80	9.57
Ethyl butyrate	116.16	2.75e3	1.85	14.60
Ethyl hexanoate	144.21	3.09e2	2.83	1.80
Linalool	154.25	6.84e2	3.38	0.08
Limonene	136.24	4.58e0	4.83	1.45

Co-solvents function by reducing intermolecular interactions in solution, especially hydrogen bonding and therefore their effectiveness depends on their lack of polarity and reduced hydrogen bonding ability with respect to water. They must however, still be soluble in water and its polar portions must be able to interact with water to keep the non-polar regions in solution (Yalkowsky 1999b).

For a solute to become dissolved in a solvent, a number of stages needed to occur. First a cavity within the water structure needs to be formed to accommodate the solute, which involved the disruption of the solvent-solvent intermolecular forces. Dispersion forces will then act to enable the compounds to gain access to the cavities. These forces will be applicable to all solutes. Finally, interactions between similar regions will occur and final solubility will be dependent on these solute-solvent interactions. Examples of characteristics are the polarity, and hydrogen bonding capacity of both the solutes and solvents (Marcus *et al.* 1988). Ruelle and Kesselring (1998a; 1998b; 1998c) suggested that the cavities produced in solution may not be static systems and therefore at 1% of time the solute will not be in solution and will be free to move around. This may have implications for the concentrations of aroma compounds detected above the solutions with the number of molecules available for partition altering with the movement of the molecules in solution. This will apply to dissolution of

all compounds in co-solvent systems and so will be relevant to all future sections. Additional information on solubility and co-solvent effects is discussed in Chapter 1).

The hypothesis was that the solvents would increase aroma compound solubility by decreasing the polarity of the aqueous system thereby retaining a higher concentration of aroma compound within the solution.

5.1.1 Effects of Propylene Glycol

Propylene glycol is the most commonly used carrier solvent used for dispersing and diluting of flavours. Solutions were prepared as per Section 2.2.1.1 and static headspace concentrations measured using APcI-MS (Section 2.3.1.1).

The effect of increasing propylene glycol concentration produced significant effects on the headspace of the different aroma compounds investigated. Figures 5.1 - 5.2 showed decreases in headspace concentration with increasing solvent concentration for linalool, diacetyl, ethyl butyrate and hexanal, no significant changes for ethyl hexanoate and ethyl acetate and an increase for methyl acetate and limonene.

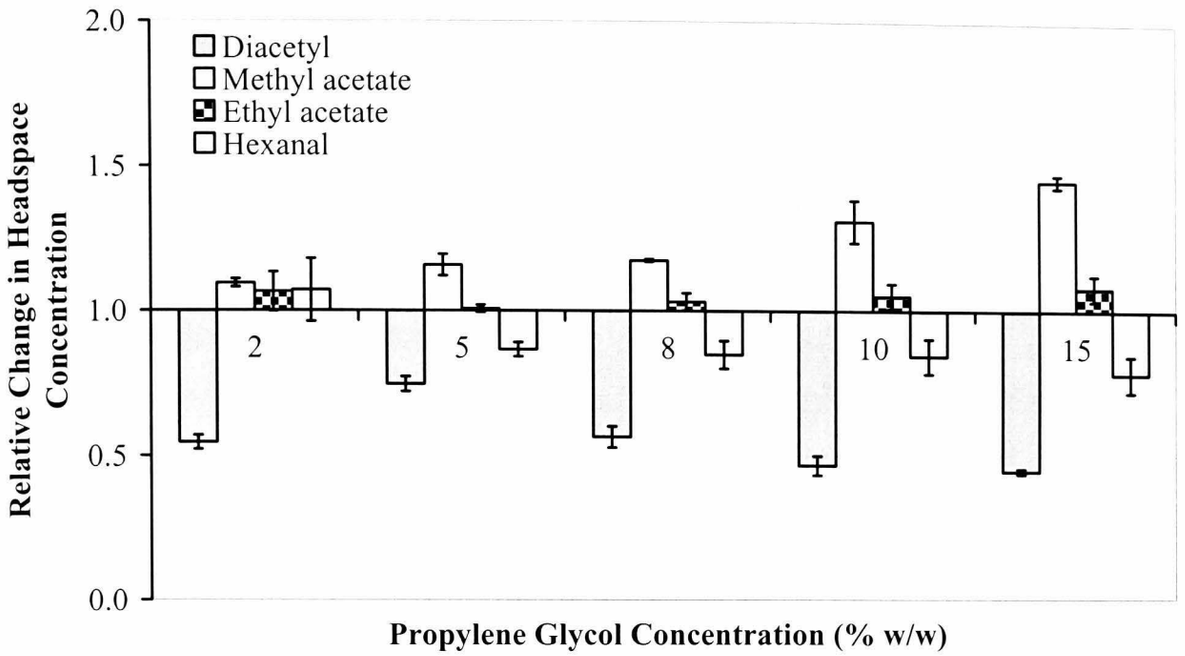


Figure 5.1 Effect of propylene glycol on headspace concentration of volatiles between 0 and 15 % w/w propylene glycol. Four aroma compounds; diacetyl, methyl acetate, ethyl acetate and hexanal. Headspace change is expressed relative to the water sample. Means of 3 replicates.

Diacetyl, the most hydrophilic compound, showed a significant retention within the solution and a trend towards a higher retention with increased solvent levels. The Log P value of propylene glycol is closer to diacetyl than to that of water and so the decreased polarity of the system may be responsible for the increased retention of diacetyl. However, this contrasts to the other highly hydrophilic compound methyl acetate. For methyl acetate, increased propylene glycol concentration brings about an increase in the headspace concentration. This suggests that the solubility of this solute was reduced by increasing concentrations of propylene glycol causing a higher degree of mass transfer and an increase in the partition coefficient. This "salting out" effect (Friel *et al.* 2000) may be due to the solvent in the aqueous phase associating with some of the water molecules thereby reducing the number available for solubilising the hydrophilic compounds. This is in accordance with propylene glycol causing a decrease in the polarity of the aqueous phase and thus a reduction in polar compound solubility (Yalkowsky 1999b). Polar solutes such as amino acids

(Gekko and Koga 1984) and oxfenicine (Gould *et al.* 1984) have been shown to have reduced solubility, with increasing propylene glycol concentration in the aqueous phase.

Hexanal, ethyl butyrate and linalool (Figure 5.2) showed significant reductions in the headspace concentrations, which suggests that they had an increased solubility in the aqueous phase. These reductions, occurred across the solvent concentration, although there was no clear universal trend of an increasing effect with increasing solvent concentration. This suggests that increasing the propylene glycol concentration did not affect all of the compounds in similar ways and the results obtained are difficult to explain when subjected to theories on co-solvents regarding solublities and hydrophobicities. It is therefore likely that some other factors may also be influencing the partition in these systems.

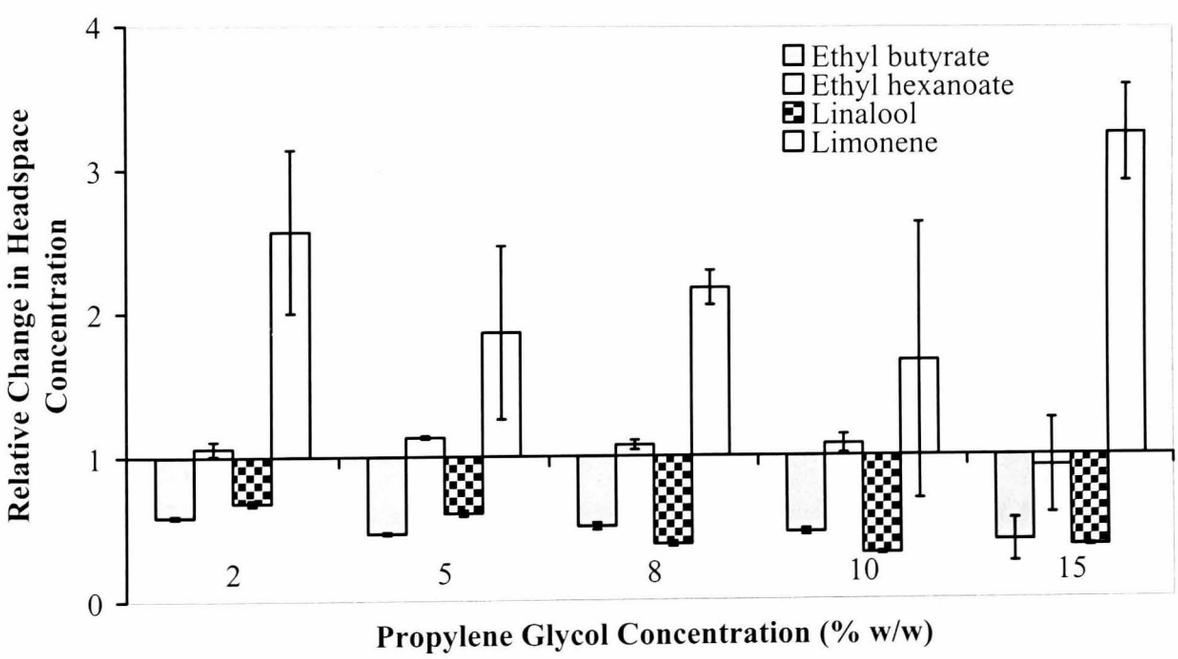


Figure 5.2 Effect of propylene glycol on headspace concentration of volatiles between 0 and 15 % w/w propylene glycol. Four aroma compounds; ethyl butyrate, ethyl hexanoate, linalool and limonene. Headspace change is expressed relative to the water sample. Means of 3 replicates.

The increase in headspace concentration observed for limonene is difficult to account for. The presence of the co-solvents should decrease the headspace partition of limonene yet large increases were observed. A potential explanation may be the poor solubility of limonene in propylene glycol (Hardinge 2002). However, limonene is equally insoluble in water. Limonene shows the highest variation for any of the samples and suggests that limonene is not truly solubilised in the aqueous and propylene glycol solutions.

5.1.2 Effects of Ethanol

Samples were produced as in Section 5.1.1 with ethanol used as the co-solvent. However, because of possible altered ionisation, by ethanol ions at the source, an adapted sampling system was used (Section 2.3.1.4). Ethanol ions were delivered to the source via a nitrogen flow thus making ethanol rather than water the ionising reagent. This eliminated the influence of ethanol in the samples from affecting the ionisation. Increasing ethanol concentrations showed different effects to propylene glycol (Figures 5.3 – 5.4). Diacetyl, ethyl butyrate and linalool showed significantly decreased headspace concentrations with increasing solvent concentration. Limonene, had high variation, but showed a decrease up to 15 %. Ethyl hexanoate showed an increase of 175-265 % at increased ethanol concentrations. Methyl acetate showed a maximum increase at 5% but subsequent decreases with additional solvent. Ethyl acetate and hexanal showed an increase with increasing ethanol concentration up to 8 % and then decreases at 10 and 15 %.

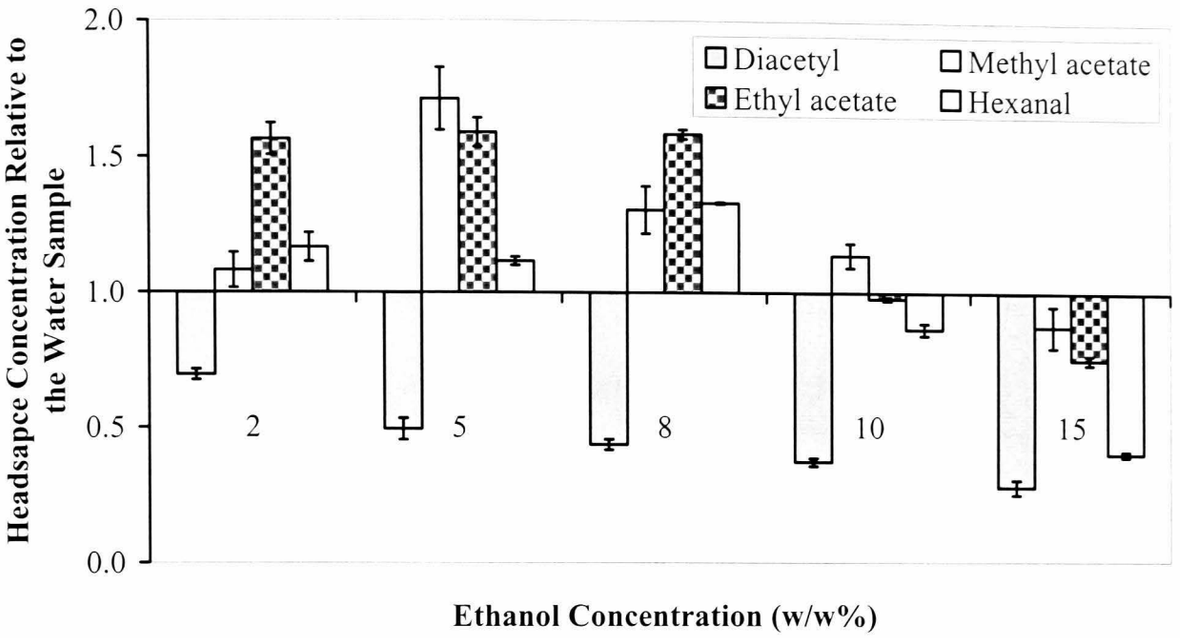


Figure 5.3 Effect of ethanol on headspace concentration of volatiles between 0 and 15 % w/w ethanol. Four aroma compounds; diacetyl, methyl acetate, ethyl acetate and hexanal. Headspace change is expressed relative to the water sample. Means of 3 replicates.

Ethanol is a less polar solvent than propylene glycol and so a higher co-solvent effect on non-polar compounds would be expected (Yalkowsky 1999b). The highly polar compound, methyl acetate, illustrates the effect by showing an increased headspace concentration which is indicative of a reduced solubility. This occurred up to 10 % ethanol after which a decrease was observed. Ethanol will increase the polarity of the solution thereby reducing the solubility of methyl acetate. Interestingly all the compounds, except ethyl hexanoate showed a reduction in headspace concentration and thus an associated increase in solubility at 15 % ethanol concentration.

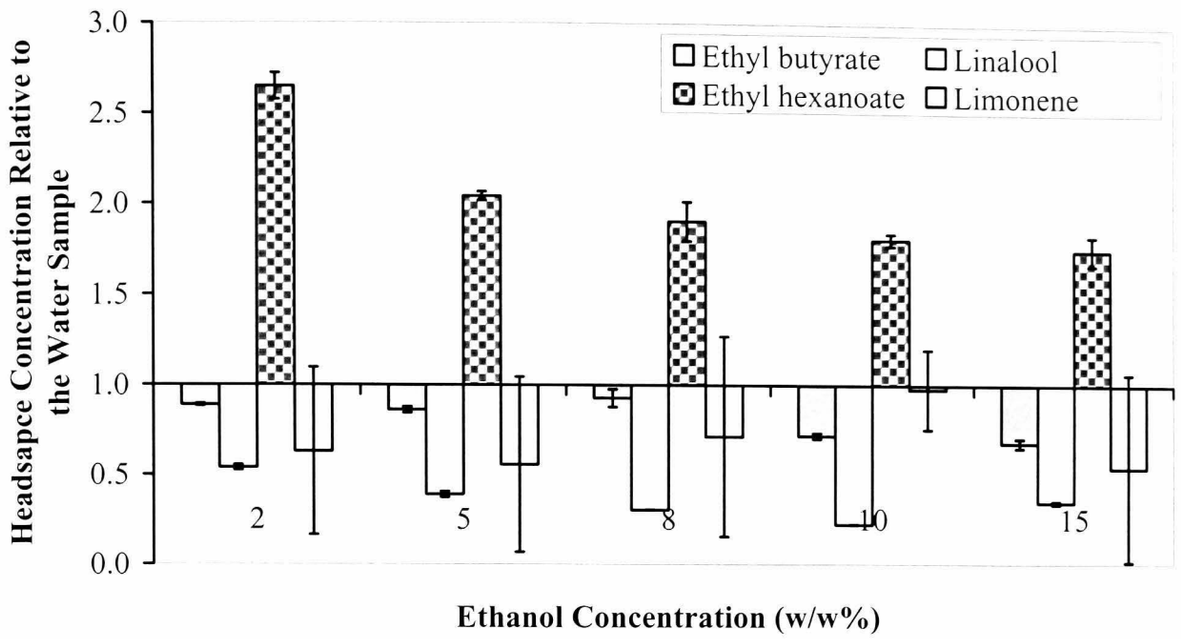


Figure 5.4 Effect of ethanol on headspace concentration of volatiles between 0 and 15 % w/w ethanol. Four aroma compounds; ethyl butyrate, ethyl hexanoate, linalool and limonene. Headspace change is expressed relative to the water sample. Means of 3 replicates.

Conner *et al.* (1998) showed that 17 % ethanol was the critical point at which partition coefficients of long chain ethyl esters were altered. They suggested that at concentrations lower than 17 % the ethanol forms a monodisperse aqueous solution and that above 17 % the ethanol forms hydrophobic clusters that reduce the activity coefficient of the esters (D'Angelo *et al.* 1994). Escalona and co-workers (1998) showed a log-linear decrease in activity coefficients for longer chain volatiles (C6-C12) at concentrations between 10 and 20 % ethanol. This behaviour is not noted with the short chain ethyl esters (ethyl acetate and ethyl butyrate) used in the current experiments which show significant alterations in partition and thus activity coefficient with ethanol concentrations as low as 2 %.

Ethyl hexanoate showed a significant increase with increasing ethanol concentration. However, a reduction from 2 – 15 % ethanol was observed. The mode of sample preparation may have led to lower quantities of ethyl hexanoate becoming dissolved and thus a lower concentration delivered to the final

samples. This would mean that the relative concentrations for the other samples would be higher. The reductions from 2.65 to 1.75 from 2 - 15 % indicate that the higher ethanol levels suggested previously are more important for longer chain ethyl esters. Other aroma compounds also show reductions in partition at concentrations of ethanol at less than 10 % confirming that this effect was not confined to esters.

Diacetyl shows a significant reduction of around 51 % in ethanol concentrations as low as 5 w/w %. This is supported by the findings of Da Porto and Nicoli (2002), who showed that diacetyl partition coefficient decreased with increasing ethanol concentration from 0-95 %. However, the degree of reduction was not as high (19 % reduction at 5 % ethanol).

Another factor that may have contributed to the observed reduction in diacetyl partition is related to the use of APcI-MS for measuring the headspace concentration. Aznar *et al.* (2004) suggested that the lower proton affinity of ethanol could displace water as the ionising reagent in the source, thereby influencing the degree of ionisation occurring. Subsequently, these authors showed that the presence of ethanol ions, at the ionisation source, decreased the APcI-MS signals of diacetyl and limonene. This may contribute to the reduction in both diacetyl and limonene partition observed with increased ethanol concentration. The headspace concentrations were measured using the APcI-MS in 'normal' mode to assess the affects of the ethanol reagent ions. Comparisons for diacetyl and limonene confirm the findings of Aznar *et al.* (2004). At 15 % ethanol concentrations the reductions in headspace concentration were 94 and 66 % respectively for diacetyl and limonene when measured in normal mode. These values compare to the 72 and 46 % reductions for the same samples measured using the 'ethanolic' system.

Aznar *et el.* (2004) also showed that the detected signal of some aroma compounds was increased in the presence of ethanol. They suggested that these differences may be due to cluster ion distribution. An increase in the number of

ethanol dimers present in the ionisation source was observed as the ethanol concentration in the sample increased. The proton transfer from the reagent ion to diacetyl thus becomes endothermic and becomes unfavourable thereby reducing the ionisation. Ethyl butyrate has a proton affinity similar to that of the dimer. They reported that this produced a 300-400 % increase in signal detection for ethyl butyrate and 150-200 % for linalool. Results comparing the 'normal' to the 'ethanolic' modes also confirm these findings. Again at 15 % ethanol, 56 and 22 % reductions were observed for linalool and ethyl butyrate respectively. This compares to reductions of 65 and 32 % observed using the 'ethanolic' mode. Ethanol may change the fragmentation pattern of some ions. Water proton transfer was very exothermic causing a higher level of fragmentation. The reduction of the exothermic nature of transfer will reduce the level of fragmentation and thereby increase the signal detected.

Overall, the effects of ethanol ions at the ionisation source may affect different aroma compounds in different ways. Increasing the ethanol concentration in the sample will increase this effect and thus data interpretation is difficult when ethanol was present within the samples without the use of the system as described in this Section. The system used, although a suitable technique for measuring headspace concentrations, may be subject to degrees of competition that will influence the ionisation of the compounds by APcI-MS. A more robust technique such as GC-MS would give an indication of any elements of competition that might be occurring. Attempts have been made when using APcI-MS to reduce the levels of competition in this experiment by measuring single volatile compounds and assessing the levels of solvent ions produced at the source. This is especially important for ethanol where a new system was developed to try to eliminate the effects of solvent ion competition.

5.1.3 Effects of Glycerol

Glycerol was the most hydrophilic solvent investigated. It has a Log P value of -1.65 (Table 5.1) and is readily miscible with water. This coupled with its high viscosity suggested that it may be the least efficient at solubilising the generally hydrophobic aroma compounds and was anticipated to have similar partition coefficients to those of water.

Figures 5.5 – 5.6 show no definite trend between the aroma compounds for their partition above the solutions. Diacetyl and hexanal show decreased partition with increased glycerol concentration. Ethyl butyrate shows an initial increase at 2 % glycerol followed by reductions with increasing solvent. Linalool shows a reduction of about 50 % in headspace concentration at 2 % glycerol but subsequent increases in solvent concentration do not significantly affect the partition. Conversely, methyl acetate and ethyl acetate demonstrate increases in partition with increasing solvent concentration. Ethyl hexanoate again shows minimal effects of the solvents and limonene again shows a substantial increase in headspace concentration (3 fold at 15 % glycerol).

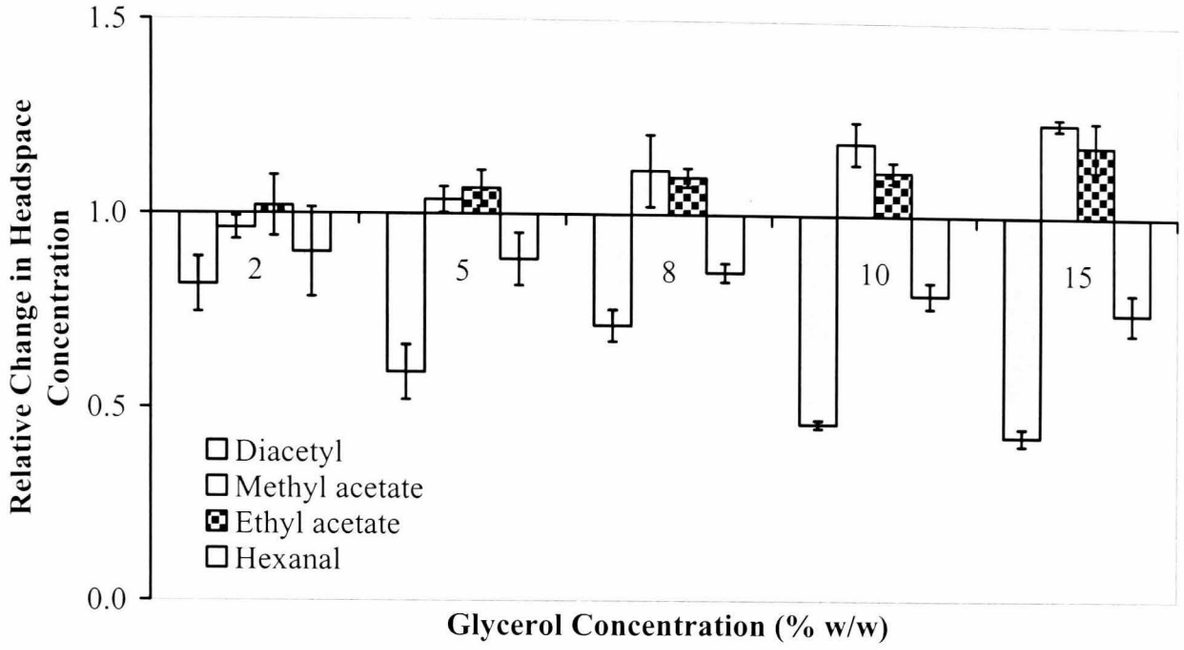


Figure 5.5 Effect of glycerol on headspace concentration of volatiles between 0 and 15 % w/w glycerol. Four aroma compounds; diacetyl, methyl acetate, ethyl acetate and hexanal. Headspace change is expressed relative to the water sample. Means of 3 replicates.

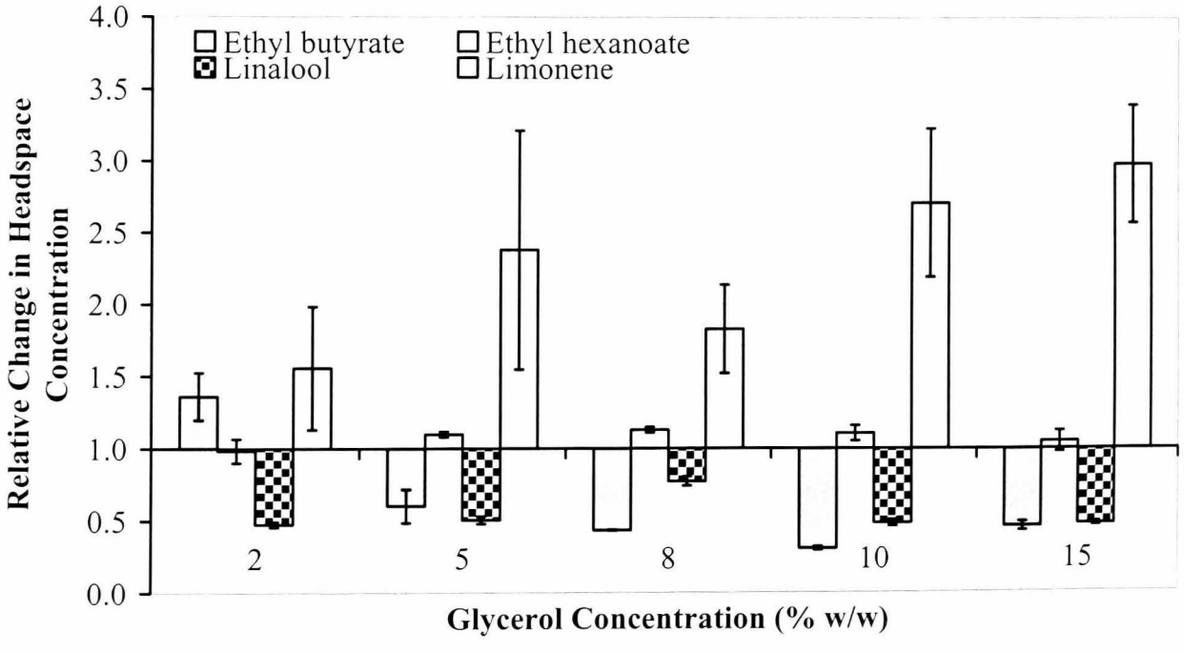


Figure 5.6 Effect of glycerol on headspace concentration of volatiles between 0 and 15 % w/w glycerol. Four aroma compounds; ethyl butyrate, ethyl hexanoate, linalool and limonene. Headspace change is expressed relative to the water sample. Means of 3 replicates.

The increase in limonene concentration of 2-3 times that in the absence of glycerol suggests that glycerol was not increasing the solubility of limonene but was actual decreasing its retention in the aqueous phase. Glycerol may reduce areas that limonene would occupy within the water structure thereby increasing limonene mass transfer and increasing its concentration in the headspace. Yalkowsky (1999b) has suggested that the co-solubility effect of glycerol is about 50 % that of propylene glycol and this may explain the lower effects of glycerol observed for all of the aroma compounds. This will especially be the case for the most hydrophobic molecules such as limonene.

Increased concentrations of glycerol will increase the viscosity of the solution. Nahon *et al.*, (1998a) suggested that hydrophobic compounds showed less release because the viscosity of the solution increased. With increased viscosity, the time to reach equilibrium increases as the Stokes-Einstein law states that the movement of a material is inversely proportional to the viscosity. However, these studies are at static equilibrium therefore diffusion effects due to viscosity can be disregarded in this case.

5.1.4 Effects of Triacetin

Triacetin exhibited a low aqueous solubility and therefore at the concentrations used reached its solubility limit and separated out from the aqueous phase. Owing to its density (1.155 g cm^{-3}) being greater than that of water, it formed a separate phase at the bottom of the solution (i.e. not in contact with the gas phase). This will have implications for partitioning of the aroma compounds. This phase separation process became visibly apparent between 5 and 8 % triacetin concentration, although the solubility of triacetin in water has been suggested to be up to about 10 % (Hardinge 2002).

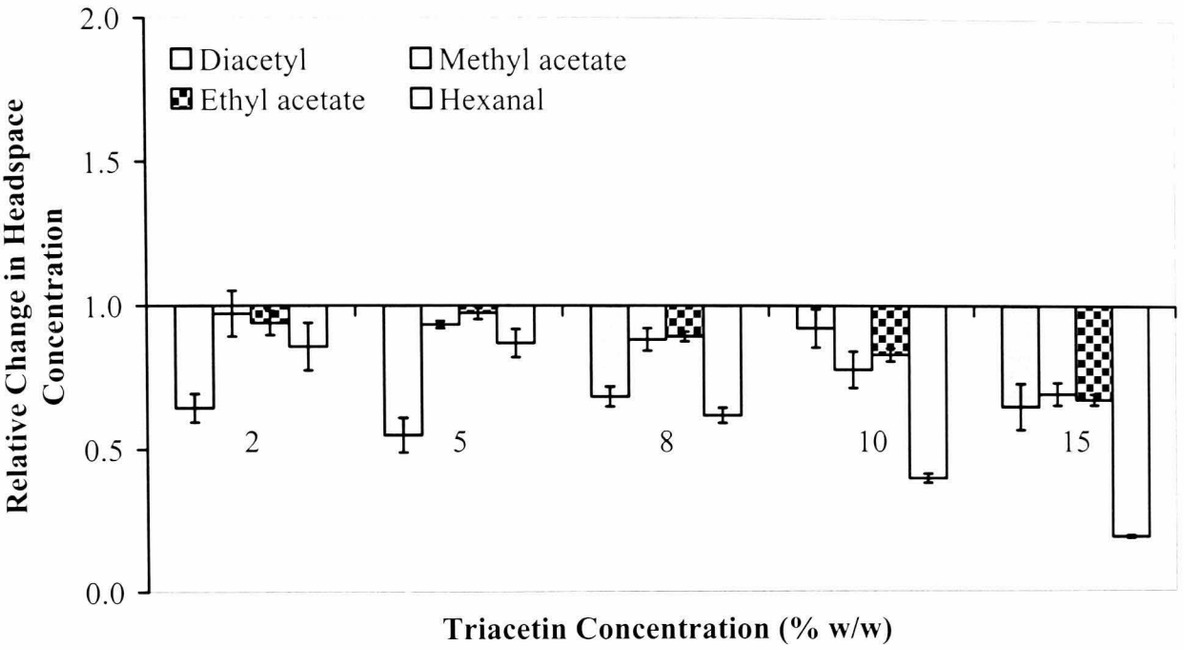


Figure 5.7 Effect of triacetin on headspace concentration of volatiles between 0 and 15 % w/w triacetin. Four aroma compounds; diacetyl, methyl acetate, ethyl acetate and hexanal. Headspace change is expressed relative to the water sample. Means of 3 replicates.

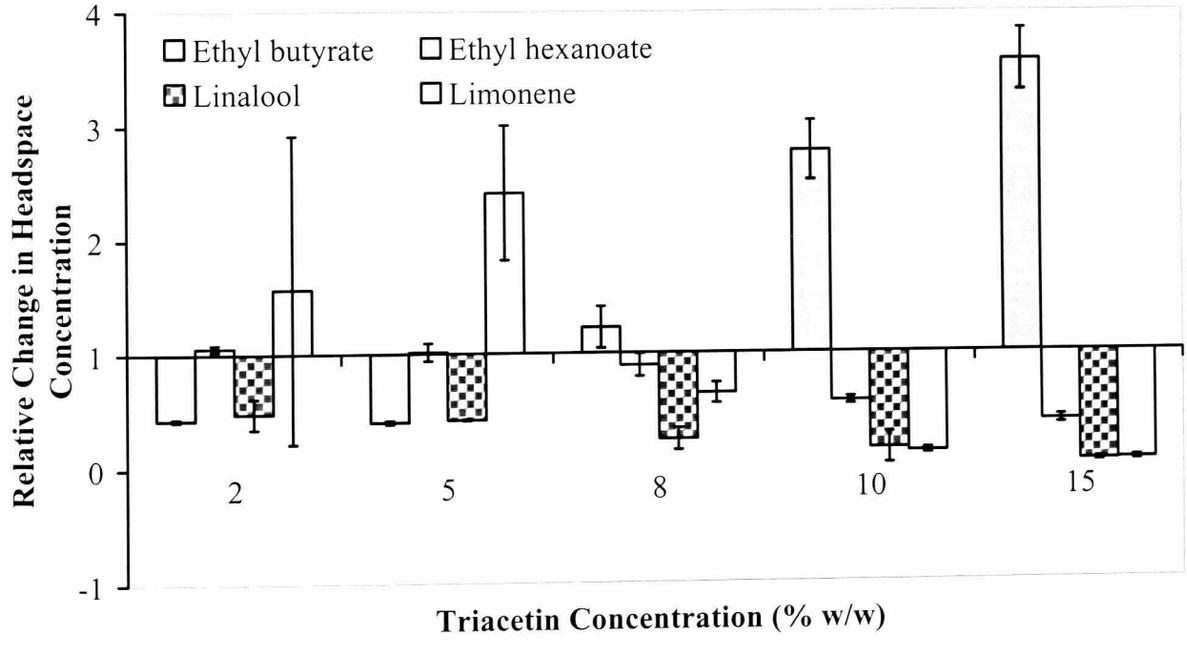


Figure 5.8 Effect of triacetin on headspace concentration of volatiles between 0 and 15 % w/w triacetin. Four aroma compounds; ethyl butyrate, ethyl hexanoate, linalool and limonene. Headspace change is expressed relative to the water sample. Means of 3 replicates.

The use of triacetin (hydrophobic solvent) again showed a very different response. Due to its hydrophobic nature it was expected that triacetin would have a significant effect on compounds with more hydrophobic properties. In real food situations, triacetin is generally used to solubilise water soluble compounds in lipid phases.

Figures 5.7 and 5.8 show that high triacetin concentrations (>8 %) led to a decrease in the headspace concentration for all of the compounds except ethyl butyrate which showed a very high increase. The hydrophobic compounds showed the greatest effects. This suggests that these compounds were preferentially soluble in the triacetin and at higher concentrations will partition into the triacetin phase and will have less access to the headspace. Diacetyl, ethyl acetate and methyl acetate showed a slight reduction and this may be due to some partitioning into the solvents phase.

In contrast, ethyl butyrate showed an increase in the headspace concentration, which suggests that triacetin was forcing it out of solution. However, this does not fit with the previous hypotheses. It may be the case that ethyl butyrate was less soluble in triacetin however; the decline in the 2 and 5 % samples suggests that this was not the case.

At lower triacetin concentrations (2-5 %) linalool, diacetyl and ethyl butyrate were significantly lower and limonene was significantly higher, with the other compounds having a similar headspace concentration to that of water. This suggests that limonene was forced out of solution by triacetin, which may be due to the fact, that in order for the triacetin to be soluble in the phase, it was not capable of solubilising limonene. This is however, seems unlikely to be the case as limonene should be much more soluble in triacetin than in water which suggests some other factors may be playing a role in the solubility of limonene in this system.

5.1.5 Conclusions

Overall these results suggest that the solvents increase the solubility of volatile compounds in the aqueous phase, thereby reducing their partition and activity coefficients however; there are certain exceptions which may be due to properties of the compounds or ionisation parameters of the APcI-MS. Solvent effects vary significantly depending on the aroma compound in question.

The importance on the properties of the co-solvents cannot be ignored and there is a suggestion that ethanol is the most efficient co-solvent for increasing retention of aroma compounds in solution. This is followed by propylene glycol and subsequently glycerol and this is in agreement with the prediction by Yalkowsky (1999b) based on solvent parameters. However, the difficulties in data interpretation associated with the ethanol samples make any conclusions difficult to confirm.

Sample preparation may also be an important aspect to consider. Samples dissolved in a 50 % solvent solution which are subsequently diluted slowly down to 5 % total solvent concentration will show an increased solubility effect than samples prepared in 5 % solvent solutions (Schober and Peterson 2004b). This has implications for the way in which aroma compounds are added to the solution and the subsequent dilutions made. In many food flavourings, the pure aroma compounds are dissolved in the pure solvents and so this was the technique used in this study however, dilutions from stock solutions of aroma compounds may provide a more robust data set.

In the interpretation of the results, hydrophobicity has been assumed to be the major driving force for solubility but this may not necessarily be the case as different functional groups may affect solubility. For example, functional groups such as hydroxyl groups may show better solubility than described by Log P values. Calculation of Log P values may also be a source of error with each

software package using different parameters and many previous studies employing different techniques for calculating the values. Overall size of molecules may be important to fit into cavities in the water structure. Solubility parameter, dielectric constant and surface tension of the solvents may also be important characteristics determining a compound partition coefficient from co-solvent solutions.

If increasing co-solvent concentration brings the polarity of the aqueous solution close to that of the solute, deviation from the log linear approach can be expected. The log linear approach suggests that the co-solvents in water generate a logarithmic increase in solubility with increasing co-solvent concentration (Pinal *et al.* 1990). A parabolic model occurs if the addition of high levels of co-solvent makes the solution polarity less than that of the solute. Concentrations of solvents are reasonably low in this experiment and higher concentrations may be required to produce more marked effects. Many authors have shown that ethanol had no effects on partition of many aroma compounds up to levels of 15 %+ and then only on the most hydrophobic compounds (Conner *et al.* 1998; Escalona-Buendia *et al.* 1998), however, these authors used GC-MS to measure the data and this would eliminate any of the problems associated with competition experienced when using APcI-MS.

5.2 Static Headspace Analysis of Gas Phase Concentration Above Sucrose 'Sugar mix' Solutions

The importance of solvents on partition from aqueous solutions has been demonstrated. However, most real foods are not purely aqueous systems and contain a range of other molecules including sugars, salts and acids as well as macromolecules. This section aims to investigate how some of these other solutes affect partitioning in the presence of the solvents.

As co-solvents change the polarity of the liquid phase of the system, the effects of the presence of sugars and acids, which also affect the polarity, were investigated. A 'sugar mix' system was used which simulates the effect of a sugar slurry. Table 5.3 shows the make up of the 'sugar mix' solutions.

Table 5.3 Non-volatile compounds used to produce 'sugar mix'.

Solute 1	Concentration (% w/w)
Sucrose	30
Glucose syrup	40
Citric acid	1

The effects of sucrose in solution on aroma compound volatility has been widely studied (Wientjes 1968; Kieckbusch and King 1979a; Ebeler *et al.* 1988; Le Thanh *et al.* 1992; Nahon *et al.* 1996; Roberts *et al.* 1996; Nahon *et al.* 1998a; Nahon *et al.* 2000; Hansson *et al.* 2001a). These authors have shown a wide range of effects of sugars and acids on the partition and activity coefficients of aroma compounds. 'Sugar mix' was studied to give a representation of the dissolved sample in the mouth and how this affects the partition and release for the different solvents. Triacetin was not used as it has been shown to have mixing incompatibility in the gel systems.

5.2.1 Effect of 'Sugar Mix' on Headspace Concentration

'Sugar mix' was produced to represent the effects of a solution after the breakdown of a more complex matrix. The static headspace concentration was measured for five aroma compounds above a 'sugar-mix' solution compared to in water alone. Figure 5.9 shows that the more hydrophilic compounds, methyl acetate, hexanol and ethyl butyrate were present in the headspace at higher concentrations suggesting a "salting out" effect as described by numerous other authors (Nawar 1971; Voilley *et al.* 1977; Roberts *et al.* 1996; Nahon *et al.* 1998a; Nahon *et al.* 1998b; Friel *et al.* 2000; Nahon *et al.* 2000). Compounds

with the largest increase in headspace concentration on addition of sucrose were those of a hydrophilic nature whereas hydrophobic compounds were salted in due to a reduction in the polarity of the system (hydrophobic regions) (Nahon *et al.* 1998a). Volatility was shown to be reduced in sucrose solutions for polar compounds and increased for non-polar ones by Roberts *et al.* (1996). They suggested that due to the higher volatility of the more polar compounds they would be most affected by the increase in the solids content which would serve to hinder aroma compound movement in solution. This data however, contrasts to the results obtained in our experiments. Our results show the opposite effect at the high sugar concentrations with hydrophilic methyl acetate salted out the most and all the compounds except menthofuran salted out in this system. The presence of glucose syrup rather pure sucrose may provide more available free water allowing greater movement of volatiles than was the case in Roberts system. However, the data agree well with that of Friel (2000) who showed salting out of linalool, methyl acetate, ethyl butyrate and hexanol and a salting in of menthofuran, which is in agreement with our data.

Polarity significantly influences the solubility but partition may be a result of other properties such as the vapour pressure, size and structure of the compounds. Therefore Log P may not be only factor influencing partition especially in those samples which are from "ideal" solutions. Nahon (1998a) showed some compounds (hydrophilic) had no salting out effect at low log P values. Friel *et al.* (2000) related the effects of 65% sucrose on partition coefficients to properties of the aroma compounds including Log P, LUMO energies and a connectivity index term. Linalool shows the influence of these findings in that although it is a hydrophobic compound (Log P = 3.38) it also shows an increase in partition in sucrose rich solutions which was suggested by Friel. This is difficult to explain although some authors have shown that the "salting in" effect does not apply to all hydrophobic compounds (Friel *et al.* 2000). Linalool is an example of this, and it maybe that the hydrogen bonding effects between the sugars and water are reducing the number of linalool-water

interactions thereby reducing the solubility and subsequently causing the salting out effect observed.

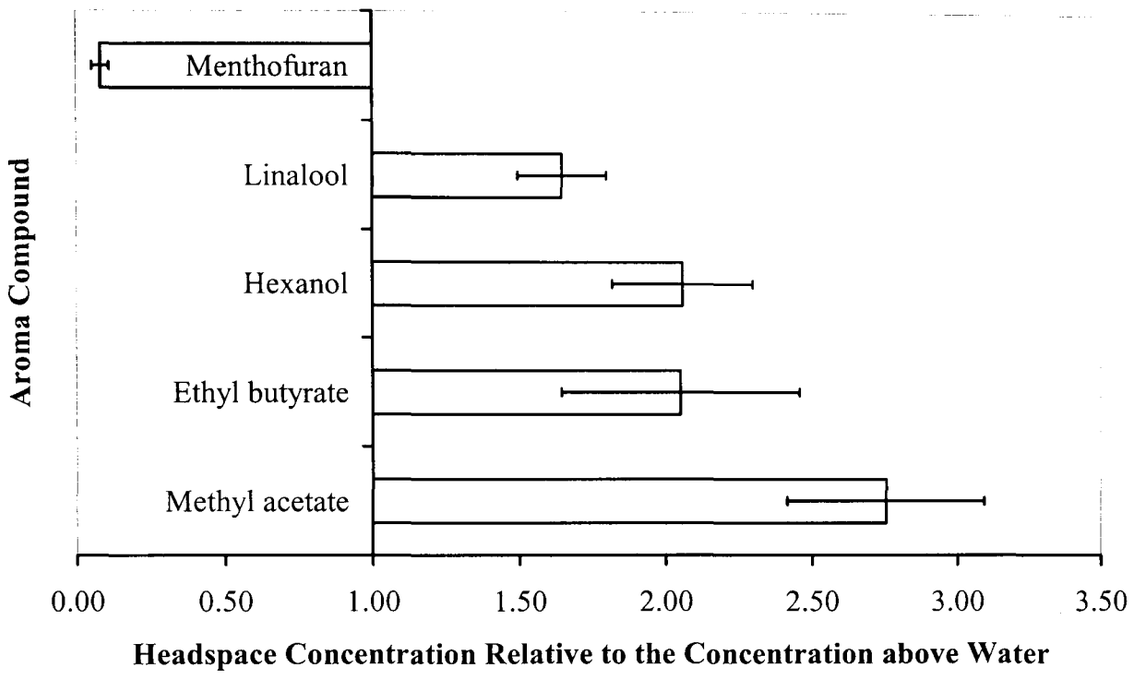


Figure 5.9 Effect of 'sugar mix' on the partition of aroma compounds compared to partition above water solutions. Means of four replicates. Data normalised to water sample.

Menthofuran, the most hydrophobic compound (Log P 4.29) used, showed a significant "salting in" effect. Non-polar compounds similar to menthofuran have been shown to be "salted in" at high sucrose concentrations (Roberts *et al.* 1996; Nahon *et al.* 1998a). This is interesting, as although the solubility of menthofuran is less than linalool in water, it does not show the non-linear effect as the free water available for dissolution decreases, instead it becomes more soluble in solution. It is possible that the increased hydrophobic nature of the solution is preferentially solubilising the menthofuran thereby increasing its solubility.

Wientjes (1968) showed reduction of strawberry volatiles in headspace when added to fructose and invert syrup. Citric and phosphoric acid decreased the release of esters and the effect was suggested to be due to the dissociated forms

of the acids (Hansson *et al.* 2001b). This effect was only observed for esters but other aroma compounds were unaffected. The other flavour compounds were not affected. Ahmed (1978) also showed that acids and sugars led to increase in threshold of d-limonene (i.e. reduced partition) which would be due to the hydrophobic nature of limonene. These data suggest that the other components of the 'sugar mix' will also influence the partition coefficient of the aroma compounds by affecting their solubilities in the solution.

At these high sugar levels, the sugar causes the solution to become more hydrophobic increasing the retention of the hydrophobic compounds (de Roos and Wolswinkel 1994). The likelihood of sucrose-sucrose interactions will increase as the sucrose concentration increases and thus the number of hydrophobic regions available for hydrophobic aroma compound retention will increase. The structure of the solution will be altered by the increase of sucrose. At low sugar concentrations the sugars will be totally hydrated and thus their impact on the water structure will be minimal. As the concentration increases, the number of sucrose-sucrose bonds will be higher. As it dissolves and is diluted the number of intermolecular hydrogen bonds will decrease from two to one and then to none at solutions of less than 20 % (Starzak *et al.* 2000). This occurs as the molecules of sucrose will have less interaction with one another and will be better surrounded by water molecules as the concentration of sucrose decreases. These changes in structure will alter the solution such that different compounds are retained based on their physical chemical properties. Yalkowsky (1999a) reported that sugars may act as co-solvents by altering the polarity of the solution. Sugars are generally highly polar molecules and when present in solution will impart this polarity to the solution, similar to glycerol, and this may explain the effects observed of reduced retention for the hydrophilic compounds.

Another important aspect to be considered in this system is the water activity and dry matter content of the systems. These values would help to explain the changes observed within the system when different components were added and would help to explain the differences in behaviour of the aroma compounds. Dry

matter content is important to ensure that the levels of sugars within the samples remain constant for each as these are the important molecules influencing partition in this system.

5.2.2 Effect of Solvent Type and Concentration on Partition from 'Sugar Mix'

Having observed effects of the 'sugar mix' on partition, two of the compounds were chosen for further investigation, linalool and methyl acetate. The same 'sugar-mix' sample was produced and aroma compounds were added at different solvent concentrations. To ensure dilution of the samples was kept to a minimum, the samples with lower levels of solvents had water added to maintain the same levels of dilution.

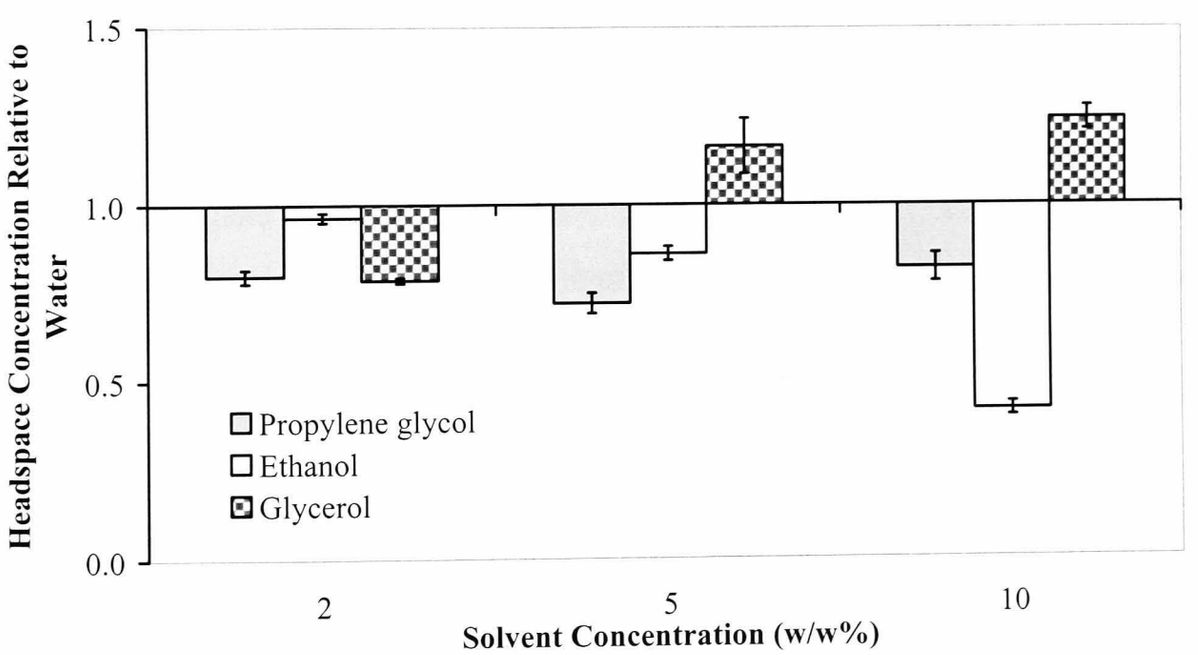


Figure 5.10 Effect of increasing solvent concentration on the relative headspace concentration (relative to water) of co-solvents for linalool in a 'sugar mix' system. Means of three replicates.

Figure 5.10 shows that the use of solvents had mixed effects on the partition of linalool. Propylene glycol increased the retention in the solution, but was not subject to further increases when the solvent concentration was increased. This suggests that propylene glycol was increasing the solubility of linalool in the mixture. However, the effect of 10 % propylene glycol was not as pronounced as for methyl acetate (Figure 5.11) and this may be due to a reduced solubility of linalool in propylene glycol. Linalool shows a reduction in headspace concentration with increasing ethanol concentration. This was again due to increased solubility which reduced its ability to partition into the gas phase. The presence of sugars will increase the hydrophobic nature of the solution and therefore it may be that the increased hydrophilicity produced by the presence of glycerol was actually reducing linalool solubility. Glycerol can also have a 'salting out effect' in solutions (Nawar 1971) which may explain the observations seen for linalool with increasing glycerol concentrations.

Figure 5.11 shows a more dramatic effect for methyl acetate. At 2 %, propylene glycol and glycerol show no significant differences in partition, however, ethanol shows an 85 % reduction in the headspace concentration. This appears to contradict the data observed in aqueous solution for ethanol, which shows an increased headspace concentration of methyl acetate. However, comparing the response for the methyl acetate partition in the 'sugar mix' compared to water (data not shown) indicated that a 325 % increase was observed. This suggested that although methyl acetate showed higher detection in ethanol solutions than water (Figure 5.3) the effect of the sugars was greater. Therefore the presence of ethanol will actually reduce the headspace concentration of methyl acetate above sugar solutions by increasing its solubility in this system. Again the importance of ethanol on the ionisation of the aroma compounds must be considered, but by comparing sets of samples from these experiments in 'normal' APcI-MS some comparisons can be made.

Exponential reductions in headspace concentration were observed with increased propylene glycol and glycerol concentrations. This suggests that these solvents

have low effects at low concentrations but significant retention of methyl acetate at 10 %. The solvents will reduce the effects of the sugar slurry by providing more regions for methyl acetate to be retained thereby reducing the headspace concentration detected.

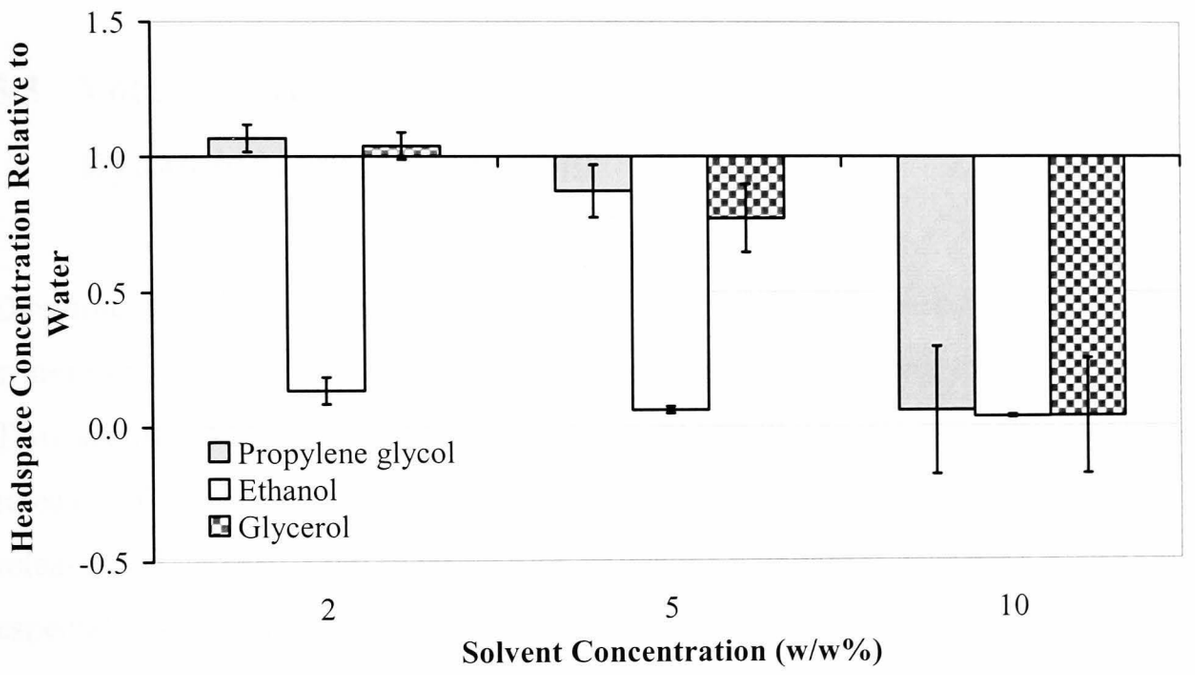


Figure 5.11 Effect of increasing solvent concentration on the relative headspace concentration (relative to water) of co-solvents for methyl acetate in a 'sugar mix' system. Means of three replicates.

Ethanol (10 %) has been shown to be structure enhancer in sugar solutions due to its hydrophobic effect (Serghat *et al.* 1992). This may explain the retention of methyl acetate in a system which essentially serves to reduce methyl acetate solubility. It is well known that the surface tension may be reduced by glucose and sucrose (Docoslis *et al.* 2000). This can influence the partition of aroma compounds into the gas phase; however, this is more likely to be the case in dynamic situations. In static systems, an equilibrium is reached with no external influences therefore the concentration will be maintained. Changes in surface tension will have more influence on dynamic systems because as the gas phase is depleted, mass transfer will occur from the liquid to the gas phase. If the surface

tension is reduced, this rate of mass transfer may well increase due to the reduction in resistance to mass transfer in dynamic systems. Presence of solvents may reduce the solubility of glucose and sucrose (Peres and Macedo 1997), and this could influence the effects of the solvents on the partition of the aroma compounds.

5.3 Volatile Release from Gels Containing Solvents in a Dynamic Headspace System

Dynamic headspace analysis was used to give a better representation of the system that occurs *in vivo*. The system was set up as described in Section 2.3.2. Two aroma compounds were assessed (linalool and methyl acetate). Dynamic release of aroma compounds is a commonly used technique for measuring release and can be used to study a range of factors that influence release. It is especially important in measuring the rate of release from thickened solutions (Bakker *et al.* 1998) or dissolution of solid matrices (Baek *et al.* 1999). These studies indicate that it is appropriate to assess the dynamic release behaviour of volatiles from gelatine-sucrose gels containing co-solvents via this technique. Release from gels-sucrose gels was investigated. Gel samples were added to heated (37°C) water solutions and stirred (magnetic stirrer; 50rpm) until the gel was fully dissolved.

Gel samples were made as per section 2.2.2.1. Aroma compounds, linalool and methyl acetate (125 mg L^{-1}) were pre-dissolved in 5 mL of pure solvent before addition to the gel mixture.

Profiles from Figure 5.12 clearly show the different effects of aroma compound release when different solvents are used. The presence of the solvents had no effect on the smooth profile of release for the polar compound methyl acetate. This may be due to its high solubility in the aqueous phase and subsequently it was released in a consistent manner as the gel dissolves in solution. Interestingly

the presence of ethanol and propylene glycol (even at only 2 %) altered the profile of release for linalool. In these cases the profiles for linalool release mimic those for methyl acetate release, with a smooth increase and a plateau phase. This can be related to the decrease in polarity of the aqueous phase induced by the presence of the solvent which will increase the aroma compound solubility in the aqueous phase. However, when water or glycerol, were used as co-solvents, the decrease in polarity was reduced and hence linalool was less soluble in the aqueous phase. This will affect the dispersion of the aroma compound throughout the solid matrix phase and will lead to areas of concentrated aroma compounds as the non-solubilised volatile compounds aggregate together (Linforth *et al.* 1999a). This will cause a more droplet like release pattern with "bursts" of aroma being released. Nahon *et al.* (2000) measured dynamic release and showed that at lower sucrose concentrations the partition coefficient of aroma compounds primarily controls flavour release, whereas at higher sucrose concentrations the influence of the mass transfer coefficient is more important.

Although there are slight differences in the maximum detected concentrations for the samples shown in Figure 5.12 for methyl acetate release, when all of the data is considered these differences are not significant. The results may have implications for the release of aroma from gels when consumed *in vivo*. However, there is no mastication event involved with these gels and therefore the release pattern will be solely related to the dissolution and melting of the gels.

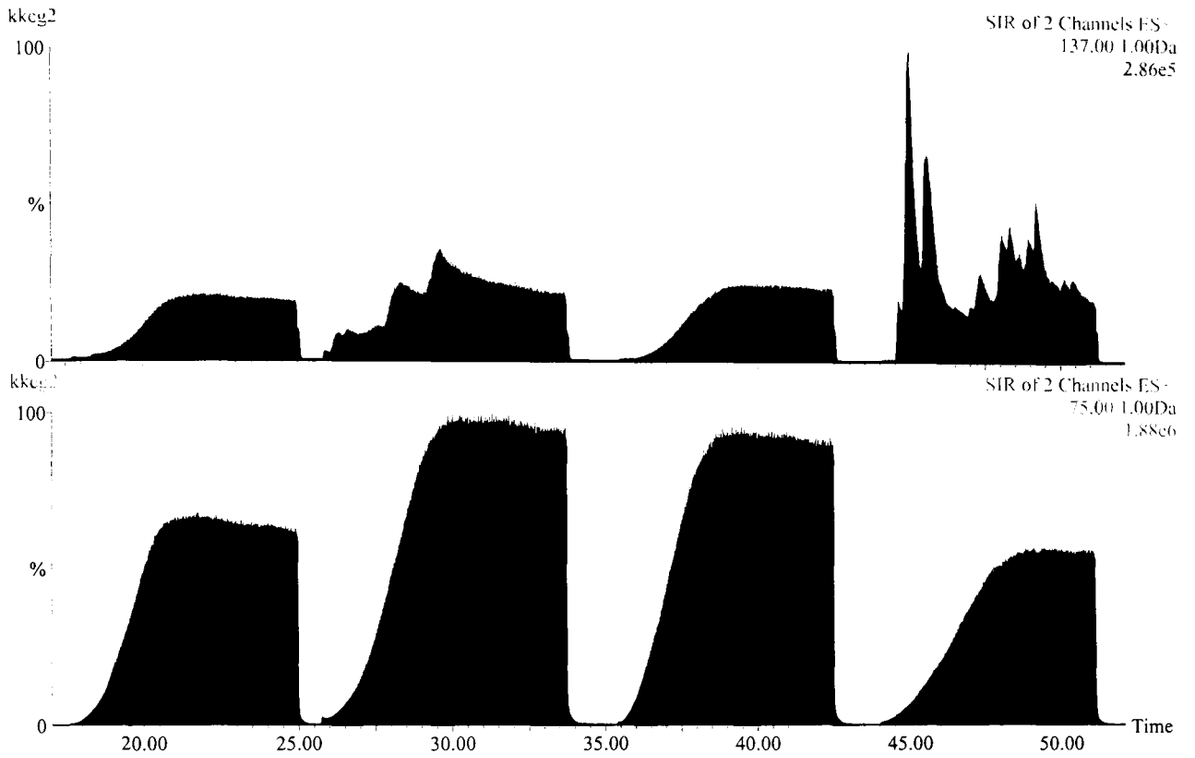


Figure 5.12 APcI-MS trace representing the release of linalool (top) and methyl acetate (bottom) from gelatine-sucrose gels as measured by dynamic headspace analysis. From left to right: ethanol (2 %), glycerol (2 %), propylene glycol (2 %) and water.

5.4 Volatile Release from Model Gelatine-sucrose Gel System *in vivo*

Gelatine-sucrose gels were investigated to analyse release from a model confectionery product which also had a melting component rather than the pure dissolution of a boiled sweet matrix. Solvent concentrations were kept at 2 % as these would be the levels likely to be found in real food systems. Solvents at these low levels were also suggested to have fewer effects on structural and melting properties of the gels.

Gelatine-sucrose gels were tested for all the aforementioned solvents except triacetin which at the concentrations used separated out and did not mix well with the hot gelatine-sucrose solution. Panellists consumed gels as per the protocol set out in Section 2.3.1.3 for a range of aroma compounds. Concentrations of aroma compounds released over time were determined by calibration of the mass spectrometer with pre-determined standards. In order to obtain a better comparison, the results were normalised for each panellist to reduce the effects of inter-panellist variation and to give a better idea of the effect of the solvents.

5.4.1 Initial Experiments

Dynamic headspace results showed a difference in aroma release profile when certain carrier solvents were used at 2 % concentrations. The main observation was that the pattern of release was significantly altered for the hydrophobic compound linalool, when added in water or glycerol, compared to ethanol and propylene glycol. Increase in the polarity of the solution as a result of the presence of the solvents caused linalool to be better solubilised and thus better dispersed. This better dispersion would reduce any concentrated regions of aroma compound thus producing the smoother release patterns observed. The maximum intensity (I_{max}) and the time to maximum intensity (T_{max}) were considered to be important parameters when measuring the aroma release from gelatine-sucrose gels (Linforth *et al.* 1999c) and these parameters were used to compare aroma release *in vivo*.

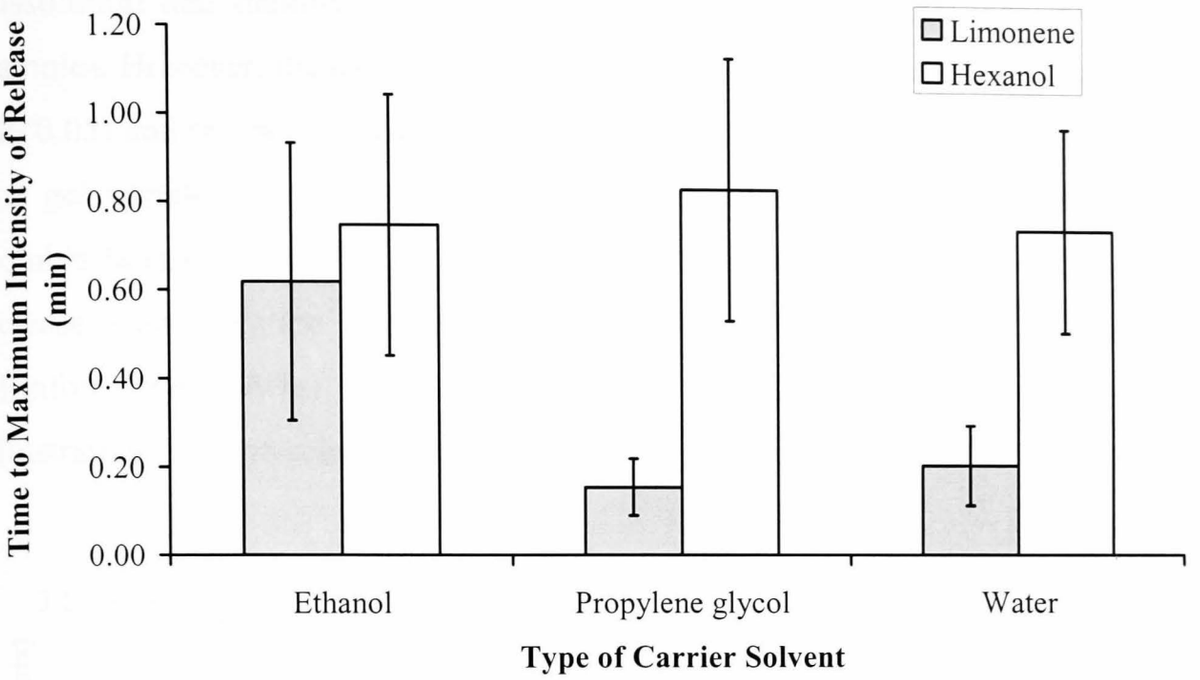


Figure 5.13 T_{max} values for the different carrier solvents and two aroma compounds. Means of 8 replicates from 3 panellists.

Figure 5.13 shows that the T_{max} value for the more hydrophilic compound, hexanol, was unaffected by the presence of solvents (due to a higher water solubility) whereas limonene T_{max} was reduced when solvents were present. Hexanol will be dispersed throughout the matrix and will be released as the gel dissolves and melts. The solvents do not affect its dispersion. This corresponds to the work of Linforth *et al.* (1999a). They showed that the release (I_{max} and T_{max}) of hexenol was unaffected by the use of hydrophobic solvents, decane and pentadecane, when compared to propylene glycol. They suggested that the hydrophobic compounds were present as undissolved droplets throughout the gel and that these would be released upon fracture leading to a burst of dissolved aroma.

This may explain the behaviour of limonene. Limonene has a high Log P value and is sparingly soluble in water. Thus when added to the aqueous gel system, it was likely to aggregate and form areas of high concentration, which were released as the gel was masticated. There would be less importance on gel

dissolution and melting and subsequently the T_{max} occurred earlier for these samples. However, the use of ethanol as a solvent significantly increased the T_{max} ($P<0.05$) and this was probably due to it increasing the solubility of limonene in the gel sample and thus production of a situation similar to that for the more soluble hexanol. A similar finding was described for decane and ethyl butyrate release from gelatine sucrose gels containing ethanol as a carrier solvent (Linforth *et al.* 1999a). These results are clearly shown by Figures 5.14 and 5.15 illustrating the time-release profiles for the two compounds.

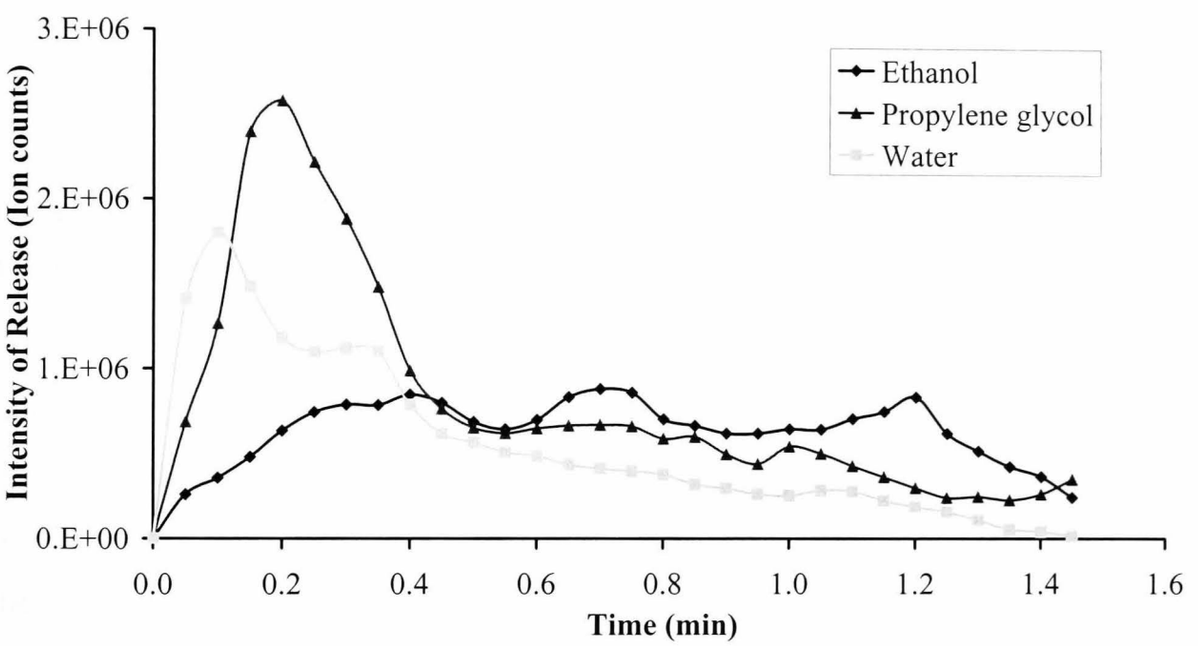


Figure 5.14 Time intensity release profile for limonene release from a gelatine-sucrose gel. Means of 9 replicates.

Limonene is often present in large quantities in lemon and orange oil preparations and the consequences of these terpenoids on other aroma compound release cannot therefore be ignored. Alteration of terpene content could be used as a mechanism to influence aroma release. Fat as a solvent has been shown to delay perception of hydrophobic aroma compounds (Tuorila *et al.* 1995) which may be just an extension of this behaviour. In contrast the use of propylene glycol showed a very low T_{max} for limonene. This may be explained by the low solubility of terpenes in this solvent (Hardinge 2002).

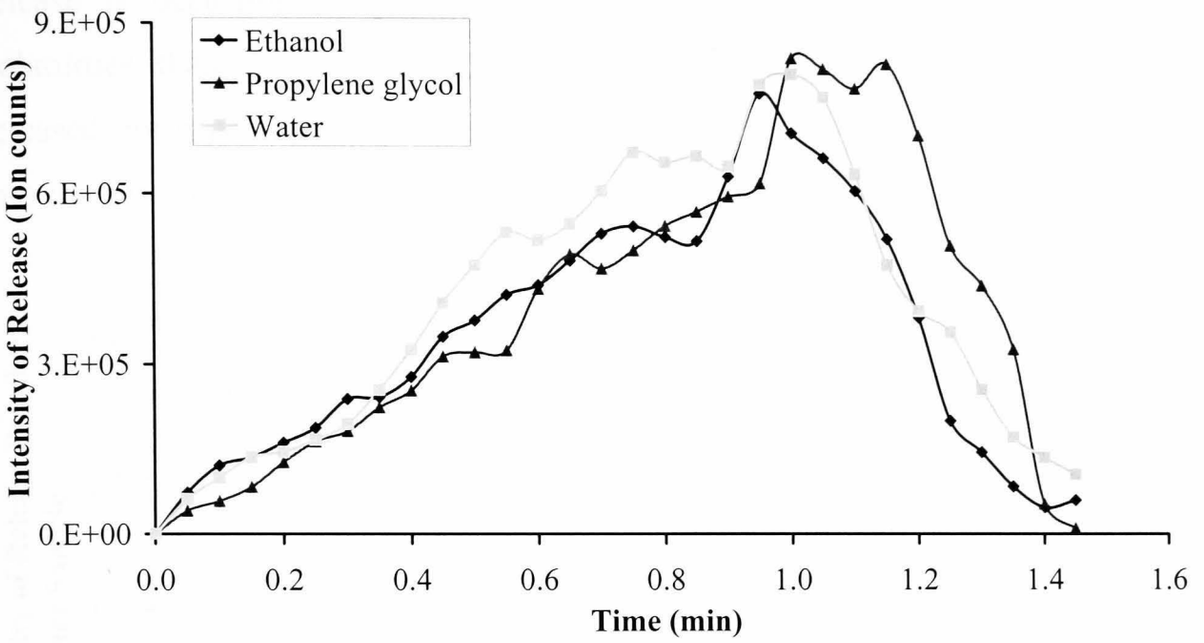


Figure 5.15 Time intensity release profile for hexanol from a gelatine-sucrose gel. Means of 9 replicates.

In contrast the solvents show no significant differences in I_{max} values for either the hexanol or the limonene ($P>0.2$) (Figure 5.16). This was not expected as it was predicted that by creating a better dispersion through the use of solvents that the I_{max} value would be reduced. Limonene in ethanol shows a lower level of release which may be due to the increased solubility and reduction in rate of partition into the gas phase in mouth.

Once the volatile is released it must partition into the saliva phase and subsequently into the gas phase (Harrison and Hills 1996). If the carrier is still present it will increase the levels of volatile that are retained in the saliva phase and will thus reduce the amount and rate at which the volatile compound can reach the receptors in the olfactory epithelium.

It has been suggested that a decrease in solubility increased the tendency to partition into the gas phase but an associated reduction in volatility reduced the amount of compound able to partition, thereby giving similar I_{max} concentrations

(Linforth *et al.* 1999c). From our results we suggest that a similar method of release is occurring as more hydrophobic compounds tend to have lower volatilities than their hydrophilic counterparts which will reduce the levels released corresponding to their lower solubilities which will promote partition into the gas phase.

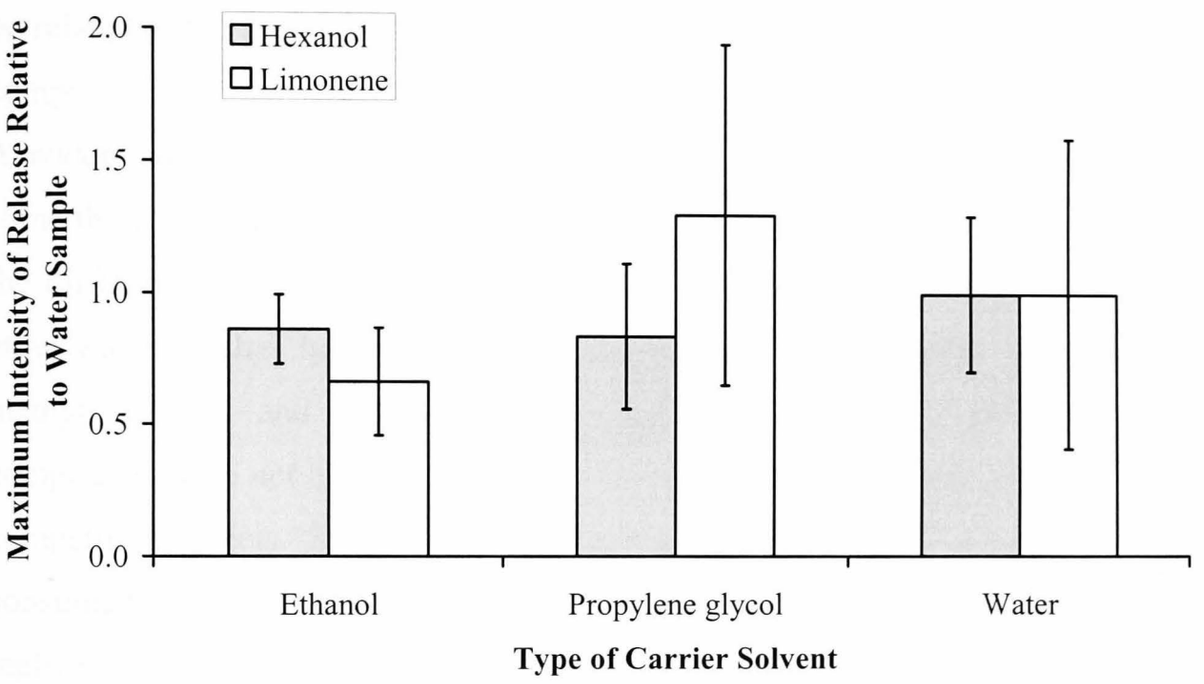


Figure 5.16 Maximum intensity of release relative to water samples. Data normalised to remove panellist variability. Means of 3 panellists with at least 2 replicates of each (total replicates 8).

The addition of solvents such as ethanol, and propylene glycol has been shown to produce microemulsions with limonene (Yaghmur *et al.* 2002b) and this may be the reason for the alteration in the release pattern shown. These may affect the release pattern and intensity. De Bruijn (1989) has suggested that vigorous mixing of organic compounds in an aqueous system may produce a microemulsion rather than a homogenous solution. This suggests that some of the hydrophobic compounds may be added as microemulsions and thus release will be related to emulsion type release patterns.

5.4.2 Effects of Compound Properties on Aroma Release from Gels During Delivery by Different Co-solvents

Section 5.4.1 demonstrated that the co-solvents, not only influence the equilibrium concentrations of the aroma compounds, but can also influence the concentration and more importantly the timing (rate) of release. This appeared to be related to the properties of the aroma compounds with the more hydrophobic compound limonene showing the greatest effects of the presence of the solvents. A wider range of aroma compounds was assessed to investigate the effects of some the compound properties on the patterns of aroma release to try to explain the differences observed. Eight aroma compounds were investigated; butanone, ethyl acetate, ethyl butyrate, ethyl octanoate, hexanal, limonene, linalool and methyl acetate; and three co-solvents (triacetin again not used). Aroma compounds were not added all together to avoid volatile-volatile interactions and competition effects. Samples were prepared as per Section 2.2.2.1 and were consumed as per Section 2.3.1.3 by 4 panellists with a minimum of three replicates of each sample.

Tables 5.4 and 5.5 show the I_{max} and T_{max} data for the 8 aroma compounds as normalised for each panellist against the gel containing only water and then averaged. I_{max} results show that ethanol generally reduced the maximum intensity of release for more hydrophobic compounds which is in accordance with the increased solubility described earlier. Although this is generally the case an exception is observed for ethyl octanoate. This is difficult to explain but may be due to other properties of the compound such as interactions with the gel structure of a boost in detection caused buy the presence of ethanol. The more hydrophilic compounds demonstrated slight increases in intensity but these were generally not significant. The T_{max} data for samples dissolved in ethanol indicate an increase suggesting increased solubility throughout the gel and a delayed release. The other solvents are less conclusive and show variations in both T_{max} and I_{max} depending on the compound which appear to be unrelated to the

solubility or polarity of the compounds. This suggests less efficient solubilisation by these solvents in the gel matrix.

Table 5.4 Effect of carrier solvents on the maximum intensities of aroma release from gels. Data are expressed relative to the gel with water as the sole solvent. Data normalised for each panellist (4 panellists). Means of three replicates.

Compound	Ethanol	Glycerol	Propylene glycol	Water
Butanone	1.10 ± 0.27	0.34 ± 0.14	1.09 ± 0.40	1.00 ± 0.30
Methyl acetate	1.13 ± 0.55	0.53 ± 0.22	1.18 ± 0.61	1.00 ± 0.32
Ethyl acetate	1.13 ± 0.62	0.46 ± 0.22	1.47 ± 0.86	1.00 ± 0.48
Hexanal	1.25 ± 0.75	0.94 ± 0.41	1.28 ± 0.48	1.00 ± 0.32
Ethyl butyrate	0.42 ± 0.38	0.69 ± 0.27	0.40 ± 0.08	1.00 ± 0.57
Linalool	0.46 ± 0.20	2.12 ± 1.05	1.19 ± 0.58	1.00 ± 0.65
Ethyl octanoate	2.55 ± 0.45	1.88 ± 1.16	3.29 ± 1.80	1.00 ± 0.64
Limonene	0.23 ± 0.13	1.31 ± 0.69	0.27 ± 0.10	1.00 ± 0.73

Table 5.5 Effect of carrier solvents on the time to maximum intensities of aroma release from gels. Data are expressed relative to the gel with water as the sole solvent. Data normalised for each panellist (4 panellists). Means of three replicates.

Compound	Ethanol	Glycerol	Propylene glycol	Water
Butanone	1.30 ± 0.78	1.08 ± 0.90	0.95 ± 0.25	1.00 ± 0.55
Methyl acetate	1.14 ± 0.33	0.96 ± 0.23	1.10 ± 0.27	1.00 ± 0.36
Ethyl acetate	1.69 ± 0.56	1.47 ± 0.60	1.53 ± 0.47	1.00 ± 0.36
Hexanal	1.32 ± 0.61	1.23 ± 0.55	1.58 ± 0.49	1.00 ± 0.37
Ethyl butyrate	1.82 ± 0.38	0.88 ± 0.46	1.86 ± 1.17	1.00 ± 0.59
Linalool	1.05 ± 0.26	0.85 ± 0.28	0.95 ± 0.32	1.00 ± 0.30
Ethyl octanoate	1.15 ± 0.17	1.03 ± 0.35	0.96 ± 0.11	1.00 ± 0.29
Limonene	4.38 ± 1.33	1.01 ± 0.48	2.08 ± 1.12	1.00 ± 0.18

Harrison and Hills (1996) predicted that aroma release from gelatine-sucrose gels occurs by the penetration theory of mass transfer. This involves the concurrent diffusion of heat into the sample with diffusion of sucrose out of it. Thereby aroma compounds will be released into the saliva as the sucrose dissolves and the gel melts. In regions of insolubility more concentrated regions maybe produced

that give rise to higher levels of release that occur earlier in the consumption period. The solvents, especially ethanol, reduce these effects by increasing the solubility in the gel matrix.

5.4.3 Confirmation Experiments

It has been suggested that solvents may affect the physical properties of the gels (ElyseeCollen and Lencki 1996) and therefore the dissolution and rate of aroma release. Other authors have recently suggested that aroma compounds themselves can influence the physical properties of gels. Lubbers and Decourcelle (2004) suggested that hydrophobic interactions are the main interactions leading to pectin gelation, and increasing the number of hydrophobic interactions there are, increased the fracture strength of the gels. They assumed that esters might increase the gel hardness through the increase of hydrophobic interactions in pectin gel network. They also showed a similar effect for solvents (ethanol, propylene glycol), and suggested that a co-solute effect could explain the increase in firmness for pectin-based gels. This study was carried out in pectin gels and may not have the same effects in gelatine-sucrose gels however, the potential that aroma compounds may also influence gel hardness should not be ignored.

Initial studies with Differential Scanning Calorimetry (DSC) and TAXT2 Texture Analysis indicated that the solvents did not affect the textural or melting characteristics of the gels at the concentrations used in these experiments. DSC data showed that the melting point for the gels was between 45-50°C, which was higher than the in-mouth temperature. This suggested that the sucrose content of the gel was increasing the melting temperature to above that of the mouth. Texture analysis showed no difference in the compression measurements for the gel samples. In consequence, the gels will rely primarily on dissolution of the sucrose into the saliva which will then lead to a reduction of the gel melting temperature and hence melting will be able to occur (Harrison and Hills 1996).

Rates of aroma release will therefore be dependent on the rate at which the sucrose is dissolved and heat can transfer to the gelatine to cause melting.

5.4.4 Conclusion

The effect of aroma release from gels from *in vivo* experiments is not as well defined as for those studied by dynamic release studies. Explanations include the introduction of masticatory events that will fragment the gel more rapidly, thereby mixing the fragments and causing a more rapid breakdown of the samples. Any effects of solvents may be reduced by the effects of saliva, as mixing of the sugar slurry in-mouth will also influence the partition of the aroma compounds. Friel *et al.* (2001) showed influences of salivary components on partitioning of aroma compounds. The overall conclusion to these results is that the most significant effects on aroma release were in the timing of release with ethanol being the most influential.

5.5 Boiled Sweet System

A hard candy, or boiled sweet, matrix was investigated to study the effects of dissolution on the release of aroma compounds. Schober and Peterson (2004a) suggested that the aroma compounds from hard candy exist in "pocket-like" cavities which are extremely limited in mobility due to the sugar glass matrix. Hills and Harrison (1995) utilised the simplicity of the boiled sweet system to model flavour release based on measurements of dye release during dissolution. They suggested that a two-layer stagnant film theory provided the most appropriate explanation for the mechanism of flavour release from boiled sweets. This theory was based on a number of assumptions related to the dissolution and diffusion of sugars. Delivery of aroma compounds into the saliva is dependent on sample dissolution, and hence the ratio of volatile to non-volatile components remains constant throughout sample consumption (Schober and Peterson 2004a).

Therefore, a boiled sweet matrix was chosen to serve as a model to characterise the flavour-carrier solvent interactions over a prolonged period where no fracture of the sample is involved.

Boiled sweets were produced as per Section 2.2.6 and samples were consumed on the APcI-MS by a single panellist to reduce variation. Two aroma compounds were tested; limonene and ethyl butyrate for each of the solvents used and these were added at 125 mg L^{-1}.

Figure 5.17 shows that the I_{max} of release was significantly higher ($P<0.05$) for both aroma compounds when propylene glycol was used as the carrier solvent. The total increase was four-fold compared to that of samples when water was the solvent and, in excess of eight fold higher when ethanol was used as the solvent. Retention of the aroma compounds was estimated by re-dissolving the samples in aqueous solution, equilibrating at 20°C for 24 hours, and then measuring the headspace concentrations (Figure 5.18). The data for retention, match those for release with propylene glycol having a significantly higher level (11 fold) of aroma compared to water. Interestingly, ethyl butyrate showed a lower level of retention than limonene. This may be due to better solubility of ethyl butyrate by solvents or higher losses due to the higher volatility of ethyl butyrate.

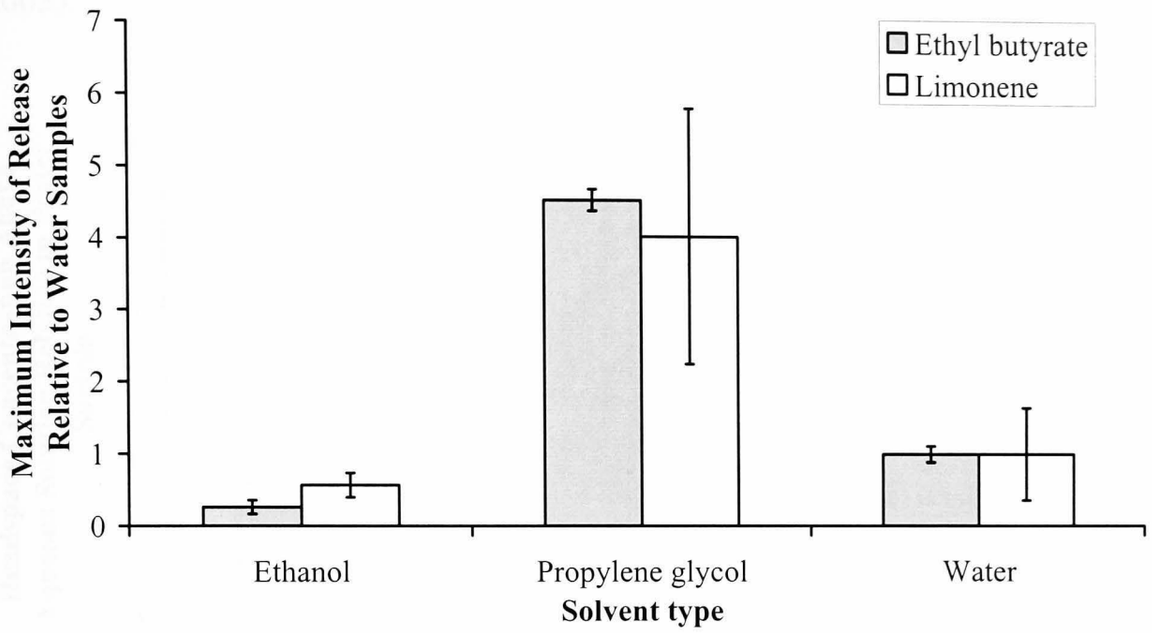


Figure 5.17 Maximum Intensity of aroma release from boiled sweet samples containing different co-solvents. Means of three replicates. Error bars represent standard deviation.

Flavourings for boiled sweets are usually dissolved in vegetable oil because the solubility of most aroma compounds is higher and subsequently the volatility of these compounds is much lower in oil than in other solvents. However, de Roos (2003) showed that addition of aroma compounds in vegetable oil had a lower retention in the final product compared to propylene glycol. They suggested that addition of oil to the hot sugar phase produces a two phase system where volatilisation is from the oil phase alone. Conversely, propylene glycol resulted in rapid mixing with the sugar phase, which subsequently reduced the compound volatilisation by increasing the resistance to mass transfer rather than by effects on the volatility itself. For the solvents used in our experiment, all of the solvents should rapidly mix with the sugar-slurry due to similar solubilities (unlike the oil), however any incompatibilities in mixing could explain the differences observed for ethanol or water when used as solvents, thus lowering the retention level. It is therefore unlikely that the solvents used for experiments will phase separate in the way that oil has been shown to do which may lead to a higher

retention in the final product for all of our samples as suggested by De Roos (2003).

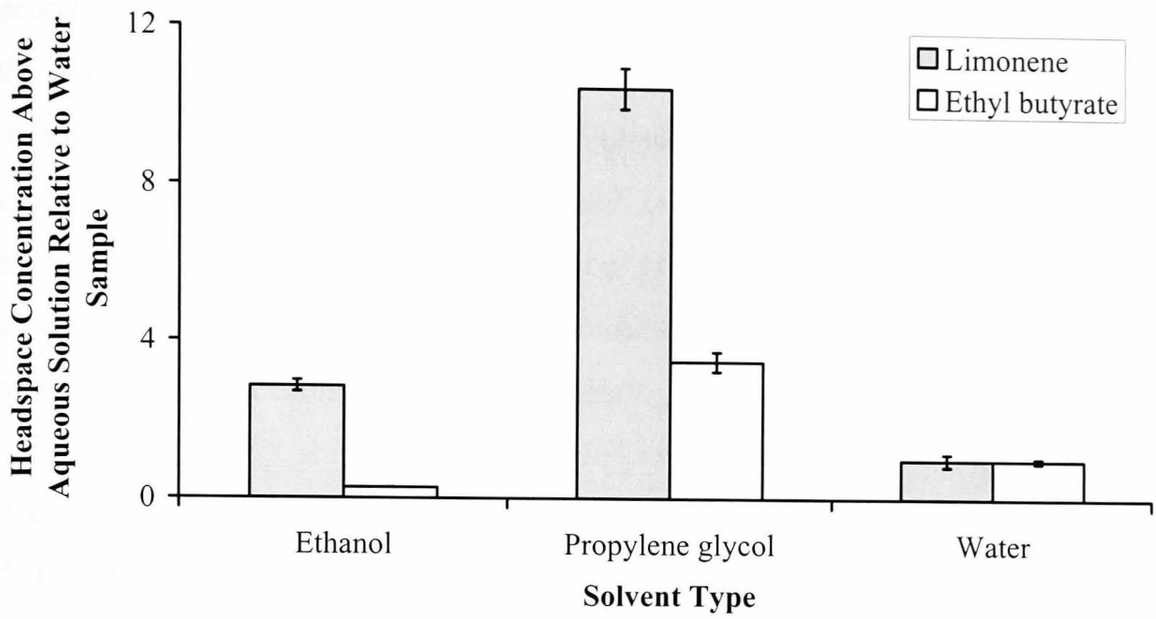


Figure 5.18 Headspace concentration above re-dissolved samples containing different co-solvents. Means of three replicates relative to the values for water.

A similar finding was shown by Schober and Peterson (2004b) for L-menthol release. They reported that the release rate from propylene glycol was more rapid and a higher headspace concentration (2 fold) was observed from propylene glycol than 1,8-cineole. This was not due to hydrophobicity of the solvent however, as another hydrophobic solvent miglyol showed the same behaviour as propylene glycol and hence a colloidal influence was suggested. Results from Section 5.1 suggest that the polarity of the solvent was an important consideration when aroma compounds are released from food matrices.

Retention may not be the only important factor when considering aroma delivery. β-cyclodextrin was showed to have an 86-fold higher retention of aroma compared to propylene glycol, however, the low levels of moisture meant that propylene glycol had a better release and subsequently the final samples were perceived to be mintier (Reineccius *et al.* 2004). This suggests that although the

aroma compounds could be retained at a higher level it is still important to allow these compounds to be released. β-cyclodextrin allowed higher levels of aroma compounds to be delivered into the boiled sweet and reduced the losses during production compared to propylene glycol but this aroma became trapped within the matrix and was not available for release during consumption. This shows the importance of not only delivering the highest amount flavour possible but also ensuring that this aroma can be released *in vivo* during consumption. In our experiments it was clear that propylene glycol not only delivered a higher concentration of aroma to the sweet than the other solvents, but it also allowed a higher intensity or aroma to be released during consumption.

Figures 5.19 and 5.20 show the profiles for volatile release from the three different carrier solvents. These data show that the release rate was higher from propylene glycol than for the other two solvents. This data correspond to that of Schober and Peterson (2004b), who reported that hydrophobicity of the solvent affected the release rate. They showed that propylene glycol released L-menthol at a faster rate in an aqueous system than the hydrophobic solvents. However, they showed that no difference in release rates was observed between the solvents for release *in vivo.*

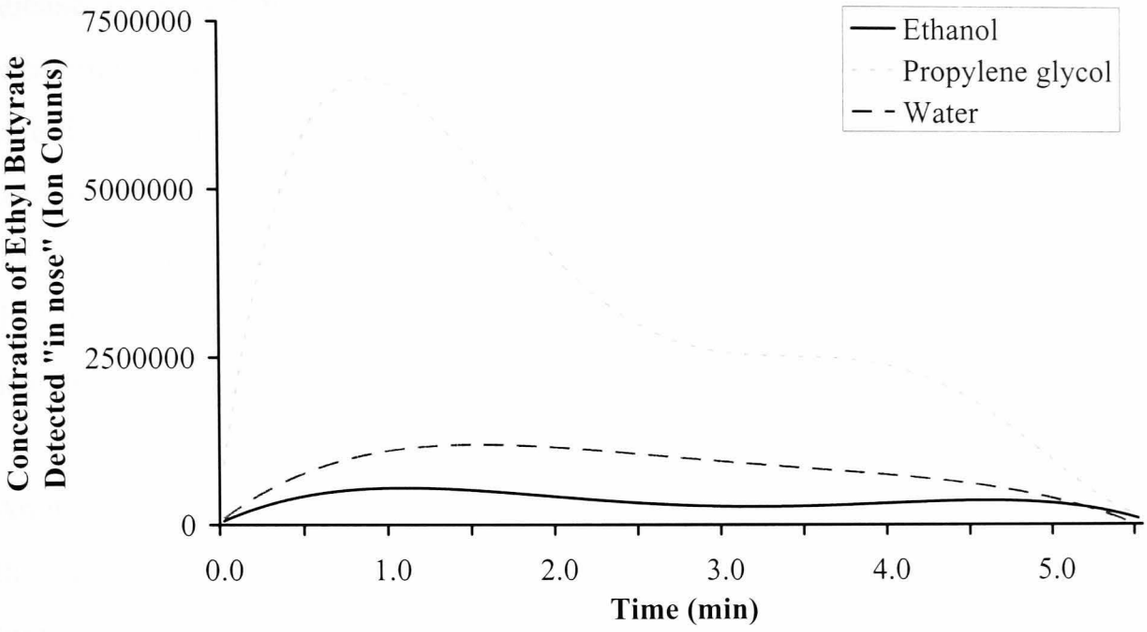


Figure 5.19 Ethyl butyrate release from boiled sweets over time when consumed by a single panellist. Mean of three replicates. Data smoothed by a 6-point polynomial trendline.

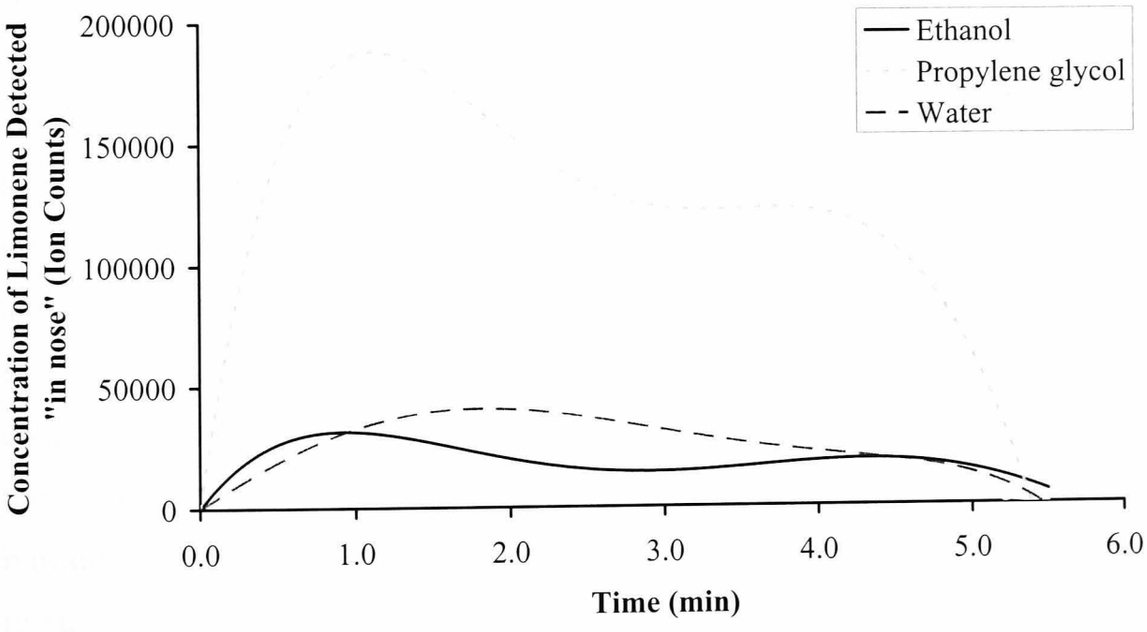


Figure 5.20 Limonene release from boiled sweets over time when consumed by a single panellist. Mean of three replicates. Data smoothed by a 6-point polynomial trendline.

These previous studies may explain the increased retention and subsequent release observed from propylene glycol however, the solvents used in our experiments all have higher polarities than vegetable oil, miglyol and 1,8-cineole. If it is not the polarity of the solvent, or the volatility of the aroma compound that is important other factors must be considered. The solubility of the two aroma compounds should be higher in ethanol than propylene glycol yet; they will be equally less soluble in water, thus discounting solubility of the aroma compounds as an important factor for release in this case.

Another potential explanation is related to the boiling points of the solvents themselves (Table 5.6). Addition to the hot-slurry had to occur at high temperatures to prevent the slurry from setting (90-100°C) before aroma compounds could be dispersed. Although the aroma compounds have boiling points in excess of the temperature at which the flavour-solvent mixture was added, the solvents do not. Propylene glycol, which showed the highest retention and release, has a boiling point of ~187°C which is far in excess of the sugar-slurry and will therefore be unaffected at these temperatures. Ethanol and to some extent water however, have boiling points below (in the case of ethanol, 78°C) or similar (water, 100°C) to that of the sugar-slurry. These solvents will be subjected to degrees of boiling when added to the molten sugar phase and thus greater losses of aroma compounds would result. It is well documented that additions of solutes to a solution can affect its boiling point. This is best evidenced by the reduction in aqueous solution boiling point in the presence of ethanol, or the increase in boiling point in the presence of sodium chloride. It is therefore likely that the higher delivered aroma was a consequence of higher boiling point ensuring a higher level of aroma compounds in each of the samples rather than any increased solubility engendered by the differences in co-solvent parameters.

Table 5.6 Boiling Points of carrier solvents and aroma compounds: * means experimental data as retrieved from the EPI Suite Database.

Compound	Boiling Point (°C)
Ethanol	78.2*
Propylene Glycol	187.6*
Water	100.0
Ethyl butyrate	121.5*
Limonene	178*

The raw data from this experiment show spikes in the aroma time-release profile, throughout the consumption process, as aroma is released. This suggests that flavour compounds are not truly dispersed throughout the matrix of a low water system but are present as concentrated regions within cavities in the matrix as described by Schober and Peterson (2004a). This has implications for the dispersion techniques and the release of aroma compounds from these types of system. Release from these concentrated regions may change volatile release rates and subsequently change perception when these regions are present compared to systems where they are not. Further discussion of systems with concentrated aroma is discussed in Chapter 6.

5.6 Conclusion

Increased aroma dispersion has been shown to be an important practise when delivering flavours to foods; however the implications for the effects on release need to be recognised. Carrier solvents possess different properties and this will affect their efficiency in dissolution, dispersion, partition and release of aroma compounds. Propylene glycol is the most commonly used carrier solvent due to its absence of any taste/flavour as well as a reasonable solubility for a range of aroma compounds. Ethanol, along with its associated acceptance problems, shows a higher solubility for many hydrophobic compounds but subsequently shows reductions in partition and release of some aroma compounds. It is important to note at this stage the caveats when using APcI-MS for samples

containing ethanol. The influence of ethanol on the ionisation of aroma compounds must be considered when this technique is used. Glycerol and triacetin do not show suitable characteristics to be used in the sugar systems studied, due to incompatibility and insolubility in the case of triacetin, and poor solubility and high viscosity of glycerol.

Increasing solvent concentration increases the solubility of aroma compounds in the aqueous phase by a corresponding increase in the hydrophobicity of the system, however, this leads to higher retention and a reduction in the partition into the gas phase. The presence of solvents reduces the impact of flavour delivery. Results from gels confirm this behaviour and this is particularly evidenced by the extension to the T_{max} value. This suggests that a better dispersion of aroma is produced as concentrated regions produce spikes in detection. These spikes are indicative of a high concentration of aroma being rapidly a stages through the eating process. These contrast with results from dispersed aromas which show steady levels of aroma being released throughout consumption. I_{max} results confirm that reductions in release are also observed.

Studies using very low water systems, such as boiled sweets, have shown that aroma compounds may not truly be dispersed and that they may aggregate into "pocket-like" regions within the matrix (Schober and Peterson 2004b). These "aroma rich" regions will only be released when accessed when the matrix is sufficiently broken down and may produce "bursts" of aroma release.

6 POTENTIAL OF AROMA RELEASE FROM DROPLETS – A MECHANISM FOR RELEASE

Chapters 4 and 5 demonstrated the impact of the dispersion of volatiles on a high mixing length scale as well as the importance of dissolution of volatiles throughout a food system. This Chapter aims to expand on these findings by investigating the effects of delivering these aromas in a concentrated form, i.e. as droplets suspended within a food. It was shown in Chapter 5 that carrier solvents increased aroma compound solubility, however, this generally decreased the partition and release of the volatile compounds with an extension in the time-release profile. The conventional mechanism for aroma release assumes that during mastication or consumption, the aroma compounds are solubilised in the liquid phase and are transferred to the gas phase in the mouth through partition (Taucher *et al.* 1995). Gas from the mouth is then transported to the tidal air flow in the throat due to chewing or swallowing (Hodgson *et al.* 2003a) and "pulses" of aroma find their way to the olfactory epithelium where the sensing process starts.

Interestingly, when a compound was poorly soluble, e.g. linalool in glycerol, the release was significantly higher with a shorter T_{max} value. In food systems it has been generally considered that aroma needs to be homogenously dispersed to produce the optimum release, yet flavour 'bursts' have often been described as important for improving perception by reducing the effects of adaptation. When other solutes are present (e.g. sugars), then the solubility of many aroma compounds is compromised. The same effect occurs during food processing when water is removed and it is pertinent to question in what form the aroma compounds exist in products like hard boiled candies (water content less than 1 %) or in dry cereal foods (water content below 10 %). For foods that contain fat, it is likely that the aroma compounds are located in the fat phase. However, for some food products, it is probable that the aroma compounds are not in solution

but phase separated. A number of authors have noted that reduction of the fat phase can lead to "flavour bursts" due to a reduction in aroma compound solubility (Malone *et al.* 2000).

Potential examples of phase separation are confectionery products where the initial solutions of aroma compounds meet high sugar concentrations and encapsulated flavours where the initial aroma solution is spray dried. Encapsulated flavours are claimed to give a "flavour burst" a phenomenon that is very difficult to describe using the conventional physicochemical release mechanisms. Firmenich have developed a number of encapsulated products one of which is called Flexarome ®, which has been reported to give a "flavour burst" in the initial stages of chewing when incorporated in chewing gum. The aroma in this case is protected by its encapsulant and is only released when crushed by chewing or dissolved by saliva (Harvey and Barra 2003).

This section describes the effects of incorporating phase separated aroma on release from defined model systems including solutions, thickened solutions and two model confectionery systems. Static equilibrium headspace, dynamic headspace and breath-by-breath analysis were carried out to investigate how aroma compounds were released and some of the findings were evaluated by sensory testing. A model was produced based on the physicochemical properties of the aroma compounds to describe the changes in observed intensity.

6.1 Assessment of Droplet Behaviour in Aqueous Systems

Initial studies investigated the mechanisms of aroma compound release in aqueous solutions when added as a droplet. Studies from our laboratory showed that volatile compound droplets of 2 μl, injected into aqueous solution, migrated rapidly to the surface. At this point they spread out across the surface and evaporated (Linforth 2003). Observation of the behaviour of a range of volatile

compounds showed that different compounds possessed different behaviours based on their physico-chemical properties. Charcoal was placed on the surface of the solutions to observe the behaviour of the droplets when they reached the air-water interface (Linforth 2003). The charcoal was solely used to allow a better visualisation of the behaviour of the aroma compound droplets at the surface. A system was developed to measure the intensity of release from these droplets when injected into an aqueous sample using dynamic headspace monitoring. The samples were monitored as per the apparatus described in Section 2.3.1.5. Droplets (0.2 µl) were injected into the system whilst the headspace above the solution was sampled into the APcI-MS. For solubilised aroma, 0.2 µl was injected into the solution and agitated vigorously for 30 seconds to produce a solution. This sample was a used to compare the droplet results to data for solutions. The headspace above these solutions was assessed. Three replicates of each sample were tested and the mean values for the droplet results divided by the mean value of the dispersed results to produce the normalised data. Therefore a value of 1 represents no change in release, and positive values indicate an increase.

Figure 6.1 shows that hexanal had the highest relative increase in intensity when released as a droplet compared to when in solution (140 fold). The other compounds showed a 20 to 70 fold increase in intensity. For all compounds tested, the release from the droplet was significantly higher than when in solution. Conventional models are based on the principle that; aroma release occurs by compound dissolution in the liquid phase followed by subsequent partition (via mass transfer) into the gas phase above the liquid (Harrison 1998). It is apparent that this is not the case in this instance. The rapid and significant increase in release suggests that the droplet behaves in a different way. It is likely that as the droplet migrates to the surface of the solution (due to its lower density than water) the pure volatile droplet is subjected to minimal dissolution. Once it reaches the interface the effects of the change in surface tension will permit the droplet to rupture and to form a thin layer of volatile compound on the surface of

the solution. Observation of the droplet in solution confirms this behaviour. At this stage the volatile compound is subject to rapid volatilization from this very thin layer. Further discussions of droplet mechanisms are discussed in Section 6.3.7.

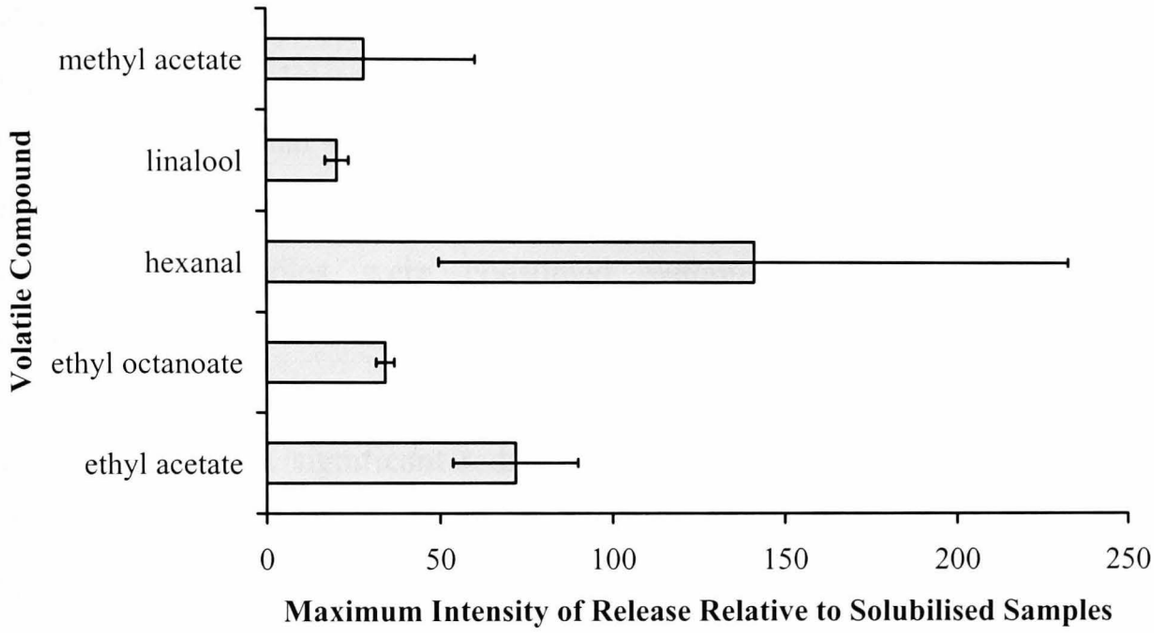


Figure 6.1 Maximum intensity of droplet release relative to the dispersed aroma when injected into solutions. Data represent maximum intensity of release from droplets relative to solubilised aroma. Means of 3 replicates. Error bars represent the standard deviation.

Ideally it would have been of interest to observe the effects of aroma droplet *in vivo* using breath-by-breath analysis. However, rapid droplet migration makes this study impractical and hence methods of immobilising the droplets within a matrix were investigated to study droplet release *in vivo*.

6.2 Droplet Release from Thickened Aqueous Samples

The next stage was to immobilise the droplets in a viscous liquid system using a thickener to restrict droplet migration. Xanthan was chosen as it is a food grade

hydrocolloid (Odake *et al.* 1998) and does not interact significantly with the compounds used.

The preliminary results described in Section 3.9 showed that increasing the concentration of xanthan increased the time taken for the volatile droplet to reach the air-solution interface. This restriction of movement is essential so that the droplet can be delivered to the mouth intact and then released during consumption. Xanthan solutions (15 mL, 0 - 0.8 %) were pipetted into glass test tubes and a droplet of pure volatile compound (1 μl) was injected at 25 mm from the surface. Samples were consumed without mastication and aroma concentrations measured using APcI-MS.

Figure 6.2 shows a significant reduction in the maximum intensity of release from droplets when the concentration of xanthan was increased. This was expected as increased viscosity would restrict the droplet from moving to the surface of the solution in the short time period that the sample was in mouth (<2 seconds). This would prevent the volatilisation of the droplet from the surface and hence reduce maximum intensity of release.

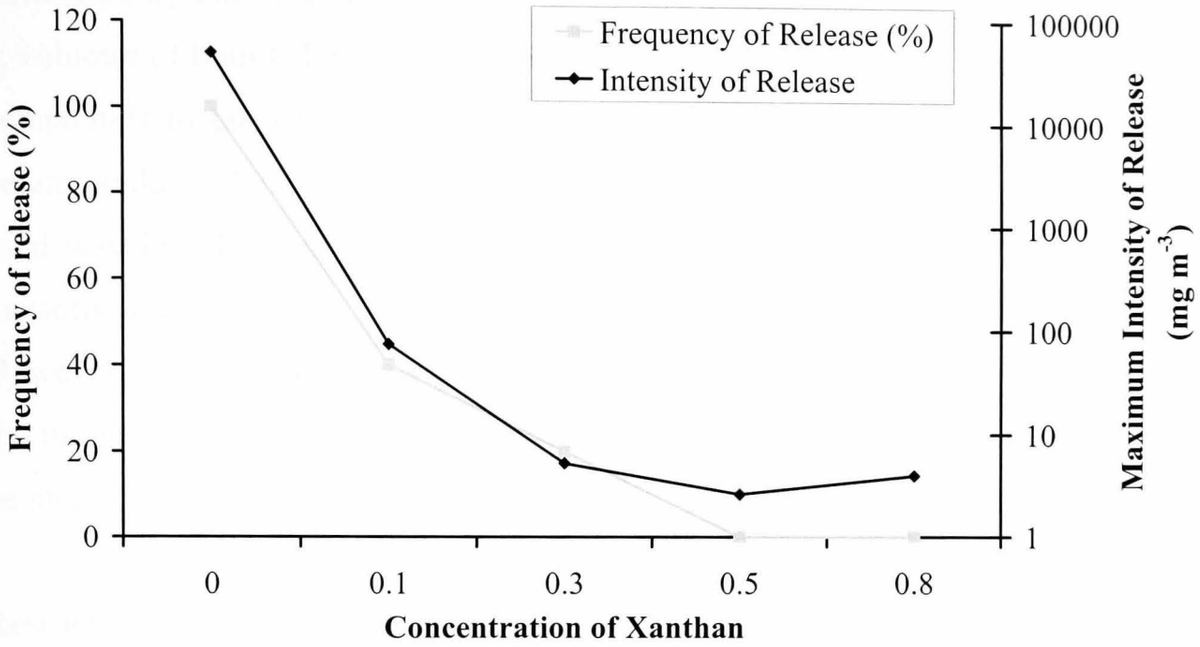


Figure 6.2 Graph of I_{max} and % frequency of release for 1µl droplet of limonene from differing concentrations of xanthan solutions. Means of 8 replicates.

Frequency of release refers to the number of times (as a percentage) that the droplet was considered to be released in mouth during consumption. This value is based on the concentration of aroma detected in nose exceeding a predetermined level. If the concentration did not exceed this level the droplet was said to not have been released thus lowering its frequency of release. Detection of lower intensities were assumed to not be due to droplet release. Figure 6.2 also shows that the frequency of droplet release changed as a function of xanthan concentration. This can be explained by the fact that the increased entanglement of the xanthan coils significantly restricted the movement of the droplet (Section 3.9) and prevented the droplet from reaching the air-solution interface. As xanthan restricted droplet migration in the sample so it also hampered the droplet access to the air-water interface in mouth. The rapid swallowing of the sample without mastication, and insignificant levels of saliva production for dilution both serve to prevent the droplet from being released.

Other studies have shown that droplet release from liquid samples can be influenced by chewing actions (Linforth 2003). Closure of the velum may occur at volumes of liquid above 10 mL in the mouth which prevents delivery of aroma compounds to the nasal airways (Hodgson, *Unpublished*). This closure of the velum could explain the low levels of droplet release as the volume of solution used was 15 mL. This volume may cause velum closure and any droplets not instantly released may not have access to the nasal airways subsequently. However, in this study only swallowing occurred and therefore the influences of the in mouth shear, and velum closure may be negligible as droplet release may be more likely to occur from the throat.

Restriction of aroma compound movement by xanthan could be causing the decrease in intensity. Steric phenomena at concentrations above c* are the most likely cause of droplet restriction. Gonzalez-Tomas and co-workers (2004) reported that increasing xanthan concentration above 0.2%, exceeds the c* value. However, the results in our experiment indicate that the limonene droplet was still capable of migration up to concentrations of 0.3% xanthan in solution.

Binding of the aroma compounds to xanthan will further reduce the release of the aroma compounds. Increasing xanthan concentration increases the number of binding sites available concentrations of 0.01 % being shown to be sufficient to bind some aroma compounds (Yven *et al.* 1998). However, Cook *et al.* (2003b) reported that *in vivo* concentrations were unaffected by increased thickener concentrations even though the aroma perception decreased with increasing thickener concentration. These authors therefore suggested a multi-modal response was more apparent for aroma release than purely the maximum intensity of aroma detected in nose. These references indicate that care must be taken when suggesting suitable droplet retention matrices. The effects of xanthan will be important in reducing droplet delivery, especially by steric hindrance. Binding to xanthan may affect the release of the solubilised aroma compounds.

6.3 Effect of Droplet Release from Model Gelatine-Sucrose Gel System

The effect of a solid, gel matrix on release was then studied. Insertion of droplets was best achieved using a micro-litre syringe and injecting the appropriate volume as the gel started to set (Section 3.10). This created a spherical droplet suspended in the viscous solution which then became immobilised in the gel. Attempts to inject the droplet after the gel had set resulted in fissures with the result that the aroma compound was in the form of an irregular shape (Section 3.10); therefore injection of droplets occurred during gelation. For control samples with solubilised aroma, propylene glycol was chosen as the carrier solvent to disperse the volatile compounds, even though it has been shown not to be the most efficient solvent (Chapter 5). It is important to note at this stage that all the gel samples in this experiment contained 2 % propylene glycol. However, it is the most commonly used solvent in confectionery products such as gelled sweets and does not produce the alterations in APcI-MS ionization observed when ethanol was used (Section 5.1.2).

6.3.1 Preliminary Experiments

Preliminary experiments showed that gels containing droplets released a very high intensity of volatile compounds into the nasal airways and the signal recorded exceeded the maximum detection limit of the mass spectrometer (MS) (Figure 6.3). MS operating conditions and droplet sizes were subsequently modified for future experiments to produce data within the APcI-MS detection limits. For all the droplet experiments, release was observed, indicating that mastication had exposed the droplet. There was concern that the droplet would not necessarily be ruptured during mastication and could be swallowed intact but this did not occur in any of the samples tested. This finding contrasts to the data

for thickened solutions, which showed poor levels of droplet delivery especially at higher xanthan concentrations (Section 6.2).

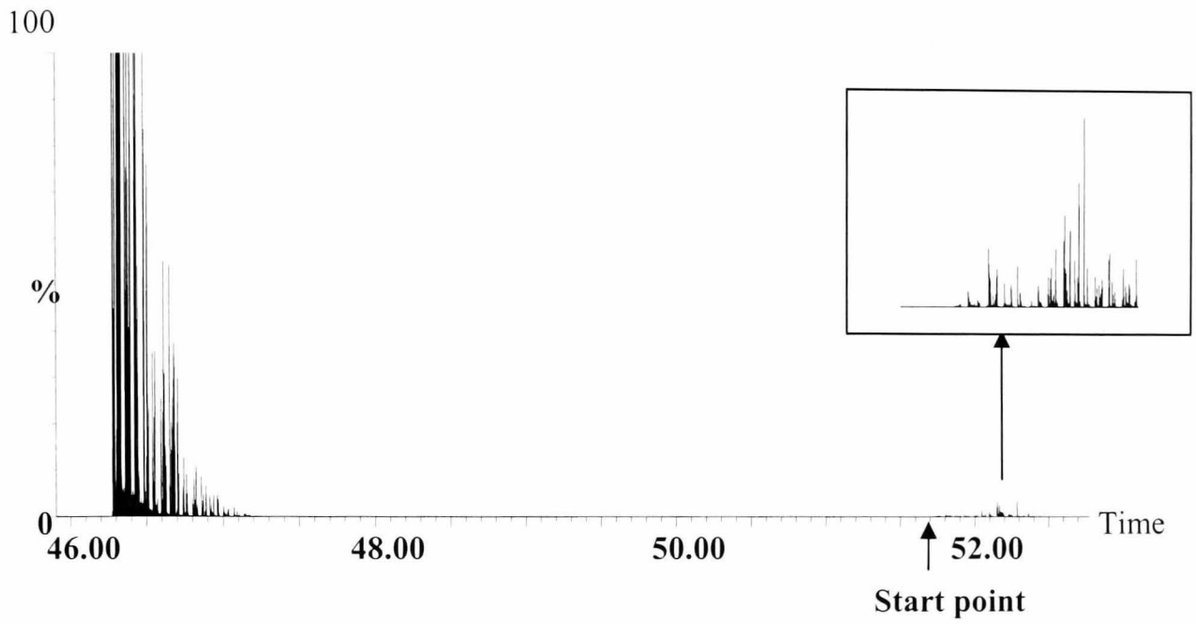


Figure 6.3 Difference in APcI-MS traces for droplet against dispersed release for ethyl butyrate. Droplet sample on the left, dispersed sample on the right (Sample has been magnified in the box to show the pattern of release).

Figure 6.3 illustrates the difference in profiles of ethyl butyrate release between droplet and solubilised aroma (both samples contained the same amount of aroma). For the droplet, there was a very high initial increase as the droplet was released compared to the solubilised aroma. This indicates that, as in solutions, the droplet was being released rapidly during the consumption period and gave a significant increase in the intensity of release. This behaviour correlates to that observed *in vitro* (Section 6.1). This contrasts to the solubilised aroma which showed a gradual release as the gel dissolved, melted, and the aroma partitioned from the saliva into the oral cavity, as suggested by the model of release from gelatine gels (Harrison and Hills 1996) or by real data obtained by Linforth *et al.* (1999b).

6.3.2 Dynamic Headspace Measurements

As a comparison to dynamic headspace release from aqueous systems, dynamic release from gels was measured *in vitro*. Gel samples containing limonene and ethyl butyrate either as 1 μl droplets or as solubilised aroma were placed into 100 mL of water in a sealed Schott bottle on a heated magnetic stirrer (37°C, 50 rpm). The dynamic headspace concentration over time was measured as per Section 2.3.1.2.

Figure 6.4 shows the concentration of volatile in the headspace with time (normalised data for comparison of droplet and solubilised limonene). It shows that the droplet releases more quickly and this reinforces the conclusion that aroma is being released from the interface. This not necessarily apparent from the image shown in Figure 6.4, however, it is important to consider that the data has been normalised to the maximum intensity for each sample. It is also important to note that there is only a minimal level of aroma release occurring from the droplet sample prior to the droplet being released. At this point, a rapid increase in aroma release is detected as this is what is meant by the rate of release being higher from droplet compared to solubilised samples. Interestingly, there was a low detection level before the apparent release of the droplet which suggests that some dissolution, either into the gel or due to the increased temperature or agitation had occurred. It also demonstrates that the droplet could be retained until the surrounding gel matrix was dissolved in the absence of mastication.

An inflection point is also observed during the dissolution of the solubilised gel sample which may be explained by a build up of aroma dissolved in solution followed by a more rapid release as the solution gains a higher aroma concentration.

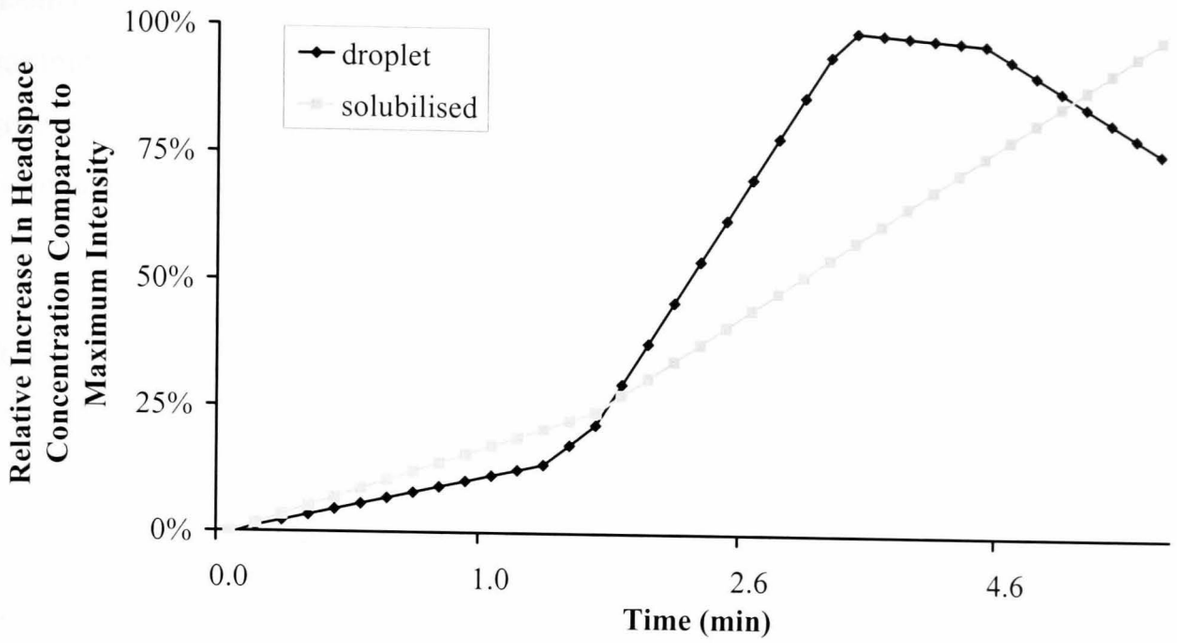


Figure 6.4 Dynamic rate of release of limonene from gelatine-sucrose gels when added as a droplet or solubilised aroma. Single sample.

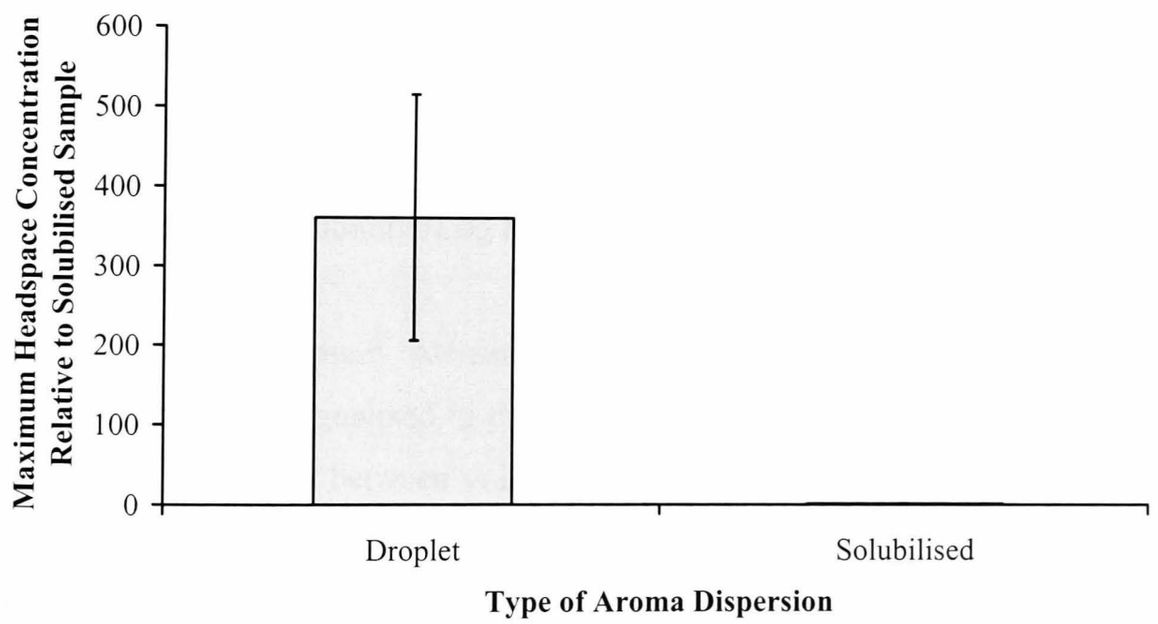


Figure 6.5 Maximum intensities of limonene release from gelatine-sucrose gels when added as a droplet or solubilised aroma. Mean of 3 replicates. Data normalised to solubilised sample. Error bars represent one standard deviation.

Interestingly these results showed a significant increase in the intensity detected, with limonene having a 360 fold increase in droplet I_{max} compared to solubilised

aroma (Figure 6.5). This was comparable to the data observed in aqueous samples where 20-150 fold increases were observed for droplets compared to solubilised aroma. Results from ethyl butyrate again exceeded the detection limits and are not shown.

6.3.3 Effect of Aroma Incorporation on Maximum Release Intensity (I_{max})

Gelatine-Sucrose gels were prepared as per Section 2.2.2.1 to compare the influence of four volatile compounds: ethyl acetate, ethyl butyrate, limonene and hexanal on the maximum intensity of release using 4 panellists. Compounds were added as 1, 2 or 4 droplets or solubilised using propylene glycol and incorporated into the gel (Section 2.2.2.4) with same overall aroma concentration for each sample. It was expected that 1 droplet would show an increased $I_{max,}$ for all the aroma compounds tested, and that increasing droplet number would decrease the I_{max} as the droplets would not all be released simultaneously. These volatile compounds represent a range of different aroma compound properties including differences in hydrophobicity (Log P), solubility and vapour pressure.

Results for the maximum intensities of release are shown in Table 6.1 as normalised values (normalised to the sample with the solubilised flavour), such that all the differences between volatiles could be readily compared. Therefore, values >1 showed an increased release in maximum intensity when droplet samples were consumed. For all droplet samples and all aroma compounds there was an increase in the intensity of release ranging from 3-660 times that of the solubilised sample.

Table 6.1 Intensity of *in vivo* release (I_{max}) from gelatine-sucrose gels containing droplets In nose aroma concentrations were measured using APcI-MS and normalised to the solubilised sample. Data from four panellists, each consuming three replicate gels.

Compound	1 drop	2 drops	4 drops	Solubilised
Ethyl acetate	14 ± 9	10 ± 7	9 ± 7	1 ± 0.93
Ethyl butyrate	667 ± 635	381 ± 290	240± 194	1 ± 0.87
Hexanal	84 ± 70	67 ± 60	42± 32	1 ± 0.63
Limonene	11 ± 12	5 ± 4	3 ± 3	1 ± 0.55

Limonene showed the lowest increase in intensity ratio (11 fold) for a single droplet compared to solubilised aroma. In contrast, ethyl butyrate showed a far greater increase of around 660 times the I_{max}. Ethyl acetate showed an increase of 14 times and hexanal an increase of 84 times. The ratios between the droplet and solubilised samples varied widely depending on the compound assessed. These observed differences can be explained by variation in the behaviour of the individual compounds. Further discussion of the effects of aroma compound physicochemical properties is described in Section 6.3.7.

The relative intensity of aroma release from samples containing 1, 2 and 4 droplets is shown in Figure 6.6. For the four compounds studied the results show that 1 droplet had a higher intensity of release than 2 droplets which was higher than 4 droplets. The ratios show a reduction of 20-60 % from 1 droplet to 2 droplets and 35-70 % from 1 droplet to 4 droplets. The large variation in standard deviations depended on the aroma compound studied but overall variation in I_{max} was high, with a percentage coefficient of variance of 58 % for ethyl butyrate, 38 % ethyl acetate, 54 % hexanal and 70 % for limonene. Despite these relatively high levels of variation there was a clear statistical difference in release between samples.

Limonene release from droplets showed the same decline from 1 to 4 droplets as the other compounds, although the intensity of release was only 3-11 times

higher than the solubilised aroma. The lower volatility of limonene means it will not volatilise as intensely into the oral cavity leading to lower concentrations detected at the nasal epithelia. Limonene shows reduced solubility in propylene glycol (the dispersant used in the mixed sample) and thus may be present within the 'solubilised sample' as a number of small droplets dispersed throughout the gel sample (Garti *et al.* 2001). This may lead to release from a series of micro-droplets which would give a higher intensity of release. Reduced solubility of limonene in propylene glycol has been shown to affect the intensity and profile of release from gelatine-sucrose gels (See Section 5.4). This may be a reason why the intensity ratio was lower for limonene release than other compounds. If a "solution" of limonene is in reality a dispersion of a large number of smaller droplets then the release from these may be relatively higher than for a compound which produces a homogenous solution.

The observed decline in intensity as the droplet number decreased mimics more the situation that is probably occurring when aroma is dispersed in real foods. In most food systems, aroma compounds are unlikely to be dispersed as one or two droplets. Due to the nature of food matrices, they are also unlikely to be present as homogenous solutions. The presence of fat may well inhibit the formation of aroma droplets depending on the compound solubility in the fat phase, but the potential for release as droplets may still be apparent if the oil is available in droplet form (perhaps as an emulsion). The real situation will be somewhere in between, with regions of inhomogeneity throughout a matrix. This level of inhomogeneity will depend not only on the matrix itself but also on the properties of the aroma compounds with their physicochemical properties influencing their solubility and release kinetics.

In encapsulant systems, aroma compounds are retained within pockets in a matrix (usually polysaccharide) and are released when this matrix is broken down by fracture or dissolution (Reineccius 1991; Whorton 1995; Gunning *et al.* 1999; Harvey *et al.* 2000a; Shefer and Shefer 2001). These matrices are likely to

have low levels of aqueous phase and it is possible that higher releases in intensity observed from encapsulants may be due to the release of aroma as droplets.

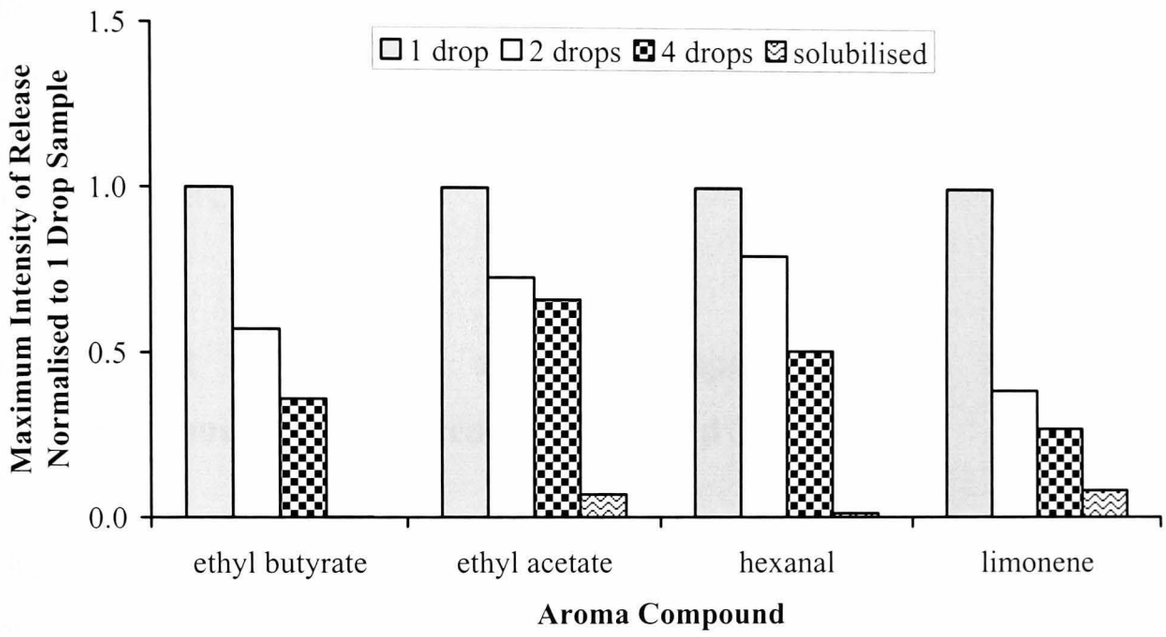


Figure 6.6 Maximum Intensity of aroma release for 4 compounds from 1, 2, 4 droplets or solubilised aroma. Data was normalised to highest sample (1 drop) for 4 panellists with 3 replicates of each.

Increased droplet number showed a reduction in aroma compound intensity (Figure 6.6). This effect can be explained by the process by which the droplets were released from the gel. Upon mastication, the gel was fractured and any droplets accessed by this fragmentation would be rapidly released. However, the probability of fracturing all 4 droplets simultaneously was low. Therefore release of the total aroma from 4 droplets was unlikely to occur in the same mastication event. Subsequently, the quantity of aroma released was lower than for 1 droplet. Increased droplet number causes a more gradual release, which corresponds to a reduction in the maximum intensity of release.

Significant differences (ANOVA) were still observed between the intensities of release when the solubilised samples were removed from the data set for ethyl

butyrate and limonene ($p<0.001$ and $p<0.05$ respectively). This was not the case for ethyl acetate or hexanal ($p>0.05$ and $p>0.10$ respectively) which suggested that the variation between droplet samples was less significant for these compounds than compared to the solubilised aromas. However, in all cases, the droplet samples released at significantly higher intensities than the solubilised aroma. As the number of droplets increased, the intensity of release declined. This suggests that larger aroma droplets release at higher intensities, *in vivo*, than smaller droplets.

6.3.4 Effect of Droplets on the Temporal Release of Aroma Compounds Compared to Solubilised Samples

The profile of the release is important for perception. The time to maximum intensity (T_{max}) is an important indicator of release rate. Figure 6.7 compares the T_{max} values for the 4 different compounds and illustrates the same trend that was seen for ethyl butyrate (Section 6.3.1), with the solubilised samples having a significantly longer time to maximum intensity than the droplet samples ($p<0.05$).

The earlier T_{max} from 1 droplet may be explained by an increased probability that 1 droplet will be released, rather than 2 or 4 droplets simultaneously, in a single masticatory event (Prinz 1999; Prinz and Heath 2000). Reduction in T_{max} indicated a reduction in aroma compound solubility in the sample. This produced a 'burst-like' pattern of release.

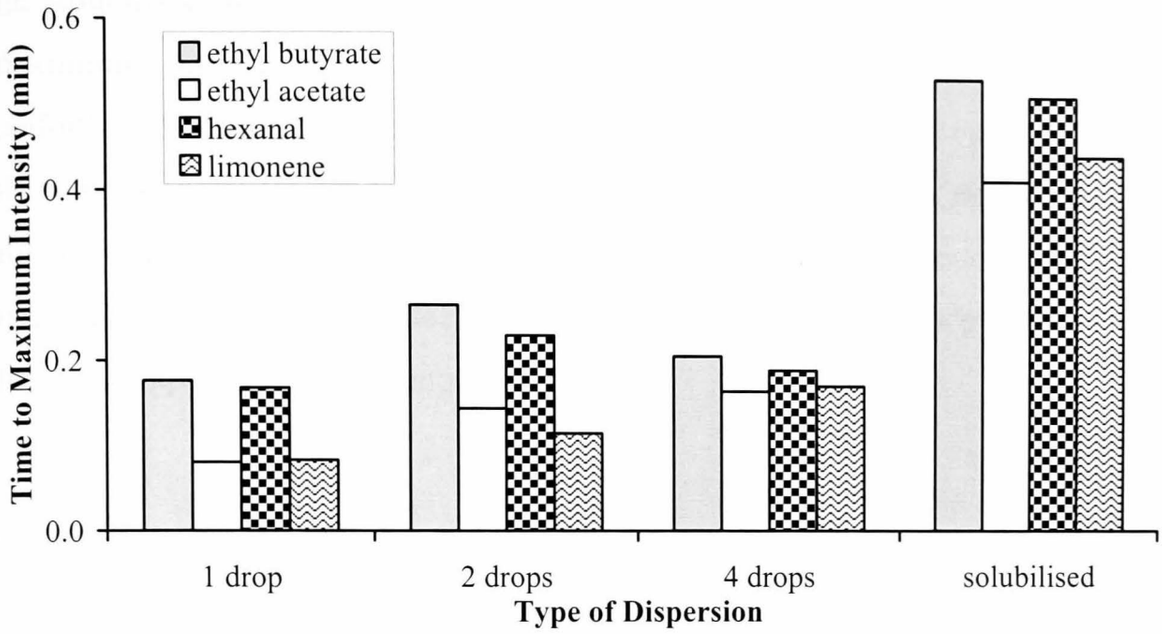


Figure 6.7 Time to Maximum Intensity (T_{max}) of aroma release for 4 compounds from 1, 2, 4 droplets or solubilised aroma. Means for four panellists consuming 3 replicates of each sample.

Different patterns of release (Figure 6.8) are exhibited by different compounds with two hydrophobic compounds (limonene, and ethyl octanoate) showing high early peaks but a rapid reduction in release intensity. The more hydrophilic compounds (ethyl butyrate and 2,3-diethyl pyrazine) show a longer sustained release. This may be due to increased solubility of these compounds into the saliva or mucosal membranes (Buettner 2002; Buettner *et al.* 2002a). Alternatively, the vapour pressures of these compounds may be influencing the release. The two hydrophilic compounds studied have higher vapour pressures. This means that, as the gas phase became depleted, they would rapidly re-equilibrate to a high concentration in the gas phase (Marin *et al.* 1999). The reservoir of aroma retained in the oral cavity could be a reason for the increased persistence.

This suggests that release from droplets also affected the rate of release. This is demonstrated by the fact the droplets show a rapid early aroma release as they are liberated from the gel matrix which declines rapidly thereafter. In contrast,

the solubilised aroma shows a slower more consistent release and reaches the maximum concentration at a later point during the consumption process. In Linforth and Taylor (2000) reported that low Log P or low vapour pressure (Log pl) compounds produced the greatest level of persistence due to repartition into mucous membranes, which may explain the behaviour observed for the hydrophilic compounds but does not explain the lack of persistence observed for the lower vapour pressure compounds.

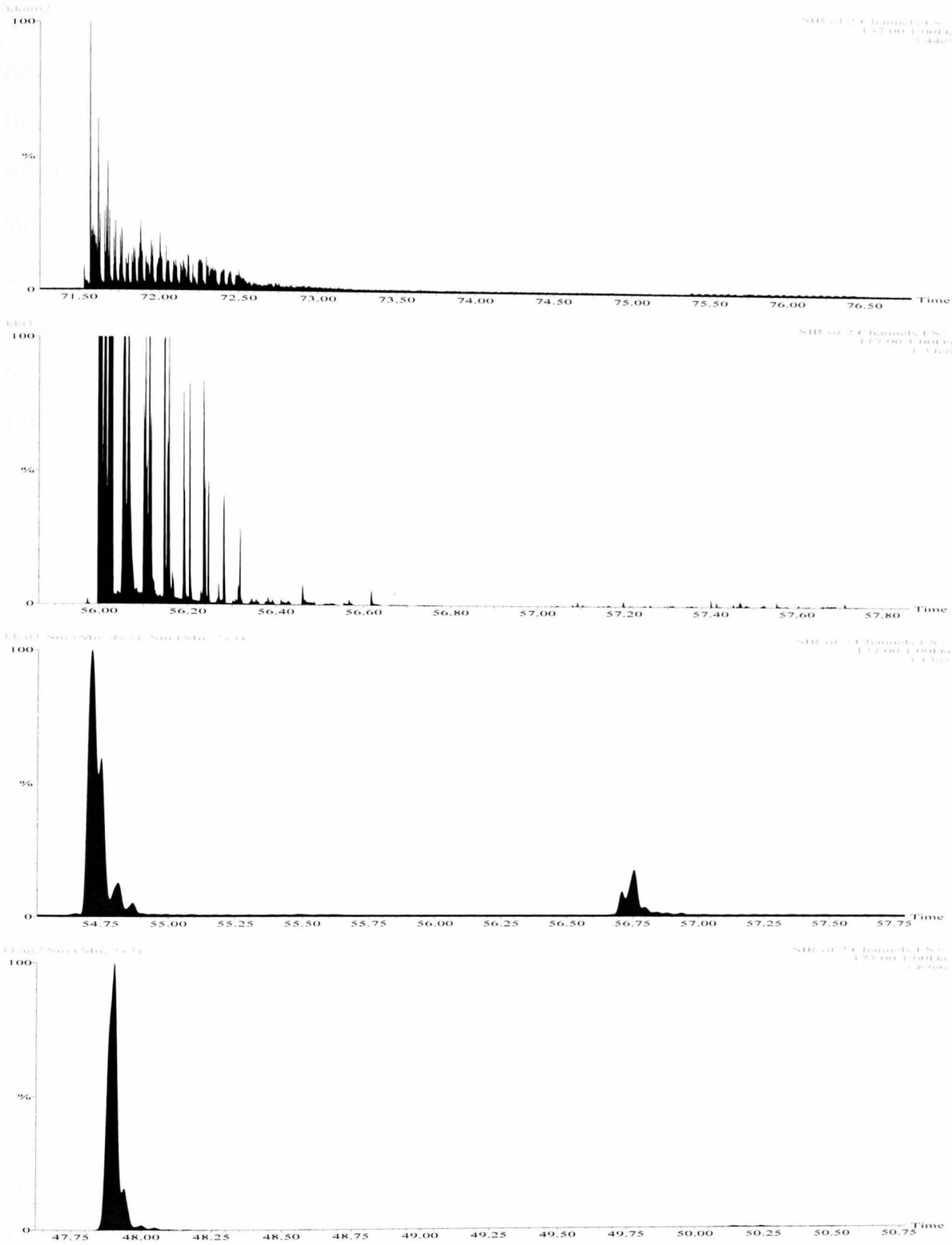


Figure 6.8 APcI – MS traces representing the difference in aroma release profiles between droplet samples (1 µl; left side) and aroma mixed in homogenously (right side) for 4 compounds (from the top: 2,3-diethyl pyrazine, ethyl butyrate, ethyl octanoate, and limonene).

Time-release profiles for ethyl butyrate and limonene illustrate the variation in release from droplets and solubilised aroma (Figures 6.9 and 6.10). Ethyl butyrate showed a rapid increase in aroma concentration as the droplet was released followed by prolonged persistence. Solubilised ethyl butyrate shows a standard release curve for a solubilised aroma from a gel, with a gradual increase as the gel is broken down. Limonene also showed the initial droplet burst, but the solubilised aroma curve does not follow the standard release observed for ethyl butyrate, perhaps due to the presence of the aforementioned microdroplets. Limonene solubilised in 2 % propylene glycol showed early "bursts" of release not dissimilar to droplets and thus a less "standard" release curve was observed (Section 5.3).

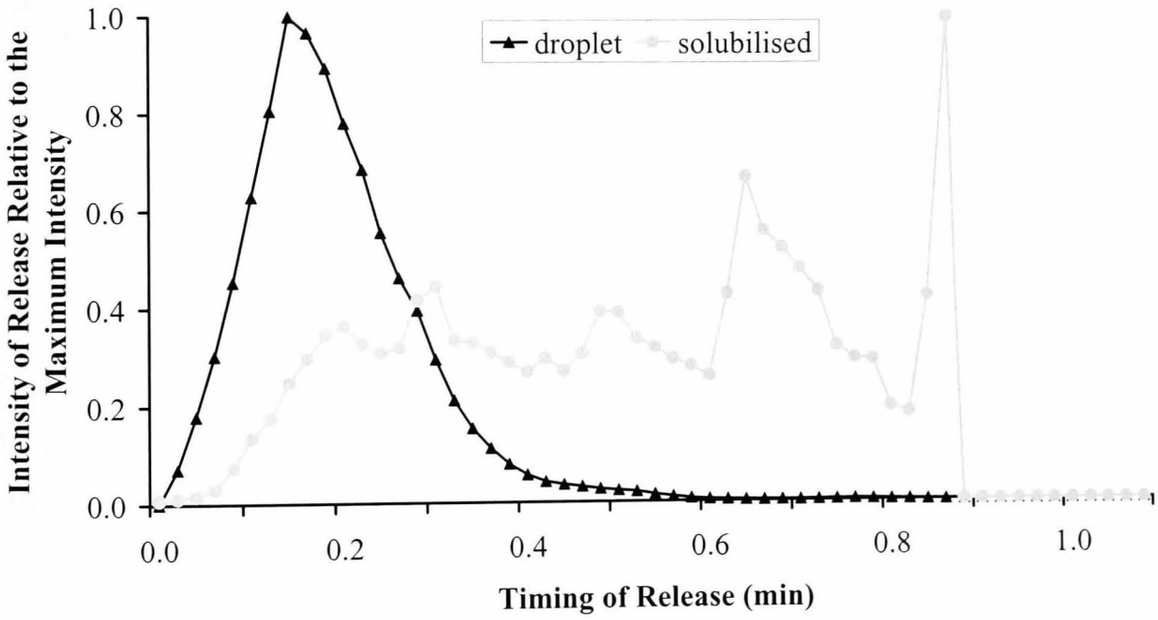


Figure 6.9 Aroma release of ethyl butyrate from gelatine-sucrose gels in the form of a droplet or as solubilised aroma. The data is expressed relative to the maximum intensity for each of the curves and is the mean of 12 replicates.

Reduction of droplet size in emulsions created an increased surface area thereby increasing the area available for volatilization (Miettinen *et al.* 2002). This may be the case for droplet release. However, a contribution from the volatility of the

compound in question may also be of importance (Linforth *et al.* 1999c). A greater amount of volatile compound will volatilise to a greater extent. These results showed that droplets have a definite impact on, not only the intensity, but also the profile of aroma release from gelatine-sucrose gels. The intensity of release from the droplets may be mainly dependent on volatility, whereas prolonged release may be more related to the hydrophobicity of the compounds.

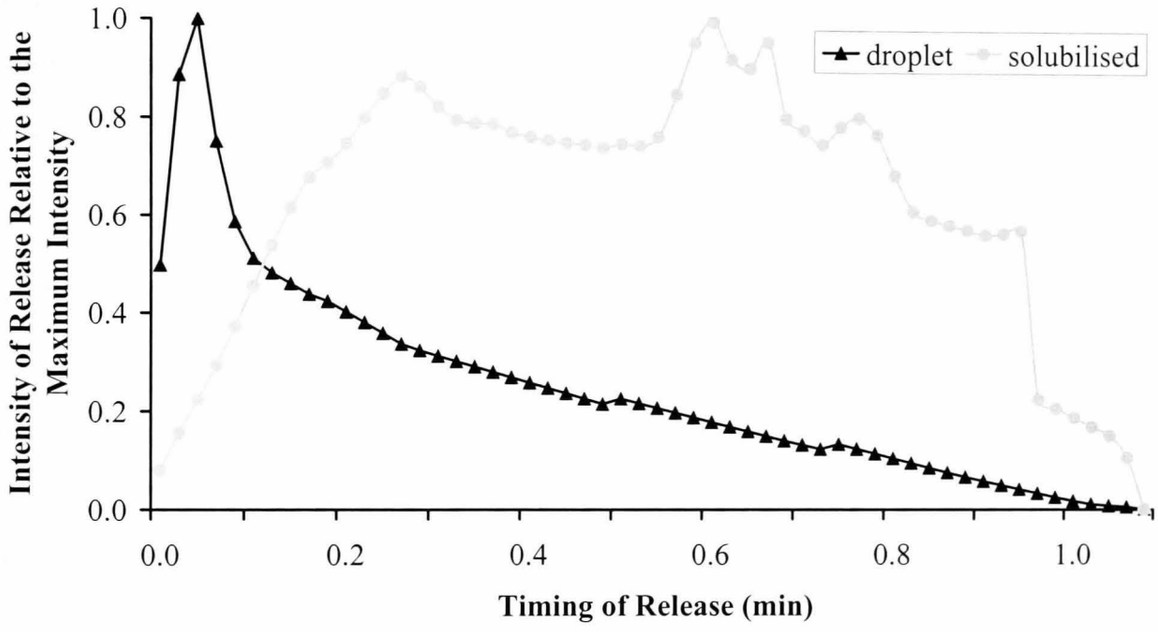


Figure 6.10 Aroma release of limonene from gelatine-sucrose gels in the form of a droplet or as solubilised aroma. The data is expressed relative to the maximum intensity for each of the curves and is the mean of 12 replicates.

Baek *et al.* (1999) found that differences in sensory perception of furfuryl acetate release from gels were not due to binding to the gelatine but were due to different rates of gel breakdown and release. However, these workers also noted that the different gelatine strengths used were causing the gels to breakdown at different rates thus releasing the tastants (acids, sugars) in an altered pattern. In this experiment, the gels will breakdown in a similar manner and tastants should be released at the same rates thus minimizing any tastant effects on perception. Too rapid release of aroma in some systems may produce a disrupted flavour profile

and this will not necessarily represent an appropriate release pattern (Whorton 1995).

Sensory analysis of release from gelatine-sucrose gels has implicated the rate of release rather than the intensity as the most important factor in perception. Baek *et al.* (1999) investigated different strength gels and measured the gradient of the release curves. They showed significant effects of gelatine concentration on the rates rather than intensities of release. These results were confirmed by observed differences in sensory perception. All the panellists in this study, noted that samples containing droplets had significantly higher intensities of aroma than the solubilised samples.

6.3.5 Effects of Droplets on Sensory Perception

A sensory difference test (paired comparison) was carried out to elucidate any sensory difference in intensity between the one and two droplet samples. Sensory difference tests are used when samples are described as confusable, that is to say perceptual differences may exist between the samples but they are not obvious. Consequently difference tests are never used when the differences are large and obvious. The paired comparison is an example of a directional difference test where the panellist is directed to identify the strongest (or weakest) sample of a specified attribute (in this case the flavour).

Results from the paired comparison analyses showed no significant differences between the intensity of release for either ethyl butyrate ($p = 0.58$) or limonene ($p = 0.86$). This suggests that, although there were differences in instrumental release for 1 and 2 droplets, the high intensities of aroma release in both samples made it possible that any sensory differences may be masked. It was proposed that the time-release profile played a more important role in perception than the actual intensity of release. The rate of release was similar between the two

samples even though the intensity varied and this may become the overriding factor for sensory perception from gels.

6.3.6 Effect of Different Panellists

Although the overall trends for droplet release suggest differences, the variation observed is reasonably high. Normalising the data reduced these effects however, differences between panellists may still have influenced the data.

Figure 6.11 demonstrates the differences between the four panellists for ethyl butyrate release from droplets and solubilised aroma. This graph illustrates that significant differences between panellists were also apparent. Panellists 1 and 2, and panellists 3 and 4, show similar intensities of release to each other but differences between the two groups. Panellists 3 and 4 had much higher intensities of aroma release for droplet samples than panellists 1 and 2 for all of the samples investigated. This is likely to be due to physiological parameters of the panellists rather than any variation in the samples.

Figure 6.12 shows the corresponding T_{max} values for each of the panellists and confirms that all the droplet samples were released earlier than solubilised aroma. The degree of time difference is again panellist dependent with panellists 2, 3 and 4 all showing similar values and only panellist 1 showing a difference in T_{max} for the 2 and 4 droplet samples. This may be due to panellist chewing rate. A reduction in chewing rate may have increased the time taken to liberate the droplets, thus delaying the time taken to reach the maximum intensity.

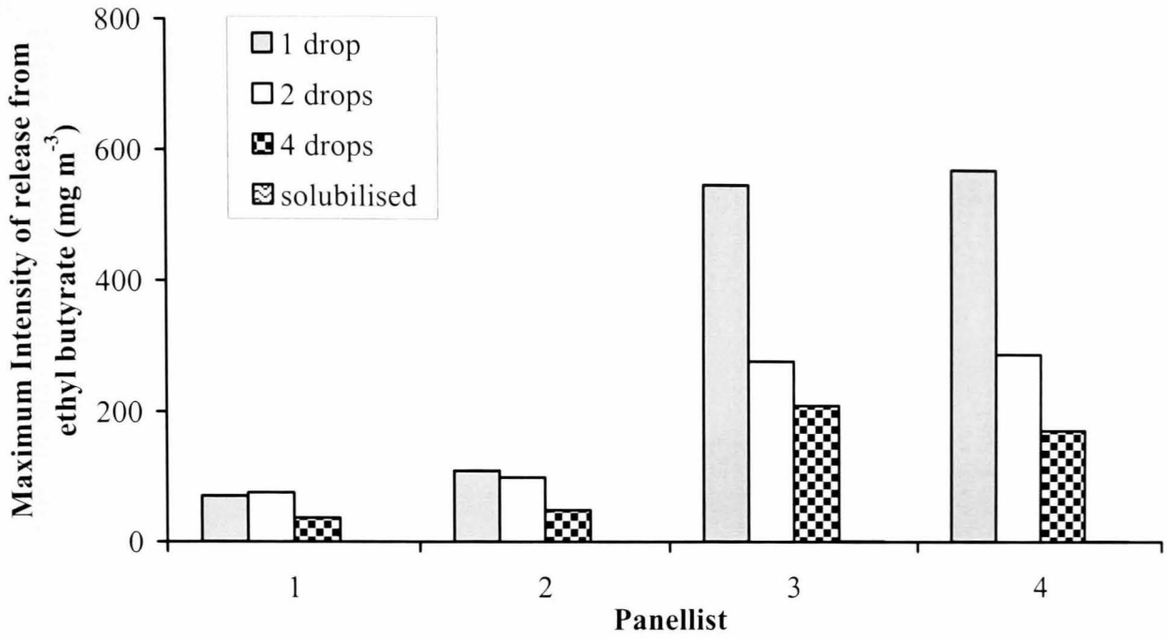


Figure 6.11 Maximum intensity of ethyl butyrate release *in vivo* from gelatine-sucrose gels for the 4 different panellists each consuming 3 replicates of each of the droplet and solubilised samples.

Similar patterns of release were a characteristic of all the panellists. However, significant differences were apparent in the intensity and timing of release (I_{max} and T_{max}). I_{max} and T_{max} values were significantly different between each of the panellists investigated (I_{max} $P< 0.01$ and T_{max} $P<0.05$) for the consumption of gels containing ethyl butyrate (Figures 6.11 and 6.12). This was also the case for the other three aroma compounds tested (data not shown). Although there is a significant inter-panellist variation, intra-panellist variation is less significant. The intra-panellist variation was lower as panellists tended to consume similar samples in a similar manner (Guinard *et al.* 1997; Buettner and Schieberle 2000b; Geary *et al.* 2002; Lucas *et al.* 2002).

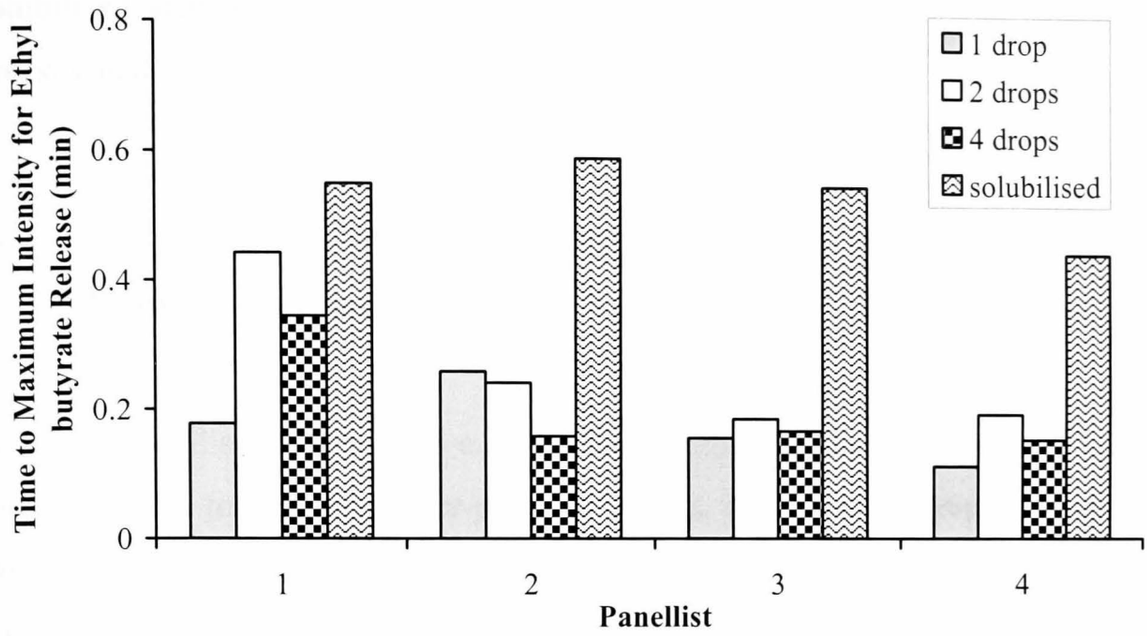


Figure 6.12 Time to maximum intensity for ethyl butyrate release *in vivo* from gelatine-sucrose gels for the 4 different panellists each consuming 3 replicates of each of the droplet and solubilised samples.

Chewing and saliva flow rate have been shown to significantly influence the rate of aroma release from solid samples (Harrison *et al.* 1998). Panellists vary in physiology and therefore different concentrations of aroma compounds will be delivered to the nasal epithelia during consumption of gel samples. Increasing the number of droplets reduced the probability that they were all accessed simultaneously during mastication. This may account for reduction in I_{max} and the subsequent increased T_{max}. The increased T_{max} may be a result of droplets being released over different mastication events.

Overall, the panellists showed different intensities of aroma release but these effects could be accounted for by differences in physiology and mastication patterns. Similar panellist behaviour was observed for droplet and solubilised aromas and therefore, by normalising the data for each panellist much of this variation could be eliminated. It was apparent from the normalised data that droplets had significantly different intensities and timings for release than

solubilised aroma and these differences occurred irrespective of the panellist investigated.

6.3.7 Effect of Physicochemical Properties on the Intensity of Release from Droplet and Solubilised Systems

A single panellist (representative of all the panellists) was chosen to consume future samples to minimize inter-panellist effects. By representative the panelist involved showed higher levels of aroma release from droplet samples than solubilised ones and was intermediate values of the panelists studied previously. All of the droplet samples showed an increase in the intensity of release so comparisons were made between, only the release from a single droplet, and the release from solubilised aroma. Twelve more aroma compounds (added singly) were tested and the data was again normalised to the solubilised sample. The properties considered to be influencing the intensity of release were the vapour pressure (volatility), hydrophobicity (Log P), and the solubility of the aroma compounds in the aqueous phase.

Vapour pressure affects compound partitioning into the gaseous phase and subsequently affects the concentration and rate at which aroma compounds will transfer into the gas phase (Marin *et al.* 1999). Log P has been shown to be an important parameter in predicting aroma compound release from sucrose solutions (Friel *et al.* 2000). It is a parameter related to compound solubility in both the gel system and saliva with more hydrophobic compounds (higher Log P values) being less soluble in both phases. Aqueous solubility influences the partition of aroma compounds into mucous membranes in the nose and throat which will relate to persistence from droplet release (Buettner *et al.* 2002a; Hodgson *et al.* 2003b). Other properties (such as polarity, surface tensions, LUMO energies, bond contributions) may have effects although they were not considered here.

Results for droplet/solubilised ratios as well as corresponding physicochemical properties are shown in Table 6.2. This shows that the increase in release intensity from droplets compared to solubilised, ranged from 4-2400 times depending on the aroma compound investigated. Initial indications showed no obvious pattern for any of the physicochemical properties, in isolation, on the values of the droplet/solubilised ratios. However, when a combination of factors were considered it appeared that a large amount of the variation in the data set could be explained by Log P (which was highly correlated with the solubility values, R^2 = 0.96, i.e. as hydrophobicity increased there was a subsequent decrease in water solubility (Figure 6.13)) and vapour pressure (R^2 = 0.73).

These two descriptors were modelled in Design Expert and this gave a quadratic model with two linear and three quadratic terms. The result produced data based on a transformation to a $\log_{10}$ of the droplet/solubilised ratio to ensure that residuals were normally distributed with a constant variance (Equation 6.1).

$\log_{10}$(droplet/dispersed) =

-7.86626

+6.49124 * Log P

+7.55882 * Log vp

-0.94078 * Log P^2

-1.82611 * Log vp^2

-2.13604 * Log P * Log vp **Equation 6.1**

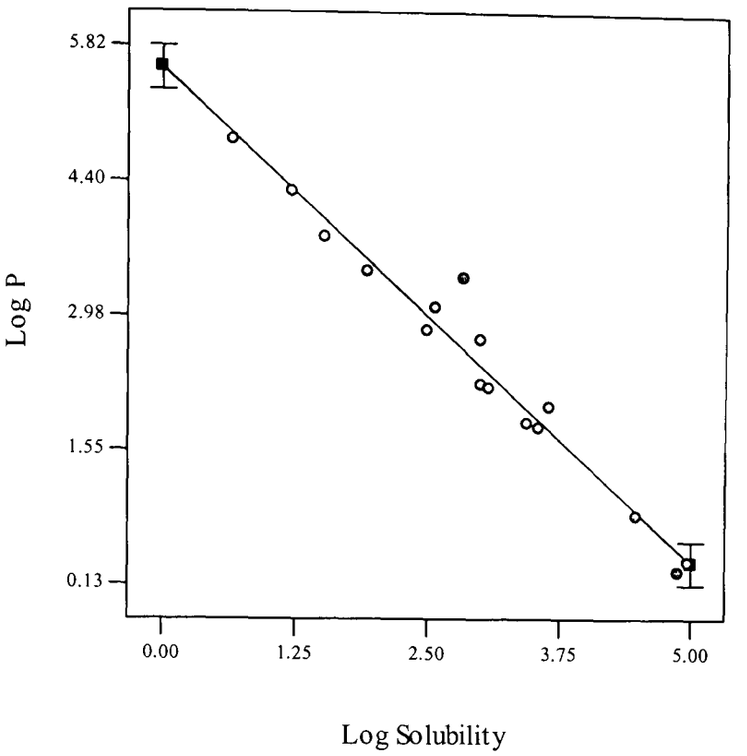


Figure 6.13 Log P values for 16 aroma compounds plotted against their Log Solubility values (R^2 = 0.96).

When Log P was low, and vapour pressure high, compounds such as butanone and methyl acetate showed only a 4-fold increase in release intensity. Increasing Log P showed an increase in the intensity ratio (Figure 6.14) up to a maximum of around 2500 fold, after which further increasing Log P showed a decline in the intensity ratio. Ethyl hexanoate (Log P = 2.83, Log vapour pressure = 0.26) had the highest ratio of release. This was thought to be due to its relatively high hydrophobicity (low water solubility) but a higher enough vapour pressure to allow rapid transfer to the gas phase. Compounds with higher Log P but correspondingly low vapour pressures showed a lower intensity release ratio with carvone and citral showing increases of only 7-10 fold. Interestingly, limonene with a very high Log P value and a relatively high vapour pressure showed an increase of only 11 times.

Table 6.2 Properties of the aroma compounds used in this study. The physicochemical parameters were calculated using a group contribution type software package (EPI-Suite, Syracuse Research Corp, USA). Drop : solubilised ratio describes the mean I_{max} from the droplet sample divided by the mean I_{max} from the solubilised samples.

Compound	Log P	Log vapour pressure	Log Aqueous Solubility	Drop : Solubilised ratio
2-butanone	0.26	1.99	4.88	4.2
Methyl acetate	0.37	1.72	4.97	4.3
Octanol	2.73	-0.62	3.00	6.6
Carvone	3.07	-0.89	2.56	7.6
Citral	3.45	-1.04	1.93	9.9
Limonene	4.83	0.16	0.66	11.4
2,3-diethyl pyrazine	2.02	-0.11	3.65	12.5
Ethyl acetate	0.86	1.99	4.48	14.3
Hexanal	1.80	0.98	3.55	83.7
Menthofuran	4.29	-1.09	1.21	88.1
Linalool	3.38	-0.80	2.83	388
Octanone	2.22	0.37	3.07	421
Ethyl butyrate	1.85	1.16	3.44	666
Ethyl octanoate	3.81	-0.63	1.52	764
Amyl acetate	2.26	0.62	3.00	1794
Ethyl hexanoate	2.83	0.26	2.49	2433

A similar effect was observed, for increased Log vapour pressure (Log vp) values. Increasing Log vp demonstrated an increase in the potential fold difference of release up to an intermediate vapour pressure. At which point, further increases in vapour pressure exhibited a decline in release ratio (Figure

6.15). It is important to note that these two factors taken in isolation are not sufficient to predict the fold increase in delivery, due to droplets, but by calculating both parameters the fold increase can be predicted.

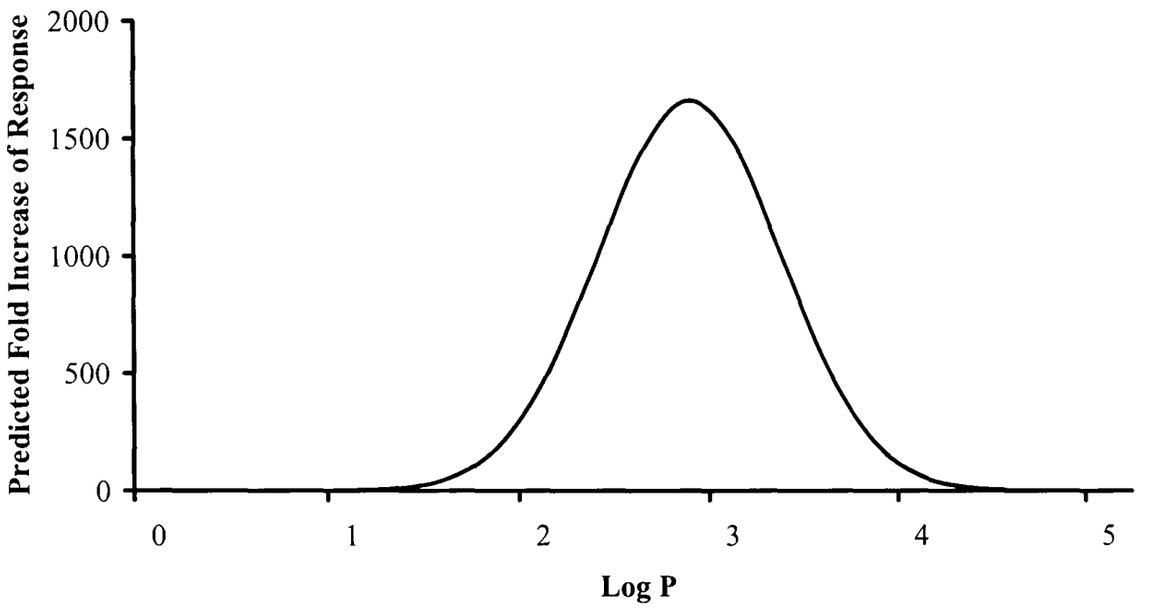


Figure 6.14 Effect of varying Log P on the increase in the droplet : solubilised ratio for I_{max} values of aroma release at a fixed vapour pressure (Log vp = 0.6).

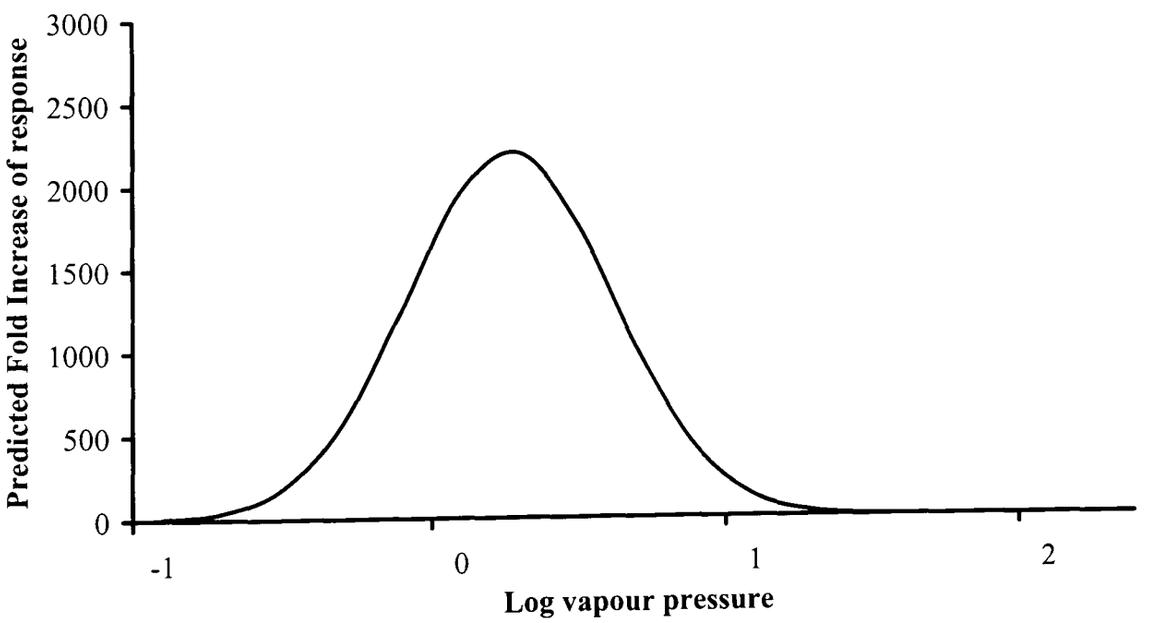


Figure 6.15 Effect of varying the Log vapour pressure on the increase in droplet : solubilised ratio for I_{max} values of aroma release at a fixed Log P (Log P =3.25).

A parabolic effect was shown for increased Log P values of aroma compounds and their partition into the headspace, when investigated in ethanol containing solutions (Aznar *et al.* 2004) and is common in many other biological systems (Smith and Williams 1998). The parabolic effect described in this system can be explained by the balance between the two properties to give optimal release into the gas phase from droplets. At low Log P values, compounds were highly soluble in the liquid saliva phase and so were subject to some degree of dissolution. This reduced their potential to rapidly partition into the vapour phase even at very high vapour pressures. As Log P increased, there was a concurrent decrease in vapour pressure. However, those compounds with higher vapour pressures showed the largest increase in release whereas those with lower vapour pressures had a much lower release. The compounds with higher Log P values were less soluble and so were less likely to dissolve in the aqueous phase. However, the lower vapour pressures made them struggle to volatilise into the gas phase even though they do not mix well with the saliva. Those compounds with very high Log P values had a low increase in intensity even when the vapour pressure was higher. This may be due to other interactions within the system. Compounds with lower vapour pressures may be dependent on mass transfer-type mechanisms which will reduce the concentration detected.

Mass transfer is a major factor affecting partition into the gas phase in solutions. Emulsions of smaller droplet sizes showed higher release due to changes in mass transfer (Linforth *et al.* 2002). Miettinen *et al.* (2002) showed that this occurred for the non-polar compound linalool but not for the polar compound diacetyl, inferring that a reduction in the solution reservoir (solubility) of aroma (provided by the emulsion) would increase the release of compounds at higher Log P values. The presence of the dissolved gel fragments and sugars will affect the dissolution and release patterns observed depending on the compound investigated. Friel *et al.* (2000) reported 'salting in' of more hydrophilic compounds and 'salting out' of hydrophobic compounds at 65% sucrose concentrations. Nahon (2000) showed that in higher sucrose concentrations, up to

60%, the mass transfer coefficient became more important for the release rather than partition. This important characteristic needs to be considered when comparing the aroma release from gelatine-sucrose gels. Droplet behaviour may be unaffected by these processes as it avoids this mechanism of release but the results may have implications for solubilised aroma.

6.3.8 A Potential Mechanism for Droplet Release

A potential mechanism has been suggested to explain what is happening in the case of the aroma being released as a droplet rather than via conventional partition mechanisms.

Figure 6.16 demonstrates the behaviour of a droplet of aroma in a gel and compares it to the release of solubilised aroma. In the droplet system, fracture of the gel was a more important aspect for aroma release. Once liberated from the gel, the droplet rapidly transferred to the surface of the saliva where it formed a 'thin layer' of pure aroma compound. This 'thin layer' was subject to rapid volatilisation into the gas phase, rather than partition, thus minimising interactions with the saliva phase. This led to a much higher concentration detected at the olfactory epithelium which may have had consequences for perception. When the aroma was solubilised, the gel was fractured as previously, yet the release of aroma was dependent on the dissolution and melting of the gel matrix. This led to a gradual increase in intensity in mouth as the gel was broken down (Baek *et al.* 1999; Linforth *et al.* 1999b; Hollowood *et al.* 2000; Taylor *et al.* 2001). This was demonstrated by the larger T_{max} value for solubilised systems (Figure 6.12). Increasing the surface area by further mastication further increased the rate of gel breakdown by increasing the rate of heat transfer into, and sugar dissolution out of, the fragments. The aroma was liberated gradually and was subjected to dissolution and partition into the saliva phase prior to partition into the gas phase (Harrison and Hills 1996).

Conventional models of aroma release (Harrison and Hills 1996; Harrison 1998; Harrison *et al.* 1998; Harrison 2000) have not considered aroma release to occur in the form of droplets. These authors consider release to be dependent on dissolution into saliva and partition into the gas phase as the main mechanism for release. The mechanism proposed here suggests that an alternative mode of release may be possible if aroma compounds are present as droplets within a matrix.

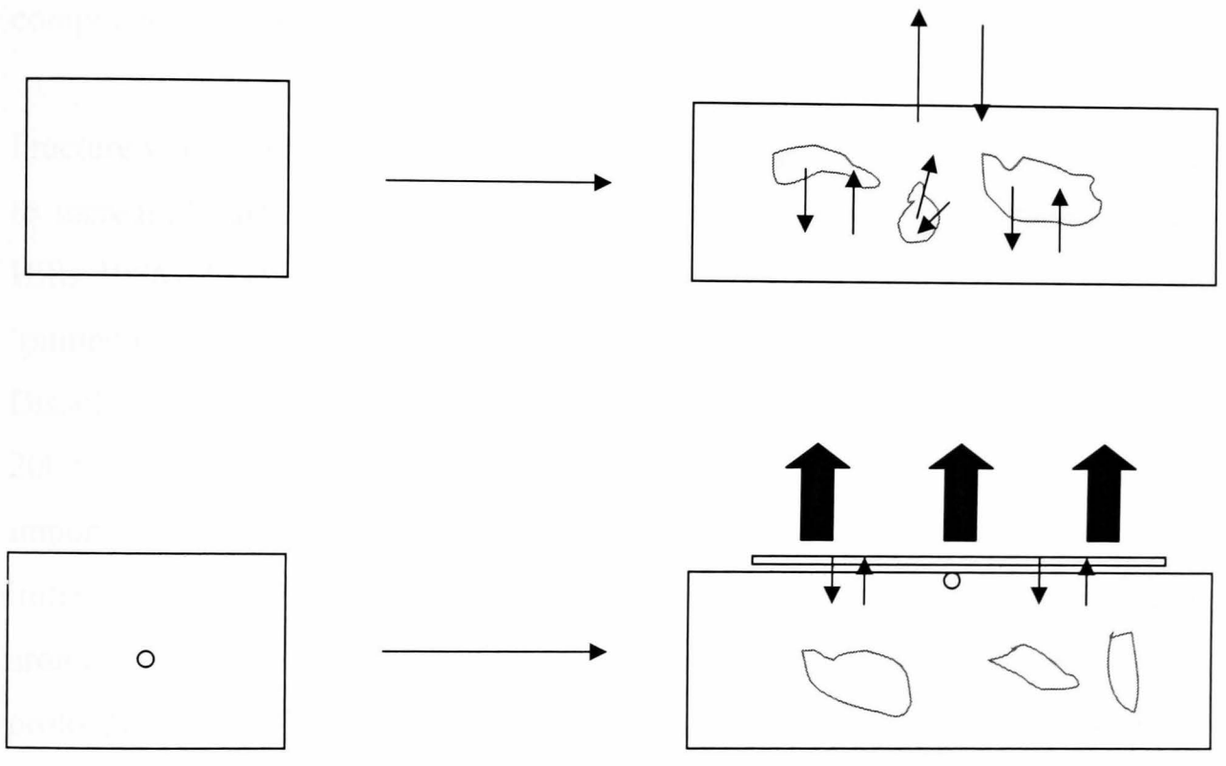


Figure 6.16 Schematic representation of gel breakdown and release of aroma. On the top; the solubilised sample shows partition into the surrounding saliva and subsequently into the headspace above the sample. On the bottom; the droplet sample shows the breakdown of the gel and the rapid release of the droplet and subsequent forming of a thin layer on the surface of the saliva where volatilisation occurs.

Fracture of the gels was an important aspect in the liberation of the droplet from within the gel matrix. A model by Harrison *et al.* (1998) predicted that aroma release from solid matrices was dependent on the rate of fracture and that increasing the rate of fragmentation will increase the rate of aroma release. This

will be especially important in matrices which contain droplets, not only in this artificial situation, but also in other food products with aroma present in concentrated forms. They also predicted that initial release rates would be less dependent on chewing and saliva flow rates and more dependent on fracture and mass transfer coefficients (Harrison *et al.* 1998). This suggests that release from droplets may be less dependent on variation in panellist physiology compared to more recognized mechanisms of aroma release, such as from solubilised aromas. In these cases, aroma release may be more dependent on the behaviour of the compounds and the matrix components.

Fracture will also increase the release from solubilised aroma but this will be due to increased surface area available for dissolution and partition (Harrison and Hills 1996). Mastication ensures that a greater concentration of compounds are 'pumped' into the nasal cavity (Buettner *et al.* 2002a; Hodgson *et al.* 2003b). Dissolution of hydrophilic compounds into mucosal linings (Buettner *et al.* 2002a) will affect the persistence of aroma release. Persistence may be especially important for perception of release from droplets. A "burst" of aroma may initially influence the sensory response however, the absence of any further aroma stimulus may reduce subsequent perception. This may reduce the prolonged perception of the droplet and may result in a disrupted perception profile.

The density (buoyancy) of the compounds will affect the speed at which droplets reach the surface of the saliva layer reducing the time available for dissolution and affecting the timing of droplet release. This will be important for compounds that have densities greater than that of the aqueous phase. No compounds in this category have been investigated in this study.

The interface between an aqueous and vapour phase is not a static system and orientation of molecules at the interface will affect the behaviour of droplets, especially when macromolecules and sugars are present. Both sucrose and

glucose dissolved in water cause a measured increase in the surface tension of the system (Docoslis *et al.* 2000). Adsorption of proteins at the air/water interface may be important for the delivery of the droplets as they will affect the surface tension of the system and thus their ability to gain access to the gas phase. Dickinson (2003) has reviewed the effects of hydrocolloids at air-water interfaces. This may be another factor to be considered when assessing the timing of droplet release as aroma compounds may differ in their abilities to access the surface.

The key differences comparing droplet release to emulsions are that, unlike emulsions, the aroma is not dissolved in an oil phase and therefore will be subject to more rapid volatilisation on contact with the gas phase. Another important point to note is that in emulsion systems an emulsifier is generally used to stabilize the emulsion which may also interfere with the release of aroma compounds in mouth. These may have similar effects to that observed for xanthan from section 6.2 in that it reduces the ability of the droplet to be released.

If release from encapsulants occurs as droplets then it may have important implications for developing products with different flavour release characteristics. Harvey and Barra (2003) have shown that release from chewing gum containing a Firmenich encapsulant (Flexarome®) showed an initial rapid increase in release, during the early stages of consumption, compared with when the flavouring was dissolved in the gum base. Malone and Appelqvist (2003) investigated encapsulation of oil droplets rather than volatiles and showed that gelled emulsion particles can be used to manipulate temporal release profiles and that release was dependent on particle size, partition coefficient and oil phase present. They showed that they could inhibit the rate of mass transfer by increasing the time over which diffusion occurred. These processes point to a potential for the droplet release mechanism to contribute to the differences observed from encapsulated systems. This is one of the primary reasons for

development of controlled release encapsulant systems (Reineccius 1995a; Reineccius 1995b; Benczedi 2002). Controlled release encapsulants aim to ensure that aroma compounds (or other encapsulated components) are released at the correct time-point and are released a controlled levels. The encapsulant aim is to protect the aromas from processing during production and then to deliver them throughout a consumption process such that a suitable concentration is released to maintain adequate perception throughout that sample consumption. An example is that used in chewing gum such that aroma is only released from the encapsulant when it is crushed by the teeth or dissolved by saliva.

6.4 Effect of Mixed Droplets on Aroma Release

Complete flavours are not comprised solely of single volatile compounds and most include a wide range of aroma compounds. Disruption of the balance of a flavour can be easily achieved by alteration of some or all of its components. Droplet release was shown to affect different aroma compounds to varying degrees. This may have important implications for "flavour" droplets if they have different properties between their components. Two aroma compounds (ethyl butyrate and octanol), with differing release characteristics, were mixed together at a number of ratios separately to produce each of the final aroma compound ratios and the aroma release measured by breath-by-breath analysis. The droplets produced contained ratios of ethyl butyrate to octanol such that a 100% ethyl butyrate contained no octanol, and 25 % ethyl butyrate meant that 75% of the droplet was comprised of octanol.

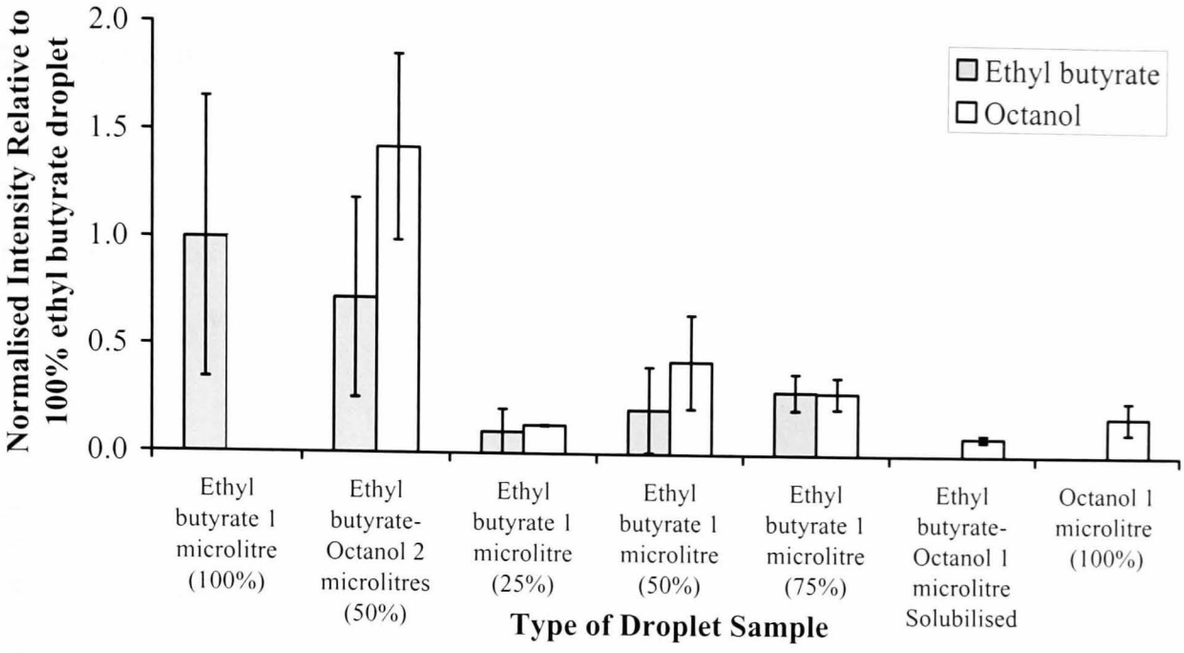


Figure 6.17 Maximum intensity of aroma release *in vivo* from gelatine-sucrose gels containing varying concentrations of ethyl butyrate and octanol in a 1 μl droplet. Data is expressed relative to the "ethyl butyrate 100%" (1 μl droplet of ethyl butyrate only). Data from one panellist consuming 2 replicates.

Figure 6.17 shows that, reduction of the percentage of ethyl butyrate in the droplet significantly reduced the intensity of ethyl butyrate released. This was expected as reduced quantities of aroma will produce a reduction in the aroma concentration available for detection. The 2 μl droplet (1 μl ethyl butyrate, 1 μl octanol showed a lower intensity of release than a single 1 μl droplet. The presence of octanol in the 25, 50 and 75 % also reduced the intensity of ethyl butyrate more than the expected ratio. Both these findings led to the conclusion that the presence of octanol was reducing the delivery of ethyl butyrate. The first effect may be due to dilution of the ethyl butyrate, moreover an influence of the octanol as a solvent may become a factor. If octanol was able to dissolve some of the ethyl butyrate then it would no longer have been available for release. The lower vapour pressure of octanol will reduce the vapour pressure of the overall mixture reducing the level of droplet delivery. The low ratios of octanol-ethyl butyrate would preclude the ethyl butyrate being totally solubilised in the octanol.

Figure 6.18 illustrates the effects of differing ethyl butyrate concentrations on the time-release profile. It shows that increasing the concentration of octanol in the sample extended the T_{max} value for 25-75 % octanol. Reduction in droplet vapour pressure led to a greater influence of mass transfer on release and hence the increased T_{max} value.

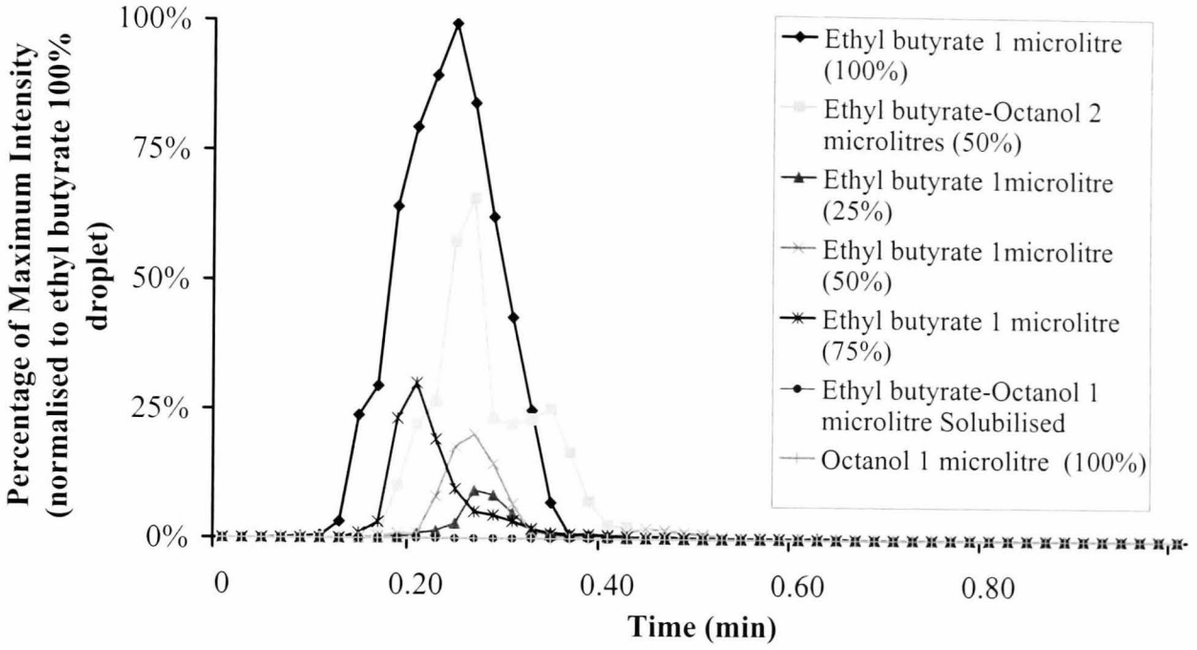


Figure 6.18 Time-release profile for ethyl butyrate release *in vivo* from gelatine-sucrose gels as a percentage of the sample with the highest intensity. Data are the means of 2 replicates from one panellist.

These results indicated that interactions between aroma compounds could influence the intensity and timing of droplets in the same way in which they influenced aroma released via partition mechanisms. These considerations should be taken into account when determining the use of droplets as a flavour delivery mechanism.

6.5 Droplet Release from Boiled Sweets

Boiled sweets represent a simple, solid matrix whereby aroma release occurs purely via dissolution of the matrix. Due to the rigid structure, no influence of mastication was involved. Hills and Harrison (1995; 1998; 2000) proposed that aroma release occurs via the two-film stagnant layer theory of mass transfer. In this theory, aroma release from solids is considered a three phase problem; from solid to saliva to gas phase, with the rate-limiting step being partition from solid to liquid (saliva) phase at the interface. However, the low water content of the boiled sweet matrix (<2 %) may cause separation of the flavour producing "pockets" of aroma (Schober and Peterson 2004b; 2004a) that may behave in a similar manner to droplets.

Boiled sweets were produced as per Section 2.2.6 and the solubilised aroma compound (ethyl hexanoate) was added pre-dissolved in propylene glycol (5 % in final sample). Droplets were injected at a suitable stage of cooling and samples were allowed to rest for 24 hours at 4°C. Samples were consumed over a 7 minute period and the exhaled air monitored by APcI-MS.

Figure 6.19 shows significantly higher release intensities for droplets compared to the solubilised aroma. Despite a different mode of consumption, whereby the droplet was released into a relatively large volume of saliva compared to the other samples tested, the same mechanism of droplet release was observed. In contrast to gels, where the ratio of maximum intensities of aroma release between the droplet and solubilised samples, increase was approximately 2400 fold for ethyl hexanoate, the results show an increase of only 93 fold for the same droplet released from a boiled sweet. The potential of smaller bursts producing an increased I_{max} level which will reduce the overall ratio was the most likely explanation. These bursts were evident from the aroma release profiles as discussed in Section 5.5. Additionally, the presence of the sugar-saliva slurry in the oral cavity may show a 'salting out' effect for dissolved ethyl hexanoate. This

would increase the level of partition in the oral cavity (Friel *et al.* 2000; Friel and Taylor 2001) causing an increase in release from the solubilised aroma.

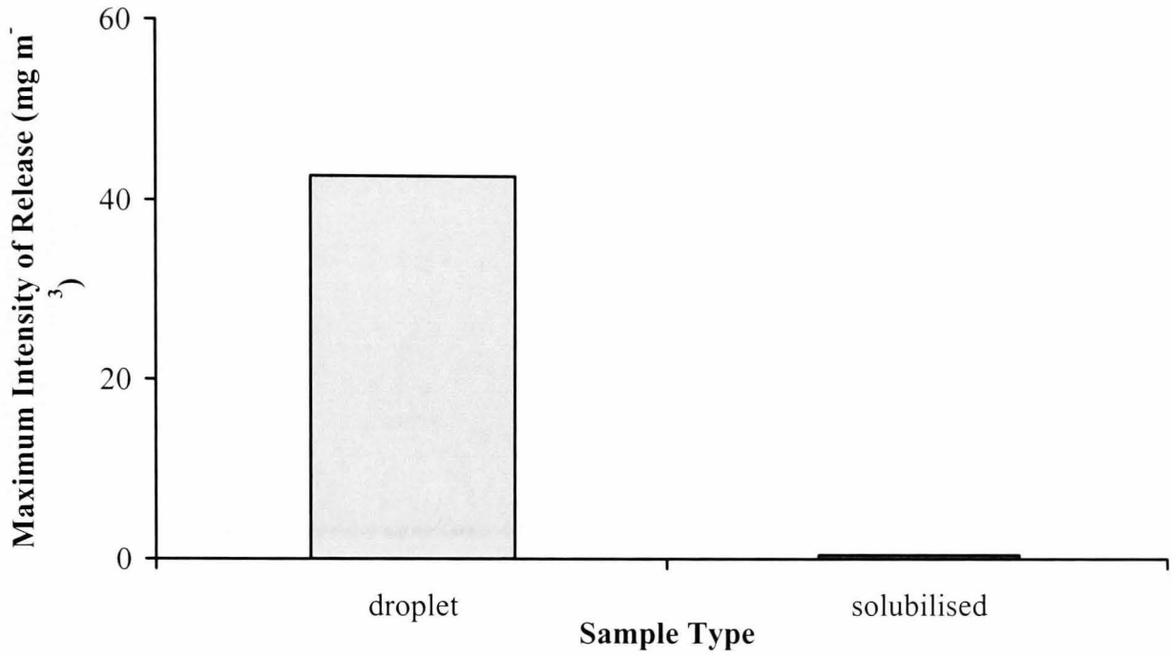


Figure 6.19 Maximum intensity for ethyl hexanoate release *in vivo* from boiled sweets for droplet and solubilised samples. Data from one panellist consuming 3 replicates.

Figure 6.20 shows the differences in the profiles of aroma release *in vivo*. The droplet was released once the matrix had dissolved to such an extent that it was free to escape from within the glassy structure. The droplet was rapidly released and a high concentration detected for a period of 45 seconds, after which the intensity declines to a negligible level. Conversely the solubilised aroma released in a 'burst-like' pattern with peaks of aroma observed throughout the consumption period. This form of release reinforces the view that so called dispersed aroma is forming localized regions which are released as the glassy structure dissolves (Schober and Peterson 2004b). It is possible that due to the low levels of water the localized regions (or "pockets") of aroma described by Schober and Peterson may be released as small droplets rather than by dissolution into the sugar-saliva phase. Further investigation via techniques such as microscopy, using fluorescent probes or chromophores may give a better

indication as to whether the conclusions drawn regarding partitioning of aroma compounds into pockets within the matrix are actually valid.

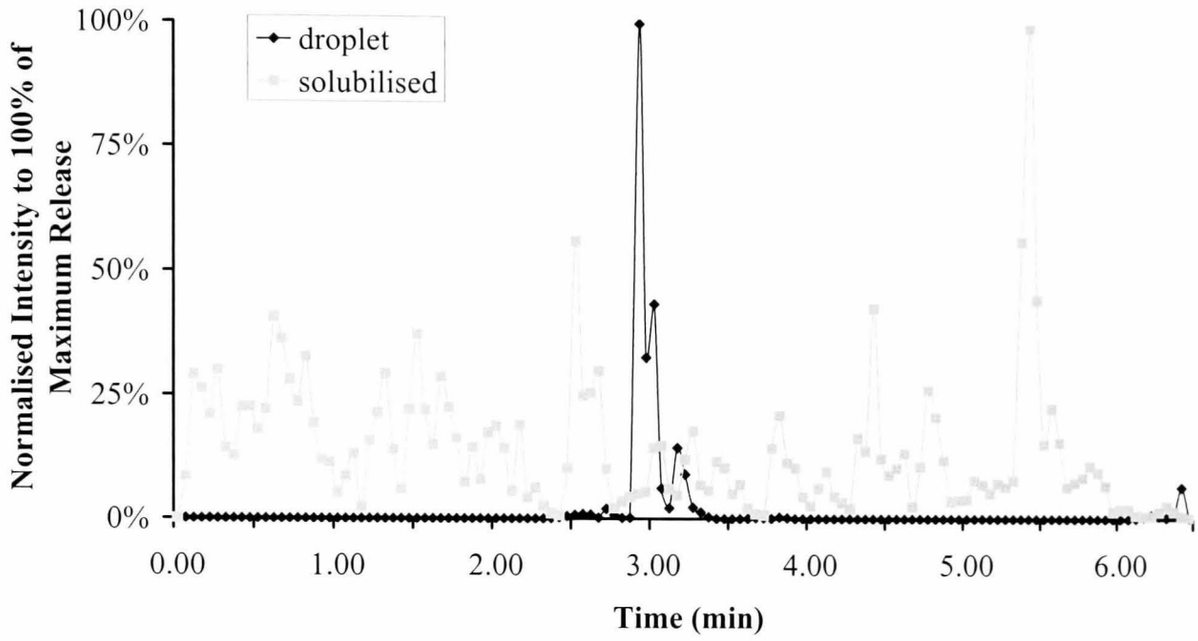


Figure 6.20 Aroma release profile for ethyl hexanoate from boiled sweets for solubilised and droplet samples. Data normalised to 100% of intensity maximum.

6.6 Conclusions

In this section a novel mechanism for release is described. The results showed that release from pure aroma compound droplets had a different release mechanism to when the aroma was solubilised in a range of liquid and solid systems. The differences involved a much higher intensity of release at a much earlier time-point. This had implications, not only for the mechanism of release, but also for the sensory profile that the consumer experienced. In many cases these so called 'bursts' are undesirable as they can lead to disruptions in the sensory profile. However, the principles observed may be important in explaining the effect of changes in release for more heterogeneous food systems. This data may provide a better insight into the mechanisms of release from encapsulants and other systems where aroma compound solubility is

compromised. Further study is required in this area to elucidate whether encapsulant systems are influenced by this mechanism of release but it is clear that release from droplets has the potential for maximizing flavour delivery.

7 OVERALL CONCLUSIONS AND FUTURE WORK

These studies into the effects of different methods of dispersion on the partition and release of aroma compounds have described the processes involved in sample preparation and their effects on release during consumption.

APcI-MS was shown to be a rapid, robust and suitable technique for quantitative and qualitative measurement of a range of aroma compounds when assessed by equilibrium headspace studies and by volatile release during sample consumption. Several protocols were developed for producing and equilibrating samples and consumption methods were developed to standardise eating of samples for 'in nose' measurement. The preliminary results on mixing and dispersion of aroma compounds suggested that these were important steps when producing systems for analysis.

In most cases throughout this thesis the data has been normalised to reduce the effects of inter-panellist differences on the overall trends in the changes in aroma release. Other methods of data treatment may hold some validity in obtaining further information from this data but would subsequently re-introduce some of the variation observed by using different panellists. A potential solution to these problems would be to have studied a single panellist in greater depth in order to get a better indication of the processes involved without the variation or need to normalise the data.

Development of a range of different dispersion systems was achieved and the effects on the partition and release from these systems were studied for a range of products ranging from solutions, to semi-solid yoghurts and gels, through to solid boiled sweet systems. Higher scale mixing based on mechanical processes such as layering, stirring and the use of a static mixer system, produced samples where aroma rich regions could be macroscopically separated from each other. The

results from these experiments suggested that although differences in the mixing patterns of the aroma compounds could be achieved, the effects on aroma release were negligible. It is suggested that the efficient, rapid, in-mouth mixing of the samples during consumption was blending the distinct aroma regions and therefore the level of aroma release was unaffected by the pre-consumption mixing pattern. Comparisons of two different matrices for layered products (gels and chewing gum) confirmed that the physical degradation of the matrix did not influence aroma release in this case.

This provides quite an interesting negative result in that it suggests that mixing of aroma compounds at this level is less important than in-mouth mixing. It may also be avenue of investigation for novel product design as the techniques used, produced samples with interesting colours and appearance.

Carrier solvents are commonly used in the food industry to disperse and dilute flavourings. Much research has been described on the effects of co-solubility but this is mainly in relation to pharmaceutical products or for solvents at high concentrations. Research into the effects of solvents on aroma partition and release from foods is sparse. The results from this thesis suggested that the use of carrier solvents can play an important role in defining the flavour release profiles detected by the consumer during consumption. Theories of compound solubility and release were based on the hydrophobicities of both the solvents and the aroma compounds. Partition studies, carried out in solutions, demonstrated the general trend towards reductions in headspace concentrations in the presence of solvents. Ethanol showed the greatest effects with results for propylene glycol and glycerol much less influential at the concentrations investigated. An important caveat is included relating to interpretation of the data from solutions containing ethanol when analysed using APcI-MS. The influence of ethanol reagent ions influencing ionisation cannot be disregarded and further studies may be required to further investigate the effects of ethanol.

Release from the confectionery products demonstrated that both the profile and the intensity of aroma release could be altered by the use of carrier solvents at 2%. The use of solvents increased aroma dispersion in the food matrix and therefore reduced the levels of concentrated aroma (droplet) regions. This caused an increase in the time to reach maximum intensity and had implications for the rate of release. As aroma release rate is a key factor determining perception (Baek *et al.* 1999), the influence of carrier solvents on perception may be an important consideration during sample production in industry.

These techniques were designed to produce dispersed, homogenous systems however, generally, this will not be the case in many real foods especially in products with low water or low fat content. Results from compounds with poor water solubility suggested that the release of these compounds may be occurring as concentrated regions or droplets of aroma as suggested by Schober and Peterson (2004b) for boiled sweets. Systems were developed where aroma was added in the form of droplets and this was compared to the aroma release when solubilised. Results showed that a single droplet produced significant increases in aroma release intensities ranging from 4-2500 fold depending on the physicochemical properties of the aroma compound. This data was modelled and a combined influence of hydrophobicity and vapour pressure was shown to be important.

Subsequently, a different mechanism for aroma release from droplets was suggested. This predicts that, when released as droplets, aroma compounds do not follow the traditional mechanisms of aroma release suggested by many authors (Hills and Harrison 1995; Harrison and Hills 1996; Harrison 1998; Linforth *et al.* 2000; Linforth 2002). Instead, upon release from a gel, droplets of aroma compounds migrate to the gas-liquid interface as pure aroma. Once at the interface the droplet ruptures and produces a very thin aroma layer at the interface from which aroma compounds rapidly volatilise into the gas phase.

Release in this case is not rate-limited by dissolution into the liquid phase and subsequent partition into the gas phase.

This alternative release mechanism may be a key factor for aroma release from encapsulants. Several authors have reported that encapsulants can give a "burst" of aroma release which can enhance perception (Harvey *et al.* 2000b; Blake 2002; Harvey and Barra 2003). It is possible, therefore, that the mechanism described could provide an explanation for this observed "burst" of release. Further study using encapsulants needs to be carried out to confirm if release occurs via this mechanism. The use of droplets may have many potential applications if it can be applied to 'real' situations with examples of these being to boost aroma perception for the elderly or those with reduced nasal responses, or to boost perception of certain aromas within a food system. Obviously the system described here has limited practicality in its present state but it should not take too much further research to unleash its potential.

Important caveats need to be made when considering the data presented in this thesis. The fact that sample concentrations were only checked via APcI-MS may lead to limitations in the actual interpretation of the results. A range of other robust methods, such as GC analysis or use of SPME fibres could have been used in conjunction with this to increase the validity of the data sets. Another important factor was the measurement of water activity and dry matter content which are important when comparing the different samples produced. The potential of competition between mixtures of aroma compounds when using APcI-Ms also provides caveats for relative comparisons however, this is overcome in many case by the use of systems that did not contain mixtures of volatile components.

Overall, this project showed that a number of aroma dispersion mechanisms could be used to produce a wide range of samples. These samples showed reproducible properties and information could be gained on the effects of the

different mixing patterns on aroma compound behaviour. The level of mixing could be investigated and relationships made between the dispersion of aroma compounds and the effects on release and perception.

Future work

The initial research developed a number of model systems that have shown the effects of the different delivery techniques and their implications for aroma release. These have shown the importance of the mode of aroma addition and subsequent dispersion to perceived flavour intensity and quality.

Further studies in layering could extend to samples which do not show rapid in-mouth mixing. If solid samples, such as boiled sweets, could be layered, the implications for this degree of mixing on release via stagnant theory mechanisms could be investigated.

The static mixer system that was developed may also have other interesting applications for assessing the mixedness of samples. The clearly defined levels of mixing that were achieved could be applied to other types of study. For example, different flows may contain different tastants (sugars, acids) and the effects on perception could be studied in this way.

The influence of carrier solvents may have implications for a wide range of aroma release studies that have used solvents. Further investigation into these effects from a range of foodstuffs may be important to determine the overall influences that solvents may impart in real food situations on release and perception.

Simple models have shown the influences of the aroma compound physicochemical properties on release as droplets. Investigation of a wider range

of compounds may give a better idea of the characteristics which influence the behaviour of the droplets and the rates of delivery.

Development of situations where perception could be maximised, via the delivery of droplets but also still maintaining a balanced flavour may be of interest. The challenge for future studies will be to utilise the mechanisms for droplet behaviour to develop novel products which could give maximal aroma delivery whilst ensuring the appropriate perceptual response.

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