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Taste-Aroma-Matrix Interactions
Determine Flavour Perception

By

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ABSTRACT

A newly recruited sensory panel was trained in magnitude estimation and time intensity sensory techniques. Sensory analysis was combined with instrumental analysis using MS Nose™ to investigate the relationship between stimulus and perception using simple model food systems.

Volatile release data was collected after swallowing aqueous solutions containing a cocktail of aroma compounds. The compounds varied in their persistence during subsequent exhalations dependent on the degree of association with the mucous lining of the throat. The rate of breathing had no effect on the shape or intensity of the release profile. The data was successfully modelled using the basic principles of interfacial mass transfer. The mechanistic model included some estimated parameters for un-measurable anatomical and physiological variables, kinetic properties of the flavour compound and terms to represent the oscillatory breathing and airflow rate. Aside from the 1st exhalation, the model fitted the data very well.

The panel rated the intensity of minty flavor in a 6% gelatine gel, containing varying concentrations of carvone. The flavor was assessed using Magnitude Estimation and Time Intensity Methods. In addition, the quantity of carvone released from the gel and reaching the assessor’s nose was measured, breath by breath during eating, using the MS Nose™. The results showed that the quantity of volatile delivered to the nose was directly proportional to the concentration in the sample, however, the absolute quantity varied greatly between individuals. Further differences were observed in the temporal dimension of their release profiles, which related to differences in their anatomy, physiology and eating habits. In some cases, these differences were mirrored by the sensory data. The relationship between perceived intensity and sample concentration was linear for both types of sensory data. Neither the speed of eating nor the concentration of volatile reached in-nose, affected an individual's ability to judge intensity. There was evidence to suggest, however, that the speed of eating affected the level of adaptation to the carvone stimulus.
The affinity of the aroma compound for water (hydrophobicity) was an important factor in influencing the temporal characteristics of the instrumental and sensory data. When aroma release was rapid (< $T_{max}$) the sensory response occurred slightly later whereas when the aroma release was much slower (> $T_{max}$), with intensity increasing more gradually, the sensory response preceded the instrumental data. These phenomena were explained in terms of a lag phase for neural processing when the stimulus was presented quickly and adaptation to the stimulus when it was delivered over a longer period of time.

A trained sensory panel assessed flavour and sweetness intensity in solutions containing varying concentrations of Hydroxy Propyl Methylcellulose (HPMC), sugar and flavour volatile. The flavour and sweetness of the viscous solutions were rated using magnitude estimation with a controlled modulus. In addition, the concentration of volatile released on the breath was measured using MS Nose™. For low concentrations of HPMC (<0.5g/100g), perceived flavour intensity remained the same, however, a steady decrease was noted at higher concentrations (>0.6g/100g). The change in perceived intensity occurred at the point of random coil overlap ($c^*$) for this hydrocolloid. The perceived sweetness of the solution showed a similar pattern with increasing HPMC concentration, although the inflection at $c^*$ was not so obvious. Despite the change in perceived flavour intensity, the actual concentration of volatile measured on the breath was not affected by the change in HPMC concentration. Low order polynomial models were produced to describe perceived flavour intensity and sweetness in viscous solutions containing HPMC and potential explanations for the changes in perception were discussed.
ACKNOWLEDGEMENTS

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Finally, I must mention all of my family and friends outside of Nottingham who for the past few months have managed, successfully, to both completely ignore me and
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1.0 INTRODUCTION

1.1 FLAVOUR AND PERCEPTION

The flavour perception we derive from eating a food product is determined by the nature and quantity of the flavour components; the availability of these components to the sensory system as a function of time; and the mechanism and strategies of perception and scaling, which determine the flavour quality and intensity over time, as recorded by the panelist.

It is generally accepted that aroma, taste, texture and mouthfeel account for the major stimuli that contribute to the perception of flavour. Stimulation occurs when compounds from the food come into contact with the receptor cells in the mucous membranes of the nose (odour/aroma) and mouth (taste) or when food structures such as emulsions or rigid cell walls affect chewing processes (texture) or interact with the mouth lining (mouth-feel) (Taylor 1996) (Taylor 2002). The flavour perceived during eating is not a simple addition of these four basic stimuli but a complex pattern that has different characteristics for particular foods (van Ruth and Roozen 2002). Whilst flavour is deemed to be a combination of these factors, aroma release is considered to be the most influential contributor to perceived flavour.

Perception of flavour shapes the hedonic responses, which are so important for consumer acceptability. Consequently, the study of flavour-matrix interaction and their effects on the release and perception of flavour is an important area of research. Many sensory studies have been conducted, but comparisons with instrumental data of quantitative and qualitative flavour composition remain difficult to interpret, and predictions regarding the sensory properties of a food from the compositional data cannot be made with any certainty.
1.1.1 Flavour delivery from foods

For aroma compounds to be perceived by consumers, they must be released from the food matrix so they can enter the airways of the nose and come into contact with the olfactory receptors (Figure 1.1).

Consumers can receive several different volatile signals from the food. Prior to consumption, the volatiles are sampled orthonasally and this first sniff often has a major impact on the overall perception of the food. Volatiles are released during eating and travel to the olfactory receptors by the retronasal route. In both situations, a combination of physicochemical parameters along with dynamic factors determines the relative distribution of volatile compounds between the food and the
air phases. As this relationship can be described in mathematical terms, various models have been proposed to predict the volatile signal that is delivered to the olfactory receptors.

When food is eaten, flavour molecules are released from the food into the mouth. Mastication and tongue movements increase the effective surface area of the food; the rise in temperature causes some components (e.g. fats) to melt; saliva dilutes the food and periodic swallowing changes the volume of the bolus. After swallowing, the volatile flavour compounds pass back up through the nasopharynx into the nose (Buettner and Schieberle 2000) (Buettner and Schieberle 2000). Flavour compounds travel, via airflow, to the olfactory epithelium, where they interact with receptor proteins, triggering a train of events which leads to perception. A sufficiently high concentration of flavour molecules has to be released from the food to stimulate the olfactory system and elicit a response. Flavour release and delivery depend on the nature and concentration of volatile compounds present in the food, as well as on their availability for perception as a result of interactions between the major components and the aroma compounds in the food (Bakker 1995) (Bakker, Brown et al. 1996) (Bakker, Langley et al. 1995). Food composition and eating behaviour determines the magnitude and time profile of flavour release, delivery and perception (Kinsella 1989) (Bakker, Brown et al. 1996).

Despite a considerable amount of research in this area, there is still a lack of models or mechanisms relating flavour composition to flavour perception. Potential reasons for this are:

- Flavour release is a dynamic process and the flavour stimuli should be measured at the receptors in the mouth and nose rather than the flavour composition in the food.
- Physiology affects release e.g. breathing, chewing, swallowing.
- Food matrix affects release e.g. binding, melting, dissolving.
Many senses are multimodal but flavour has often been studied in terms of the separate components e.g. aroma, taste.

In this thesis, some of the above factors are studied with a view to achieving a better understanding of the mechanism underlying flavour perception.

1.2 DYNAMICS OF FLAVOUR RELEASE

1.2.1 Instrumental methods for in vivo analysis of volatile flavour release.

The first attempts to measure volatile release from foods during consumption involved collecting expired air from humans and injecting it onto a GC column (Mackay and Hussein 1978). The lack in sensitivity of this technique meant that volatile compounds were not detected at the concentrations typically found in breath. To overcome this problem, different trapping methods (e.g. cryo-trapping (Linfoth and Taylor 1993) and Tenax-trapping (van Ruth, Roozen et al. 1995) (Delahunty, Piggott et al. 1994)) were employed to concentrate the flavour before GCMS analysis. These investigations highlighted the value of following volatile release in mouth but progress was slow because the trapping methods were very time-consuming and it took several weeks to obtain sufficient data to plot a single volatile-release profile (Taylor and Linfoth 1996).

The first methodology for measuring volatile release in real-time was developed by Soeting and Heidema (Soeting and Heidema 1988). They developed a membrane interface for an electron impact mass spectrometer (EI-MS), which allowed the introduction of volatile molecules into the EI-source whilst, largely, excluding air and water; both of which drastically affected its efficiency and general operation. Unfortunately, the membrane showed selective permeability and the method had poor sensitivity with detection thresholds at 25mg/kg.

The next significant development was the production of an interface that allowed the use of atmospheric pressure ionisation mass spectrometry (API-MS) to follow real-
time volatile release from subjects during eating (Linforth and Taylor 1998). The benefit of this technique was that several compounds could be followed, simultaneously, at concentrations of approx 10ppb and the response time was sufficiently rapid to analyse breath by breath release from the nose.

In this technique, exhaled air is sampled through a fused silica capillary tube by means of a Venturi effect, created by a high nitrogen gas flow. The volatiles in the breath are ionised by the corona discharge pin (4kV) and drawn into the MS analyser (Figure 1.2). This is a soft ionisation technique, which causes little fragmentation and produces mainly molecular ions by the addition or abstraction of a proton. Most molecules are ionised by the addition of a proton in positive ionisation mode (RH⁺). Ionisation occurs when charge is transferred from water (H₃O⁺) to the analyte molecules. Identification of compounds relies entirely on mass resolution so separate isomeric forms cannot be identified. API-MS has been used in a wide range of applications (Baek, Linforth et al. 1998) (Baek, Linforth et al. 1999) (Brauss, Linforth et al. 1999) (Brauss, Linforth et al. 1999) (Linforth, Baek et al. 1999) (Linforth, Friel et al. 2000) (Linforth, Hollowood et al. 2000) (Linforth and Taylor 2000) (Davidson, Hollowood et al. 1999) (Harvey, Davidson et al. 2000) (Hollowood, Linforth et al. 2002). The interface is now commercially available as the MS Nose™ (Micromass – Manchester, UK).
An alternative method to the MS Nose™ was developed by Lindinger and co-workers (Lindinger, Hansel et al. 1998a) (Lindinger, Hansel et al. 1998b). Proton transfer reaction mass spectrometry (PTR-MS) generates the reagent ion (H$_3$O$^+$) separately, and then controls the reaction between reagent ions and volatile organic compounds in a specially developed “drift tube” operated at precise temperature and pressure. All components in a mixture are, therefore, ionised to the same extent. Whilst PTR-MS has the advantage of precise control over ionisation, the sensitivities reported for flavour volatiles are similar to those obtained with APCI-MS (Taylor, Linforth et al. 2000).

The development of techniques for measuring the concentration of volatile released on the breath during eating in real-time has allowed a deeper understanding of the mechanisms governing aroma release, in particular, the temporal aspects of the release profile and the relationship between stimulus and perception.

MS Nose™ was used throughout this thesis to measure aroma release during eating.
1.2.2 Flavour release under equilibrium conditions

Flavour delivery depends on the availability of the flavour compounds in the gas phase and, therefore, on the affinity of the compounds for the food matrix. Various properties of the flavour compounds determine their interactions with food components, e.g. molecular size, functional group, shape, volatility (Kinsella 1988). Properties such as molecular weight, vapour pressure, boiling point, logP (octanol-water partition coefficient) have been used to predict the volatility of the compounds under static conditions (Roberts and Acree 1996) (Linfoth, Friel et al. 2000) (van Ruth, O'Connor et al. 2000). The presence of other components can affect the partition coefficient e.g. addition of fat, aroma binding macromolecules, salts, sugars and combinations of volatile (Bakker, Langley et al. 1995).

The equilibrium partition coefficient is an important thermodynamic property of volatile molecules in food systems. It describes the distribution of volatile (maximum concentration that may occur) between the gas and product phases at equilibrium, under defined conditions of temperature and pressure. Equilibrium exists only when the transfer of molecules at the product-gas interface is balanced and there is no net change in concentration in either the gas or product phase (Taylor 1998) (deRoos 2000).

The equilibrium partition coefficient is expressed as:

\[ K_i = \frac{C_{i_g}}{C_{i_p}} \]  \hspace{1cm} (1.1)

Where: \( C_{i_g} \) and \( C_{i_p} \) are the concentration of volatile in the gas and product phase respectively.

Whilst much of the early investigation into volatile release was carried out on static systems, its value was limited to studying matrix effects and interactions between volatile compounds and food components. By contrast, eating is a dynamic process
where the nature of the food changes over time and equilibrium is unlikely to be achieved. Food is diluted with saliva and the temperature changes; the air above the food in the mouth is mixed with fresh air during breathing. Under these circumstances, mass transfer, along with partition, will govern the movement of volatile and the rate of release becomes especially important for interpreting the perception of flavour (Taylor 1998) (Bakker 1995).

1.2.3 Flavour release under non-equilibrium conditions

When a system is not at equilibrium, kinetic factors determine the rate at which equilibrium is achieved. The movement of compounds is described by the mass transfer coefficient. The driving force for transfer of flavour compounds across an interface is the difference in flavour concentration between the two phases. The concentration gradients that exist, and the mass transfer coefficients of the flavour compounds in each of the phases, determine the rate of the unidirectional diffusion from the product to the gas (Bakker 1995). Many theories exist to describe mass transfer across an interface, with different mathematical solutions to determine the mass transfer coefficient.

1.2.3.1 Two film theory

This theory assumes that there are two interfacial layers through which the volatile diffuses. Also referred to as ‘stagnant-film’ theory, this model assumes that the boundary layers at the interface are stagnant and that mass is transported through these layers as a result of molecular diffusion. The mass transfer coefficient varies with the first power of the diffusion coefficient D and the reciprocal of the effective thickness of the stagnant layer (Hills and Harrison 1995).

1.2.3.2 Penetration theory

This theory assumes that the boundary layers are not stagnant and that there is also mass transport by eddy diffusion. It is assumed that a volume element of liquid from the bulk phase comes into contact with the interface and is exposed to the second phase for a definite interval of time. During this time, equilibrium is attained through
the surface layers through a process of unsteady-state molecular diffusion of flavour into the gas phase, before the volume element is re-mixed with the bulk liquid. In the penetration model, the mass transfer coefficient (k) varies with the square root of the diffusion coefficient (Harrison and Hills 1997) (Harrison, Hills et al. 1997).

1.2.3.3 Non-equilibrium partition model

This model assumes that mass transfer takes place only by eddy diffusion. The independence of the diffusion constant allows a multiple extraction model. It is assumed that flavour compounds are extracted from the product with infinitesimal volumes of gas. During successive extractions, full equilibrium is achieved only at the gas-product interface in the infinitesimal volumes of product and gas phase. After each extraction, the initial flavour concentrations at the surface of the product are restored by diffusion and turbulence before the next extraction takes place (deRoos and Wolswinkel 1994).

1.2.4 Modelling flavour delivery from foods

Models have been constructed to describe varyingly complex scenarios of flavour release from foods. The simplest of models may describe the equilibrium partitioning of volatiles in a static system. These provide valuable information about matrix effects and the degree of interaction between food components and volatile compounds. More complex models describe dynamic partitioning where the gas phase above a matrix is disturbed or diluted to simulate real life. These include a temporal dimension and the exact conditions used e.g. flow rates, volumes, etc. can result in differing degrees of depletion in the headspace and mixing in the matrix. Finally, there are the models that attempt to describe flavour release during eating. Factors such as dilution by saliva, temperature changes, bolus breakdown, swallowing and breathing all act in concert, making these models the most complex. They are difficult to validate due to lack of experimental data (Linforth 2002).
1.2.4.1 Developing a model

There are two main approaches to constructing a model (Linfirth 2002). The theoretical or mechanistic approach uses the principles of physics and chemistry to describe how a system is likely to behave. Equations include all parameters relating to the flavour, matrix and surrounding environment that act independently or interact to influence flavour release. One of the major advantages of the theoretical approach is that it allows the development of models, in the absence of analytical data. On the contrary, however, one of the criticisms is that they are rarely validated and therefore, their predictive accuracy is impossible to determine.

The second approach is empirical and relies on data generated from well-constructed experimental design (including necessary replication). Data are collected for a range of flavour compounds and/or food matrices. A model is constructed with sufficient significant components to describe the variation in the data set. These models may contain similar terms to those from mechanistic models. The experimental approach requires a data set to build the model and another one for validation.

One empirical method of model building that is gaining popularity in flavour research is Quantitative Structure Property Relationships (QSPR). Widely used for drug design and toxicological studies, QSPR models the behaviour of compounds from their molecular properties. To date, this technique has been successfully used to estimate partition coefficients (Dearden and Bresnen 1988); model the release of volatiles from gel systems during eating (Linfirth, Friel et al. 2000); predict the gas-liquid partition behaviour of aroma compounds in aqueous sucrose solutions (Friel, Linforth et al. 2000) and to model the persistence of volatile compounds on the breath after swallowing (Linfirth and Taylor 2000).

1.2.4.2 A review of flavour release models

A review of the literature reveals a plethora of models describing flavour release from a variety of different matrices, using a host of different experimental or
theoretical conditions and mechanisms. Some of the more significant empirical and theoretical attempts to model flavour release in recent history are listed in Table 1.1.

There are, generally, fewer models that describe volatile release during the eating process, mainly because of the number of variables that need to be considered and the limited amount of experimental data available. The more variables present in an equation, the harder it is to solve mathematically (Linfoth 2002).

Many of the dynamic headspace models were generated in an attempt to describe the behaviour of volatiles under non-equilibrium conditions as would be found in the mouth. Some of the simulated systems include dilution from saliva, breakdown from mastication and airflow from respiration. Despite attempts to recreate in-mouth conditions, previous work has shown that the volatile concentrations in the breath during the consumption of standard foods (cheese, biscuits) were substantially lower than that in the headspace (Deibler, Lavin et al. 2001). Further work has shown similar results following consumption of a simple solution (deRoos and Wolswinkel 1994). This might be due to the liquid or gas phase dilution in-mouth or as volatile-laden breath passes through the upper respiratory tract.
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</tr>
<tr>
<td>DeRoos and Wolswinkel 1994</td>
<td>Mechanistic</td>
<td>Non equilibrium partition</td>
<td>Chewing gum</td>
<td>Kgw, Kaw, saliva gum volume ratio</td>
<td>In vivo release from chewing gum</td>
</tr>
<tr>
<td>Roberts and Acree 1996</td>
<td>Empirical</td>
<td>-</td>
<td>Range of matrices (relative to water) containing thickeners and/or oil.</td>
<td>Kaw, logP, viscosity, temperature</td>
<td>RAS – Dynamic headspace sampling mimicking mouth</td>
</tr>
<tr>
<td>Harrison, Hills et al. 1997</td>
<td>Mechanistic</td>
<td>Penetration theory</td>
<td>emulsions</td>
<td>Kae, initial conc. volatile in emulsion, mass transfer coefficient, area of the air/emulsion interface, oil fraction and droplet size</td>
<td>Model describes the rate of volatile equilibration in the dynamic headspace above emulsions</td>
</tr>
<tr>
<td>Harrison and Hills 1997</td>
<td>Mechanistic</td>
<td>Penetration theory</td>
<td>emulsions</td>
<td>As for Harrison et al 1997 with additional factors dilution by saliva and gas flow rate</td>
<td>Extension of previous model to describe in vivo release</td>
</tr>
<tr>
<td>Harrison 1998</td>
<td>Mechanistic</td>
<td>Penetration theory</td>
<td>Liquids including addition of aroma binding molecules and oil</td>
<td>Mass transfer across liquid gas interface, viscosity, dilution by saliva (if food stays in mouth)</td>
<td>In vivo release of flavour from liquid foods</td>
</tr>
<tr>
<td>Reference</td>
<td>Methodology</td>
<td>Theory/Mechanism</td>
<td>Substances/Conditions</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Bakker, Boudaud, et al. 1998</td>
<td>Mechanistic</td>
<td>Penetration theory</td>
<td>Liquids containing gelatine</td>
<td>Dynamic headspace study</td>
<td></td>
</tr>
<tr>
<td>Marin, Baek et al. 1999</td>
<td>Mechanistic – convective model</td>
<td>Thermodynamic model</td>
<td>Solutions, viscous solutions and gels</td>
<td>Kaw, and mass transfer in liquid and gas phases (dependent on turbulence i.e. Reynolds number) Non stirred liquid system at equilibrium and a stirred headspace to mimic orthonasal delivery of volatile when opening food containers</td>
<td></td>
</tr>
<tr>
<td>Harrison 2000</td>
<td>Mechanistic</td>
<td>Two film theory</td>
<td>Chewing gum (made modelling easier as no losses in volume)</td>
<td>In mouth partition and the transfer of volatiles through the upper airway</td>
<td></td>
</tr>
<tr>
<td>Linforth and Taylor 2000</td>
<td>Empirical</td>
<td>QSPR</td>
<td>Solutions including addition of HPMC</td>
<td>Persistence of volatile on the breath post swallow</td>
<td></td>
</tr>
<tr>
<td>Linforth, Friel et al. 2000</td>
<td>Empirical</td>
<td>QSPR</td>
<td>Gelatine/sugar gels</td>
<td>Release characteristics measured in vivo</td>
<td></td>
</tr>
<tr>
<td>Nahon, Harrison et al. 2000</td>
<td>Mechanistic</td>
<td>Penetration theory</td>
<td>Aqueous sucrose solutions</td>
<td>Liquid and gas phase stirred (nitrogen bubbled through)</td>
<td></td>
</tr>
<tr>
<td>Linforth, Martin et al. 2001</td>
<td>Empirical</td>
<td>QSPR</td>
<td>Solutions including addition of HPMC</td>
<td>Retronasal aroma delivery post swallow</td>
<td></td>
</tr>
</tbody>
</table>
In a study investigating retronasal aroma delivery (Linforth, Martin et al. 2001), the authors concluded that the concentration of volatile in the headspace was significantly higher than the concentration of volatile exhaled via the mouth or nose following consumption – 8 fold differences in concentration were observed. The differences were primarily related to the rate of equilibration (mass transfer) although dilution of volatile in the upper airways and adsorption of volatile to nasal epithelia also contributed towards the differences. Dilution by saliva, and increasing viscosity of solution, were shown to be insignificant factors.

Common to all models is the necessity to account for the major differences between compounds. This can be achieved using partition coefficients, mass transfer coefficients or physicochemical parameters, one or more of which can be found in every equation describing the behaviour of volatiles. The air water partition coefficient, despite describing the relative distribution of volatile in static systems, is also an important parameter in models describing dynamic and in vivo release.

One of the aims of this thesis was to generate flavour release, perception and physiological data to validate models proposed by Hills and Harrison (Harrison 1999) (Harrison 2000).

1.3 THE EFFECT OF MATRIX ON FLAVOUR DELIVERY

1.3.1 Effect on partition coefficient
Foods are a complex mix of water, carbohydrates, lipids, proteins and other organic compounds all of which can interact with aroma compounds (Nahon, Roozen et al. 1996). The nature of this interaction can be chemical binding (irreversible covalent bonds), physicochemical binding (van der Waals forces, hydrogen bonds, hydrophobic interactions or ionic bonds) or simply, the differing propensities for aroma compounds to be absorbed into different phases of the food (van Ruth and Roozen 2002).
Any process that changes the concentration of ‘free’ volatile, or shifts the distribution of volatile in the food, will alter the partitioning of the aroma compounds.

1.3.1.1 Carbohydrates
The addition of simple sugars effectively reduces the molar concentrations of volatiles in the aqueous phase and has also been shown to affect the activity coefficient. Consequently, the addition of sugars will result in an increase in headspace concentration ‘salting out’ whilst others result in a decrease (Voilley, Simatos et al. 1977) (Nahon, Harrison et al. 2000) (Buttery, Bomben et al. 1971).

In a study investigating the effect of sucrose (0 – 60%) on gas-liquid partition of 40 different volatiles (Friel, Linforth et al. 2000), the headspace concentration of some volatiles increased with increasing sugar concentration, some remained unchanged whilst others were reduced. The magnitude of the effects increased linearly with sucrose concentration, although little difference was seen below 20% sucrose.

Hydrocolloid thickeners (added to foods to enhance textural properties) can bind to aroma compounds, dependent on the chemical nature of the thickener and aroma molecule. The binding is probably through hydrogen bonding (Roberts, Elmore et al. 1996) (Yven, Guichard et al. 1998).

Starch (one of the most common food components) has been shown to form inclusion complexes with a wide range of compounds including alcohols, ketones, phenols and many more (Godshall and Solms 1992). The formation of a helical arrangement of amylose molecules has been recognised as responsible for inclusion complex formation; the flavour is located in the free space between the helices (Escher, Nuessli et al. 2000).
1.3.1.2 Lipids

Lipids have the greatest impact on gas-product partitioning compared to any other food component. In lipid-containing foods, lipophilic flavours are bound to the lipid molecules by weak van der Waals forces and hydrophobic interactions (van Ruth and Roozen 2002). Lipids act as a solvent for lipid-soluble hydrophobic compounds and, therefore, the addition of lipid to an aqueous solution would reduce the headspace concentration of the more lipophilic compounds, whilst the hydrophilic compounds would be largely unaffected. When lipid is removed from a system (the production of low fat foods) the food matrix retains lipophilic flavours poorly and the resulting headspace concentrations are high (Plug and Haring 1993).

Carey and co-workers (Carey, Asquith et al. 2002) developed a QSPR model, which described the concentration of different volatile compounds in the headspace above a low lipid (0-0.2%) concentration emulsion relative to that of water. Hydrophobicity (LogP), solubility in water, dipole vector term and oil fraction were key parameters in the model.

1.3.1.2 Proteins

Proteins can interact with flavour compounds in two ways, either by reversible non-covalent interactions or, alternatively, non-reversible covalent linkages. Aldehyde groups can react reversibly with free amino acids or amino groups of proteins to form Schiff bases or, alternatively, can bind covalently with primary amino acids.

(Fischer and Widder 1997) showed that increasing the casein concentration from 0-12%, decreased the headspace concentration of ethyl 2-methyl butanoate, heptanal and ethyl hexanoate. In the same study, heat denaturation of protein reduced the concentration of volatiles in the headspace by increasing access to hydrophobic binding sites.

Changing the pH of the food can cause a considerable change in the interaction of aroma and protein (Lubbers, Landy et al. 1998) (Jouenne and Crouzet 1996).
1.3.2 Effect on mass transfer

Changes in the physical properties of the food matrix may affect the release kinetics of aroma compounds (van Ruth and Roozen 2002) (Bakker, Boudaud et al. 1998) (Bakker, Langley et al. 1995). The addition of compounds such as sugar, thickener and fat (droplet size and fat melting temperature) will change the rheological properties of the food.

1.3.2.1 Viscosity

Viscosity is an important parameter of flavour release as it determines the diffusion coefficient that, irrespective of the preferred theory, has a direct effect on the mass transfer coefficient (see section 1.2.3). It is defined as the internal friction of a fluid or its tendency to resist flow (Bourne 1982) and is caused by the work necessary to overcome the frictional forces exerted by the dissolved molecules on the fluid and, in concentrated solutions, by entangling chains (Bohdanecky and Kovar 1982).

Historically, an increase in viscosity is often related to a decrease in release of volatile flavour from the solution. This is often explained as a decrease in the rate of diffusion, which is said to be inversely proportional to the solution viscosity, as defined by the Stokes Einstein and Wilke Chang equations (Wilke and Chang 1955).

Dynamic headspace studies showed a decrease in the release rate of several volatile compounds from carboxy-methyl cellulose (CMC) solution (1%) compared to the release of the same compounds in water (deRoos 1997). The greatest decrease in release rate was seen for the most volatile compounds, which were rapidly depleted from the surface.

In a comprehensive study investigating the effect of thickener composition and viscosity on dynamic flavour release (Roberts, Elmore et al. 1996), a decrease in the release of highly volatile compounds was reported as viscosity increased. Less volatile compounds showed little or no effect with increasing viscosity. The extent
of the decrease was dependent both on thickener type and viscosity, which the author suggested were due to some sort of binding mechanism and the physical inhibition of volatile mobility, although the relative contribution of these could not be determined.

Although these effects can be easily observed in model systems the situation in mouth is more complex and it is difficult to predict whether viscosity will have an effect on aroma release.

To date, much of the research into the effect of viscosity has focused on the effect of thickener on the perception of volatile and non-volatile flavour. It is generally understood that increasing viscosity results in a decrease in perceived intensity of volatile and non-volatile components (Vaisey, Brunon et al. 1969) (Moskowitz and Arabie 1970) (Pangborn, Trabue et al. 1973) (Pangborn and Szczesniak 1974) (Christensen 1980) (Baines and Morris 1987) (Malkki, Heinio et al. 1993). Furthermore, the decrease can be dependent on thickener type (Pangborn, Trabue et al. 1973) (Paulus and Haas 1980).

Previous studies showed that the perception of sweetness and strawberry flavour was greatly affected by the addition of guar gum at concentrations higher than c*, the point of random coil overlap (Baines and Morris 1987) (Baines and Morris 1988). For any given hydrocolloid, c* is the concentration at which individual polymer chains interpenetrate and start to form an entangled network (Morris, Cutler et al. 1981). It is dependent on the number and space occupancy of the polymer molecules and is associated with a sharp increase in viscosity. Below this concentration, the individual polymer chains are free to move independently. Baines and Morris discovered that guar gum had no significant effect on perception of sweetness or flavour below c* but above this concentration, the perceived intensity of both attributes decreased steadily with increasing polymer concentration. They concluded that the decrease in flavour perception was due to inefficient bulk mixing leading to a lack of regeneration of surface volatile, as the polymer chains became obstacles to
diffusion, rather than direct binding of flavour molecules to the polymer, or restricted diffusion (Morris 1987). The conclusion was based on the fact that suppression in random coil solutions was dependent on the extent of coil overlap rather than on polymer concentration alone.

Other studies have shown that binding is dependent upon the chemical nature of the thickener and aroma compound. An investigation into the effect of polymer composition (oat gum, CMC, guar gum) on sensory perception revealed that the nature of the hydrocolloid had more effect on perceived sweetness than viscosity (Malkki, Heinio et al. 1993). Guar had the greatest effect on sweetness, and oat gum the least. The reduction in sweetness due to the addition of thickeners was dependent on the sweetener used (aspartame, fructose, sucrose). The same study looked at the effect of thickener type on the perception of flavour intensity. The physicochemical properties of the compounds used were more important than the type of polymer. Any differences in perception from equi-viscous solutions of oat, guar and CMC constituted evidence of binding or interaction between the polymer and the flavour compounds.

1.3.3 Breakdown of food matrices

1.3.3.1 Liquid foods

For volatiles in aqueous solution the release is dependent solely on the extent of saliva dilution. Dilution reduces the aqueous phase concentration, and hence lowers the breath volatile concentration.

Dilution will shift the binding equilibrium in solutions containing aroma bound with macromolecules, such as proteins and polysaccharides. In emulsions it will shift the aroma partitioning and change the release kinetics (Harrison, Campbell et al. 1998).

McNulty 1987 (McNulty 1987) proposed that during consumption, saliva dilutes the sample, and since the air-emulsion partition coefficient of an emulsion is dependent
on the oil and water fractions, the partition coefficient will change during the eating process, thereby affecting volatile release. For this to happen, the aroma compounds need to partition readily between the oil and water phases (otherwise the aroma would be trapped by the lipid), a phenomenon that was observed experimentally (Doyen, Carey et al. 2001). The greater the dilution, the more significant this factor will become in determining the breath volatile concentration. The difference between water and emulsion upon dilution can be substantial for a compound that readily partitions in the oil phase. For such a compound in a water system (with no lipid present), dilution of the aqueous phase would result in a decrease in headspace concentration. Whereas in an emulsion, aqueous dilution has little effect on the headspace concentration as the volatile is distributed in the lipid phase which remains unchanged.

1.3.3.2 Semi solid foods
For semi solid foods such as gels, which possess melting points below the mouth temperature, the driving force for flavour release is the rate at which heat can diffuse into the gel matrix and initiate melting. For harder gels with melting points above mouth temperature, the diffusion of sucrose from the surface of the gel into the saliva phase lowers the melting temperature of the surface and facilitates flavour release (Harrison and Hills 1996) (van Ruth and Roozen 2002).

1.4 PERCEPTUAL INTERACTIONS
As discussed previously physical interactions readily occur between aroma compounds and components of the food matrix. Whilst these are of particular importance in understanding the release profiles from certain foods, the interaction of input from different sensory modalities provides valuable information about an individuals percept of flavour.

For research purposes, sensory modalities are often studied individually despite the fact that human integration with the world occurs on a multimodal level (Breslin 2001). With the multimodal concept, it is implicit that interactions occur at a
cognitive level. The problem is identifying and segregating physical interactions so that these cognitive effects can be studied in more detail.

1.4.1 Taste-Aroma interactions
For many years, sensory scientists have investigated the interaction of taste and aroma to learn more about their mechanisms and the locations at which the interactions are processed. These studies have engendered considerable debate as to whether flavour is an analytic or synthetic perception.

The advocates of an analytic percept would argue that flavour is made up of individual, recognisable component parts and that the overall intensity of a flavour mixture can be determined from adding the respective perceived intensities of the independent aroma and taste components (additivity) (Murphy, Cain et al. 1977) (Murphy and Cain 1980).

By contrast, those in support of a synthetic percept would argue that the dissection of flavour into its component parts is unnatural, and that taste and aroma perceptions ‘synthesise’ to produce a totally new sensation that is flavour.

It is extremely unlikely, however, that taste-aroma interactions occur at receptor sites and that more probably, they are functions of cognition occurring at the central processing level (Noble 1996). Studies with primates have shown that in the orbitofrontal cortex, olfactory inputs converge onto neurons with taste input, forming representations of flavour (Rolls 1997).

A somewhat ‘middle of the road’ theory was proposed by McBurney (McBurney 1986) who suggested that flavour was neither synthetic nor analytic but rather a ‘fusion’ of its component sensations and that the components of the fused sensation were still perceptible by careful analysis. So although a mixture produced a new perceptual quality different to any from the unmixed components, recognising the individual qualities was still possible with a well-trained panel.
Numerous scientific papers highlight the importance of taste-aroma interactions from both academic and industrial perspectives. Table 1.2 lists some of the more significant contributions to the debate in recent history.

One of the problems with making sense of all the scientific evidence is the differing methodologies employed by the researchers. In studies assessing overall intensity, in addition to the intensity of the component parts, no interactions were observed. By contrast, interactions were common in studies where panellists were instructed to concentrate on specific qualities of the flavour e.g. sweetness, fruitiness. The size and direction of the interaction was shown to be dependent on the number of appropriate response scales used (Frank, Wessel et al. 1990) (Frank and van der Klaauw 1993). These differences were explained in terms of a ‘dumping’ effect where panellists dump important stimulus information in the available response categories if a separate scale is not provided (Clark, Lawless 1994).

Taste qualities can be attributed to odours when sniffed, assessors typically use terms such as sweet or sour even though the olfactory system contains no receptors (equivalent to in mouth) sensitive to such tastes (Voirol and Daget 1986).
Table 1.2: Summary of papers researching interactions between taste and aroma

<table>
<thead>
<tr>
<th>Reference</th>
<th>Odour and tastant</th>
<th>Flavour constuct</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murphy and Cain 1980</td>
<td>Citral, sucrose, sodium chloride</td>
<td>Analytic</td>
<td>Lack of 100% additivity explained as noise</td>
</tr>
<tr>
<td>Murphy, Cain et al. 1977</td>
<td>Ethyl butyrate, sodium saccharin</td>
<td>Analytic</td>
<td>Lack of 100% additivity explained as noise</td>
</tr>
<tr>
<td>Enns and Hornung 1985</td>
<td>Benzaldehyde</td>
<td>Analytic</td>
<td>&lt;100% additivity explained as greater noise at higher intensity</td>
</tr>
<tr>
<td>Garcia-Medina 1981</td>
<td>Coffee, acetic acid</td>
<td>Analytic</td>
<td>&lt;100% additivity explained as noise</td>
</tr>
<tr>
<td>Berglund, Berglund et al. 1973</td>
<td>Pyridine, linalyl acetate, linalool, lavandin oil, hydrogen sulphide.</td>
<td>Analytic</td>
<td>Odour-odour interactions. Proposed vector addition</td>
</tr>
<tr>
<td>Hornung and Enns 1994</td>
<td>Ethyl butyrate, sucrose</td>
<td>Synthetic</td>
<td>Intensity of aroma and taste enhanced</td>
</tr>
<tr>
<td>Frank and Byram 1988</td>
<td>Strawberry flavour, peanut butter, sucrose, sodium chloride</td>
<td>Synthetic</td>
<td>Enhancement was dependent on congruency</td>
</tr>
<tr>
<td>Frank, Shaffer et al. 1991</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Tastants</td>
<td>Type</td>
<td>Effect</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Schifferstein and Verlegh 1996</td>
<td>Strawberry, lemon, ham, sucrose</td>
<td>Synthetic</td>
<td>Enhancement was dependent on congruency</td>
</tr>
<tr>
<td>Stevenson, Prescott et al. 1999</td>
<td>Caramel, maracuja, strawberry, lychee, angelica oil, damascone, sucrose, citric acid</td>
<td>Synthetic</td>
<td>Enhancement and suppression – dependent on congruency. Exposure can modify perception</td>
</tr>
<tr>
<td>Bonnans and Noble 1993</td>
<td>Fruity odour, sucrose, citric acid</td>
<td>Synthetic</td>
<td>Tastant enhanced fruity odour</td>
</tr>
<tr>
<td>Matysiak and Noble 1991</td>
<td>Orange flavour, sucrose, aspartame</td>
<td>Synthetic</td>
<td>Enhanced fruitiness persistence for aspartame</td>
</tr>
<tr>
<td>Kuo, Pangborn et al. 1993</td>
<td>Citral, sucrose, citric acid Vanillin, sucrose</td>
<td>Synthetic</td>
<td>Acid enhanced citral odour Sucrose enhance vanillin retronasally</td>
</tr>
<tr>
<td>Davidson, Hollowood et al. 1999</td>
<td>Mint, sucrose</td>
<td>Synthetic</td>
<td>Perceived mintyness in chewing gum modulated by sugar release</td>
</tr>
<tr>
<td>Stevenson, Prescott et al. 1995 Stevenson, Boakes et al. 1998</td>
<td>Lychee, water chestnut, sucrose, citric acid</td>
<td>Synthetic</td>
<td>Enhancement of tasting ratings following associative learning</td>
</tr>
<tr>
<td>Dalton, Doolittle et al. 2000</td>
<td>Benzaldehyde, saccharin, monosodium glutamate</td>
<td>Synthetic</td>
<td>Tastant and aroma presented below threshold.</td>
</tr>
</tbody>
</table>
Apparent interactions have been explained as taste-smell confusions where subjects have recorded ‘no smell’ when smelling a tastant but have detected a smell sensation with the same sample in mouth. Conversely, subjects have recorded a taste whilst smelling an odourant but did not perceive the taste with the sample in mouth (Hornung and Enns 1994). In the studies (Murphy, Cain et al. 1977) and (Murphy and Cain 1980) taste-aroma confusions were used to explain any enhancement of tastant by aroma. Rozin (Rozin 1982) suggested that olfaction can be seen as two functionally distinct senses, one that senses the outside world (orthonasal) and the other that senses what is in the mouth (retronasal). The information delivered by each may differ in its cognitive impact. Humans are more sensitive to retronasal stimulation by sipping than orthonasal sniffing (Voirol and Daget 1986).

In common with many studies, is the conclusion that enhancement is dependent on the congruency of the taste and aroma stimulus (Frank and Byram 1988) (Schifferstein and Verlegh 1996) (Stevenson, Prescott et al. 1999). Stevenson (1999) found that certain odours enhanced the tasted sweetness when added to sucrose solutions whilst others suppressed it. The degree to which an odour smelled sweet was the best predictor of taste ratings. In a further experiment, the sweetest smelling odour, caramel, was found to suppress the sourness of citric acid but enhance the sweetness of sucrose. Odours with low sweetness suppressed the sweetness of sucrose. This study demonstrated that the effects of odours on taste perception were not limited to sweetness enhancement but also applied to sour tastes. The results could not be explained in terms of dumping (which is an asymmetric response) or taste-aroma confusion, but were consistent with an explanation of the taste properties of odours in terms of cognitive interaction mediated by prior flavour-taste associations.

Little is known about how odour information is coded although the influences of selective attention and learned associations have been demonstrated (Engen, Gilmore et al. 1991). The task of processing olfactory information often involves integrating the odour with additional information. Through such processing of
odour, meaningful associations between odour and taste may be encoded, which subsequently influence taste odour interactions.

Stevenson and co-workers (1995, 1998 and 1999) demonstrated that the perceived sweetness or sourness of previously unfamiliar aroma compounds could be influenced by continual presentation of the aromas with sweet or sour tastes.

The temporal dimension of flavour release was considered to be an important factor in determining the level of interaction in a recent study by Davidson et al (Davidson, Hollowood et al. 1999). He reported that a decrease in perception of mint flavour correlated closely with decrease in sugar release from chewing gum despite constant delivery of mint volatiles to the nasal receptors, which he concluded to be evidence of interaction. However, in subsequent work with the same panel, he found that data recording the intensity of mint and sweetness in simple solutions showed no evidence of interaction between the two

More recently, Dalton and co-workers (Dalton, Doolittle et al. 2000) have shown that tastes and smells interact positively, even when their respective intensities are below absolute detection. Thus, a sub-threshold taste (sodium saccharin) and a sub-threshold odour (benzaldehyde) were detected when presented together at approximately 63% of their individual detection thresholds. These data suggest direct neural integration of the two modalities rather than the intentional or cognitive mechanisms engaged with supra-threshold stimuli.

1.4.2 Making perceptual measurements

As with all experimental studies, meaningful conclusions are dependent on the validity, accuracy and precision of the experimental data; this is no less true of sensory studies where assessors are used as the measuring instruments. The careful selection and training of panellists can be a lengthy process.
Trained panellists learn to focus attention on a specific part of the stimulus perception, a skill that many ordinary consumers do not have. In keeping with the discussion of aroma taste interaction, Bingham and co-workers (Bingham, Birch et al. 1990) showed that the ability of the odourant maltol to affect the sweetness of sucrose was dependent upon the degree of training the panel had undergone. Trained panellists did not perceive the sucrose-maltol solutions to be significantly sweeter than sucrose solutions whereas the untrained panellists did.

Another aim of this project was to study the effect of composition on flavour perception with consideration of physical or cognitive interaction. Any investigation of this type would require good quality sensory data and clear experimental design. A comprehensive account of the recruitment, selection and training of the sensory panel used throughout this thesis is given in Chapter 2.

1.4.2.1 Scales for rating perception
Scaling techniques involve the use of numbers or words to express the intensity of perceived attributes. The validity and reliability of a scaling technique is highly dependent upon:

- The selection of a scaling technique that is broad enough to encompass the full range of parameter intensities, and also has enough discrete points to record small differences in intensity between samples.
- The degree to which the panel has been taught to associate a particular sensation with the attribute being scaled
- The degree to which the panel has been trained to use the scale in the same way across all samples and across time.

For a comprehensive review of scaling techniques see (Meilgaard, Civille et al. 1999) (Lawless and Heymann 1998) (Stone and Sidel 1985).
1.4.2.1.1 Magnitude estimation

Magnitude estimation (Moskowitz 1977) is a scaling technique based on Stevens Law. The first sample the panellist receives is assigned a freely chosen number. The number can be assigned by the experimenter, in which case it is referred to as a modulus, or chosen by the panellist. Subsequent samples are rated in proportion to the first sample. Alternatively, subsequent samples can be rated in relation to the one immediately preceding it.

The data produced by magnitude estimation have ratio properties, this solves the problems associated with panellist’s reluctance to use scale ends (Pangborn 1984). A criticism of this technique is that the panel requires a good deal of training in order to produce data that are stable and reproducible. Furthermore, individuals tend to score in blocks of 5’s or 10’s thus negating the true ratio properties of the data.

1.4.2.1.2 Time intensity

Perception of sensory attributes in food is a dynamic, not a static, phenomenon. Typically, classical sensory evaluation quantifies the sensory response using a uni-point measurement. Judges must time-average or integrate their responses to provide single intensity values (Lee and Pangborn 1986). Time intensity (Ti) sensory evaluation is an extension of the classical scaling method providing temporal information about perceived sensations. By having judges continually monitoring their perceived sensations, from onset through extinction, one is able to quantify the continual perceptual changes that occur in a specified attribute. For a comprehensive review of the development of time intensity see (Cliff and Heymann 1993).

A common practice in analysing time intensity curves is to extract certain key parameters from the curve shape e.g. maximum intensity (Imax), time to reach maximum intensity (Tmax), rate of onset of flavour perception etc (Cliff and Heymann 1993). Individual differences between judges arising from differences in anatomy, oral manipulation and scaling are lost when extracted parameters are analysed using two-factor analysis of variance (Noble, Matysiak et al. 1991).
In an attempt to fully characterise the Ti traces, various methods of averaging curves have been reported. Initially curves were averaged at given times and the mean values connected, but as all judge scores (including atypical results) contributed to the mean, this method often produced irregular curve shapes e.g. multiple peaks, peak broadening and tailing (Overbosch, Vandenenden et al. 1986).

To alleviate this problem, Overbosch and co-workers normalised the data in the intensity dimension and calculated averages in the time dimension for the ascending and descending portions of the curve. Using this method the resultant average curve contains the mean values for Imax and Tmax, thus being more indicative of the typical response (Overbosch, Vandenenden et al. 1986).

However, the Overbosch method did not make provision for plateaus or stable sections because each intensity must correspond to only one time (Liu and MacFie 1990). Liu and MacFie proposed normalising Ti curves in both intensity and time dimensions, and then averaging the intensity values for ascending and descending portions at fixed time points. Using this method the main parameters for the resulting curve are the averages of the corresponding parameter on the individual curves.

An alternative to curve averaging was proposed by Dijksterhuis (Dijksterhuis and Eilers 1997). The ‘projected prototype curve model’ was based on the assumption of an underlying smooth curve, which was projected onto the data. Garrido and co-workers proposed a parametric model in which intensity is described by a continuous function of time. Using this model, an average curve may be calculated from mean parameters derived from adjusting the individual Ti curves to the model (Garrido, Calvino et al. 2001).

Several methods have been proposed for analysing Ti curves using multivariate statistical techniques. These allow analysis of the variation in response patterns.
attributable to individual judges (van Buuren 1992) (Piggott, Hunter et al. 2000). An excellent comparison of Principal Component Analysis (PCA) of Ti curves is given by (Dijksterhuis, Flipsen et al. 1994).

1.5 AIMS

The initial aim of this thesis was to recruit and train a sensory panel, which could then be used to study the relationship between volatile flavour release and perception in model food systems.

The subsequent aim was to generate data on flavour release, initially from simple solutions, to validate models proposed by Harrison and Hills describing the transport of aroma compounds (with different physicochemical properties) from the oral cavity to the nasal epithelia. The validated model was to be used in a computer program designed to predict aroma release from foods.

Further work was to be undertaken investigating the relationship between flavour release and perception from two model food systems. Studies from gelatine gel samples would provide temporal data and allow investigation into variation among assessors due to differences in anatomy and physiology. Whilst studies of thickened solutions would investigate the interactions between viscosity, non-volatile and volatile flavour.
2.0 PANEL RECRUITMENT, SELECTION AND TRAINING

Sensory analysis provides a means of investigating the relationship between stimulus and perception. A trained sensory panel is the fundamental measuring device for performing objective sensory techniques. To produce good quality data the individual assessors and the panel need to be accurate and precise in their measurements (Piggott 1995). In other words, scores from individual panelists should tend toward the panel average, which itself should be valid and close to the ‘true’ value (accuracy). Replicate values for a single product and attribute should be the same for individual panelists and for replicated panel averages (precision).

Whilst training and experience are key to improving accuracy and precision, the purpose of recruitment and selection is to gather individuals who demonstrate qualities important to a specific project and who show the greatest potential for training.

2.1 RECRUITMENT

Applicants for the sensory panel were recruited from an advertisement in the local paper. Initially, they were screened from responses to a questionnaire (Appendix 1). These responses ruled out candidates who were, not available at particular times, had a physical condition that might impair their sensory ability or disliked too many foods. The questionnaire also collected information which, whilst not excluding them from the next stages of selection, might affect their ability e.g. smoking or age (Meilgaard, Civille et al. 1991) (Issanchou, Lesschaeve et al. 1995).
2.2 SELECTION – SCREENING TESTS

Selection of panel members depends on their performance in a series of screening tests, which generally fall into three categories (Meilgaard, Civille et al. 1991):

- to determine sensory impairment e.g. Ishihara colour blind test.
- to determine sensory acuity and discriminative ability e.g. threshold screening and difference tests.
- to determine ability to communicate intensity and character e.g. descriptive work and use of simple scales.

The tests should be as varied as possible. There is no evidence to suggest that individuals demonstrating good sensory acuity alone go on to become suitable sensory assessors (Jellinek 1985). If possible, they should reflect intended methodologies and sample types as this provides a more accurate indication of potential (Issanchou, Lesschaeve et al. 1995) (Meilgaard, Civille et al. 1991).

For the purpose of this thesis the panel were expected to consume only model food systems (simple solutions, thickened solutions and gels) and record their perceptions using time intensity or magnitude estimation methodologies. Consequently, the selection activities not only tested individual’s sensory acuity, but also their ability to describe a stimulus and to discriminate between different concentrations. Particular emphasis was placed on ranking and scaling activities. Additional selection criteria were:

- availability
- interest and motivation
- the ability to follow instructions
- the ability to concentrate on a given task
- confidence in their own ability
- the ability to work well individually and as a group
- appropriate personality
When briefing the candidates, the word ‘activity’ was used rather than ‘test’. Individuals can become anxious and perform badly if they believe their answers will be judged as right or wrong (Stone and Sidel 1985). The candidates were encouraged to write as much as possible and told that all responses provided valuable information about their sensitivity and discriminative ability.

2.2.1 Basic taste identification and matching (activity 1)
Candidates were presented with a series of basic taste solutions at concentrations well above threshold (sucrose 16g/l, citric acid 1g/l, caffeine 0.5g/l, and sodium chloride 5g/l), and asked to familiarise themselves with and describe each solution. They were subsequently presented with a second series of ten samples to describe, and asked to match each one to an original sample. Duplicate and blank samples were also included. Credit was given for each correctly described and/or matched sample. The maximum total score was 18.

This activity tested a candidate’s ability to recognise and clearly describe basic tastes. It also tested their confidence in recognition, their ability to concentrate and to follow instructions. Following International Standard guidelines (ISO 8586-1), assessors would be expected to achieve >80% of the total marks, however, it is important to exercise personal judgement over borderline results in just one activity, as individuals may demonstrate excellence in other areas.

2.2.2 Magnitude estimation (activity 2)
Candidates were presented with a series of different shapes; each one had a different fraction of its area shaded. They were asked to visually determine what portion was shaded, ¼ or ½ etc., and to record their answer by placing a vertical mark on a 10cm graphic line scale, with verbal anchors at each end representing ‘no shading’ and ‘full shading’.

This activity tested an individual’s ability to make relative measurements of shaded areas and to translate that into a different type of information i.e. a distance along the
scale. This mirrors the type of translation of information necessary to convert one's own perception of flavour intensity into a score (magnitude estimation) or the movement of a lever (time intensity). Typically a result ± 20% of the true value was considered to be satisfactory. One point was allocated to each mark less than 0.5mm away from the true result. Additional points were available for answers in the correct order. The maximum total score was 20.

2.2.3 Odour identification (activity 3)
Candidates were asked to gently sniff the contents of six glass bottles containing different odour volatiles and identify and/or describe what they perceived. The compounds represented characteristic common food aromas appropriate to this thesis. One or a half point was allocated to the responses listed in Table 2.1. The maximum total score was 6.

Table 2.1: Correct description of compounds used for odour identification screening test. This is not an exhaustive list but a summary of acceptable responses given by candidates.

<table>
<thead>
<tr>
<th>Compound</th>
<th>One point</th>
<th>Half Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>Marzipan</td>
<td>Sweet</td>
</tr>
<tr>
<td></td>
<td>Almonds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyanide</td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>Lemon</td>
<td>Fruity</td>
</tr>
<tr>
<td></td>
<td>Orange</td>
<td>Sweet</td>
</tr>
<tr>
<td></td>
<td>Citrus</td>
<td></td>
</tr>
<tr>
<td>Octan-3-ol</td>
<td>Mushroom</td>
<td>Damp</td>
</tr>
<tr>
<td></td>
<td>Mouldy</td>
<td>Fusty</td>
</tr>
<tr>
<td></td>
<td>Earthy</td>
<td></td>
</tr>
<tr>
<td>Menthone</td>
<td>Minty</td>
<td>Spearmint</td>
</tr>
<tr>
<td></td>
<td>Peppermint</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>Cloves</td>
<td>Dentist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medicine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antiseptic</td>
</tr>
<tr>
<td>Anethole</td>
<td>Aniseed</td>
<td>Gobstoppers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spicy</td>
</tr>
</tbody>
</table>
This activity demonstrated a panelist’s ability to detect and correctly identify or
describe odour. It was very important that assessors made some attempt at a
description. A minimum score of 80 % was considered to be satisfactory.

2.2.4 Ranking test (activity 4)
Candidates were randomly presented with five samples of citric acid solution at
concentrations (1.0 g/l, 0.34 g/l, 0.22 g/l, 0.15 g/l, 0.1 g/l, water) and asked to
arrange them in increasing order of intensity.

This activity tested their ability to discriminate between different concentrations and
correctly order samples from weak to strong intensity. It was considered satisfactory
to have one pair of adjacent solutions transposed, this related to a minimum score of
80%.

2.2.5 Magnitude estimation (activity 5)
Candidates were presented with a series of five orange drinks at various dilutions.
One sample was designated as a reference and given an arbitrary score of 20. They
were asked to familiarise themselves with the reference (20) and then to score each
sample in relation to that reference. The colour of the orange drink was disguised
with red food colouring.

In common with activity 2, this tested an individual’s ability to make relative
measurements, only this time using a complex flavour rather than a visual stimulus.
It also introduced candidates to methodology that would be used frequently during
future experiments. The tolerance applied to this score was ± 20%; credit was also
given for drinks arranged in the correct order of increasing flavour intensity.
Maximum total score was 20.
2.3 TRAINING

2.3.1 Methodology
Assessors were trained to use the different methodologies employed during this thesis, namely Time Intensity (Ti), MS Nose™ (Micromass, Manchester, UK) and magnitude estimation. At this stage, training focused on the first two and in particular, simultaneous use of both methods. The panel was already familiar with the procedure for magnitude estimation (see Section 1.4.2.1.1).

2.3.1.1 Time intensity
Time intensity is a more complex sensory technique, which involves rating the change in perceived intensity of a stimulus in real time. It yields a series of values with a temporal dimension, unlike traditional methods which integrate the changing flavour profile into one single rating or score (see Section 1.4.2.1.2).

When recruiting the panel there were no training guidelines or procedures available for time intensity. In this instance, training was adapted from two papers by Sjostrom (Sjostrom 1954) and Jellinek (Jellinek 1964), which related back to when Ti was in its infancy and methods of measuring perception over time were just being developed.

In this simple procedure, assessors timed the eating of a sample and placed a cross on a grid (Figure 2.1) to represent the relative change in flavour every 15 seconds, or at shorter time intervals as they gained confidence. Several different foodstuffs were used (mints, mint chewing gum, wine gums, strawberry bubble gum) and replicate results were collected for each assessor. Samples were tested randomly and results collected after each sample to avoid unnecessary bias.

As their confidence grew, assessors were asked to mentally isolate different aspects of each eating event, namely:
• the onset of perceived flavour
• the rate of increase in flavour intensity in the mouth
• the maximum intensity of flavour
• the duration of maximum intensity
• the time at which the flavour intensity begins to fall
• the rate of decrease of flavour intensity in the mouth
• the time at which flavour is no longer perceived

![Time intensity grid used for assessor training.](image)

**Figure 2.1**: Time intensity grid used for assessor training. On the x-axis each square represents a 15-second interval, on the y-axis perceived intensity is rated from 1-10 (arbitrary units)

In a second session, assessors repeated the Ti exercise, only this time instead of drawing on a paper grid they moved a pivoting lever set against a ten-point scale (Figure 2.2). The positioning of the lever on the scale and the speed of movement corresponded to the changing flavour intensity in mouth.
The lever was attached to a 9-volt battery and performed as a potentiometer allowing more or less current to flow, dependent upon its movement. The output from the lever was interfaced to a computer; the electrical signal was converted to a trace, which showed perceived changes in flavour intensity in real time.

**Figure 2.2: Time intensity apparatus.** The lever pivots across the scale allowing changes in perceived intensity to be recorded. The movement of the lever translates into an electrical signal, which is recorded as a time intensity curve.

### 2.3.1.2 MS Nose™

MS Nose™ uses Atmospheric Pressure Chemical Ionisation - Mass Spectroscopy (APCI-MS) to measure the release of volatile compounds from samples in vitro (headspace) and in vivo (during eating) (see Section 1.2.1). During eating, exhaled air is introduced into the MS Nose™ by breathing through a plastic nosepiece. A small quantity of exhaled air is sampled, and the volatile compounds present are ionised and detected on the basis of their characteristic ion masses (MH⁺). For certain studies, volatile release is measured at the same time as performing the sensory assessment and, therefore, it is vital that the process does not impact on the perceptual measurements.
Initially assessors were asked to breathe through the nosepiece without eating. They were left to relax for at least 5 minutes, during which time the level of acetone was monitored on their breath. Acetone is a natural breakdown product of digestion and can act as a marker to assess regular breathing patterns (Figure 2.3). Evenly spaced peaks would indicate that the subject was breathing regularly and that they were reasonably relaxed.

![viscosity effect on vol rel 2](image)

**Figure 2.3: Typical acetone release during breathing.** The smooth rhythmic trace indicates relaxed breathing.

At the next stage, panelists practiced eating whilst breathing through the nosepiece. They were instructed to eat normally, swallow at will, keep their breathing relaxed and not to open their mouths during eating, as volatile compounds would be lost. Finally the panel practiced simultaneous MS Nose™ and Ti measurements.
2.3.2 Samples
A principal aim of this thesis was to investigate the effect of matrix on flavour release and perception. Consequently, prior to any study, the panel were trained with samples representing the appropriate matrix type (simple solutions, viscous solutions and gelatine gels). The advantage being that assessor accuracy and precision would be determined within the context of each experiment with no loss of ability or understanding prior to testing. In the same way that high sensitivity is not always an indication of overall ability, successful performance with one product is no guide to success with others (Pal, Sachdeva et al. 1995). However, for improving panel performances, evidence has shown the superiority of product specific training over a more general sensory experience (Chambers IV and Smith 1993).

2.3.2.1 Panel orientation
An integral part of the training on each matrix was to expose the panel to samples, which reflected the full range of sensory experiences likely within the activity. This would be different types of volatile compound or a range of concentrations of volatile, non-volatile or thickener. As part of this orientation, assessors discussed the differences in product attributes and their intensity. Individually they practised rating the intensity of specified attributes until they demonstrated competence with the rating technique and a clear understanding of the defined product attributes.

2.3.2.2 Flavour intensity of mint and sugar solutions
The purpose of this exercise was to determine if the panel could isolate and accurately rate the intensity of a non-volatile and volatile stimulus in a mixed solution, without interference or interaction between the two. Rating a single stimulus from a mixture of two or more is a common feature of sensory-based experiments.

The panel rated the perceived sweetness and mint intensity of several solutions using a sip and swallow procedure. The solutions were made in water and consisted of 0, 25, 50 or 100 parts per million (ppm) (v/v) of menthone (Firmenich SA, Geneva,
Switzerland) with either 0, 1, 2.5 or 5% (w/v) of sucrose, resulting in a total of sixteen different solutions.

The intensity of either sweetness or mintyness was rated for each sample using a 10cm graphic line scale with verbal anchors ‘none’ and ‘very’ at left and right scale extremes respectively. Assessors were given two references, marked at the centre point of each scale, against which the sweetness or mintyness should be rated (100ppm (v/v) menthone solution and 5% (w/v) sucrose solution). Sweetness and mintyness were assessed in separate sessions. Samples were presented in sets of three, in a balanced random order; each marked with a three-digit code. Assessors had a break of 15 minutes between each set of samples. Plain crackers and water were used as palate cleansers.

### 2.3.2.3 Flavour intensity of minty sweet gels

The purpose of this training activity was to practice magnitude estimation and to prepare the panel for time intensity assessment of mint gel samples.

#### 2.3.2.3.1 Magnitude estimation

The panel used magnitude estimation to rate the intensity of mint flavour in a series of sweet gelatine gels.

The base gel mixture was prepared using 30% granulated sugar, 33% water, 30% glucose syrup, 6% gelatine (Type A – US mesh 20, 250 bloom), and 1% citric acid. All quantities were on a w/w basis. The gelatine was hydrated, dissolved at 60°C in a water bath and then added to the sugar and glucose solution (which had been previously boiled and cooled to below 100°C). The molten gel mixture was cooled to 60°C and mixed with quantities of carvone (Firmenich SA, Geneva, Switzerland) (dispersed in propylene glycol) to give final volatile concentrations of 125, 250, 500, 750 and 1000 ppm (mg/kg).
When set, the gel was cut into individual cubes weighing 6g +/- 1g. Samples were stored at 4°C but allowed to equilibrate to room temperature (18-20°C) prior to eating.

Each of the five concentrations was tested in triplicate. The fifteen samples were presented in sets of three in a balanced order of presentation; each labelled with a three-digit code. A reference gel sample (500ppm) was also presented and given an arbitrary score of 100. Assessors were asked to rate the mint intensity of each sample relative to the reference. Mineral water and plain crackers were used as palate cleansers.

This procedure assessed the accuracy and precision of the panel in assessing mint flavour in a complex medium.

2.3.2.3.2 Time intensity
The intensity of mint flavour in gel samples containing 250ppm and 1000ppm was assessed using time intensity. Panellists consumed triplicate samples of each concentration during a three-hour session. Samples were presented randomly with a gap of 15 minutes between each. Mineral water and plain crackers were used as palate cleansers. Time intensity measurements were made using the lever apparatus described in section 2.3.1.1.

2.3.2.3 Almond flavour and sweetness intensity in viscous solutions.
To prepare for a study investigating the effect of viscosity on flavour perception, the panel was trained to use magnitude estimation to rate the intensity of almond flavour and sweetness in solutions thickened with hydroxy propyl methylcellulose (HPMC).

Solutions were prepared containing 10, 55, 75, 100 and 200ppm benzaldehyde (Firmenich SA, Geneva, Switzerland). Each sample contained 0.6% (w/v) HPMC (Methocel, DOW Germany) and 5% (w/v) sucrose (Tate and Lyle). Appropriate quantities of distilled water and sucrose were weighed into a beaker and heated to
55-60°C. The hot sucrose solution was stirred, without turbulence, using a motorised paddle and the HPMC powder carefully added to the side of the vortex. The solution was then cooled, with continual stirring, to 4°C. A flavour concentrate was prepared by mixing 600μl of benzaldehyde with 200μl of carmoisine food colour in a 10ml volumetric flask and making up to volume with 100% absolute ethanol. The flavour concentrate was added to a pre-weighed quantity of the cooled viscous solution to achieve the final concentrations listed above. Samples were mixed using a roller bed (SRT2 - Stuart Scientific, Redhill, U.K.) for 6-10 hours, prior to ingestion by the panel. The carmoisine acted as a marker for complete mixing.

Assessors rated the perceived almond intensity of each sample relative to a reference sample (100ppm benzaldehyde, 5% sucrose, 0.6% HPMC). Samples (labelled with a three-digit code) were presented in sets of three with a break of 15 minutes between each set. The design was balanced and each concentration was presented in duplicate to each assessor.

A further two sets of solutions were prepared, each one containing 1, 2, 3, 4.5, 5, 6.5 and 8% (w/w) sucrose. One set of samples contained thickener (0.6% HPMC) and the other thickener (0.6% HPMC) and volatile flavour (100ppm benzaldehyde). Samples were prepared using the method described above. Assessors rated the perceived sweetness intensity of each sample relative to a reference (either 5% sucrose, 0.6% HPMC or 5% sucrose, 0.6% HPMC, 100ppm benzaldehyde). The experimental design was identical to the one described above.

2.4 RESULTS

2.4.1 Screening tests
The results of the screening tests were calculated as a percentage of the total available score for each candidate. The candidates with the best individual scores are shown in Table 2.2. When analysing the results it was important to consider all activities, no individual was rejected on the basis of one single score. For example,
Panel recruitment, selection and training

candidates 5 and 6 scored badly on activity 4, whereas candidate 15 scored badly on activity 3. In each case, their overall performance outweighed the individual discrepancy. Individuals may be nervous about their performance in what they perceive to be ‘tests’.

All candidates had a short interview to give feedback on the results of the activities and to discuss their strengths or weaknesses. This was also an opportunity to repeat activities or attempt further work.

**Table 2.2: Results for selection activities.** The candidate score for each activity is shown as a percentage of the total available. Only results for the best 19 candidates are displayed.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Activity 1</th>
<th>Activity 2</th>
<th>Activity 3</th>
<th>Activity 4</th>
<th>Activity 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gill Blowers</td>
<td>89</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Nina Gooch</td>
<td>100</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Sally Slaney</td>
<td>89</td>
<td>65</td>
<td>67</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>Kay Gooch</td>
<td>89</td>
<td>75</td>
<td>67</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>Carl Burton</td>
<td>89</td>
<td>65</td>
<td>92</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>Tracy Marsh</td>
<td>89</td>
<td>65</td>
<td>75</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>John Barker</td>
<td>78</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>Diane Henson</td>
<td>83</td>
<td>70</td>
<td>83</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>Joyce Burton</td>
<td>89</td>
<td>75</td>
<td>83</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>David Grierson</td>
<td>69</td>
<td>80</td>
<td>50</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>Stuart Pratt</td>
<td>89</td>
<td>80</td>
<td>92</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>Carole Hackney</td>
<td>89</td>
<td>80</td>
<td>83</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>Terry Finch</td>
<td>89</td>
<td>85</td>
<td>75</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>14</td>
<td>Mike Lord</td>
<td>89</td>
<td>75</td>
<td>67</td>
<td>80</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>Maureen Lord</td>
<td>72</td>
<td>80</td>
<td>42</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>16</td>
<td>Clare Hansen</td>
<td>78</td>
<td>65</td>
<td>75</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>17</td>
<td>Susan Sanderson</td>
<td>100</td>
<td>75</td>
<td>75</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td>18</td>
<td>Dorothy Howse</td>
<td>78</td>
<td>55</td>
<td>50</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>19</td>
<td>Kathleen Norman</td>
<td>61</td>
<td>65</td>
<td>50</td>
<td>80</td>
<td>40</td>
</tr>
</tbody>
</table>

An overall score was calculated as the average percentage across all five activities (Figure 2.3). Candidates 1-17 were all selected for training. Those with a combined score of > 80% were automatically selected, whereas 3, 4, 5, 6, 10, 14, 15 and 16
performed particularly well at certain activities and showed confidence, knowledge and enthusiasm during interview. Candidates 18 and 19, however, performed badly in the selection activities, showed little enthusiasm during the interview and did not wish to repeat any of the activities.

![Graph showing overall scores for screening activities.](image)

**Figure 2.3: Overall score for screening activities.** Overall score is the average percentage score over 5 activities. Results for the top 19 candidates are displayed.

### 2.4.2 Training on methodology

#### 2.4.2.1 Time intensity training

The simple procedure of marking perceived intensity on a grid every 15 seconds proved very useful in allowing the assessors to become accustomed to the technique. Figures 2.4 and 2.5 are examples of the replicate data from individual assessors. The maximum intensity (Imax), time to maximum intensity (Tmax) and rates of increase and decrease in perceived intensity are consistent for the three replicates.
Where assessors struggled to be consistent, the time intensity curve was broken down into separate stages so that one aspect of the flavour profile was considered at any one time e.g. onset of flavour, time of maximum intensity, duration of maximum intensity etc. Once an assessor was confident with one aspect of the profile, another was introduced until, finally, they were able to produce consistent data.

**Figure 2.4: Time intensity curves for tic-tac mints.** Replicate results for assessor 10 rating perceived intensity of mint flavour at 15-second intervals.
Figure 2.5: Time intensity curves for strawberry chewing gum. Replicate results for assessor 7 rating perceived intensity of strawberry flavour at 15-second intervals.

2.4.2.2 Simultaneous Ti and MS Nose™

The panelists quickly adapted to the unusual laboratory environment and were able to demonstrate regular breathing when using the MS Nose™. Some found the procedure mentally demanding and the whole group felt that they would benefit from more experience. Assessors commented that it was hard not to think too much about their breathing and chewing, making it difficult to be relaxed. Despite these comments, the practice session yielded reasonably consistent data.

2.4.3 Training on samples

A review of the literature reveals many multivariate and univariate methods for assessing panel performance and reliability. Multivariate methods tend to be more complicated as they treat all variables simultaneously (Sinesio, Risvik et al. 1990), (Arnold and Williams 1986). They analyse the relationship among variables and are commonly used to monitor panel use of descriptive vocabulary in traditional profiling methods.
By comparison, other methods focus on each attribute separately. These are often easier to interpret and yield much more information about the interaction between assessors, attributes and use of scaling techniques (Naes and Solheim 1991), (Hirst and Naes 1994), (Dijksterhuis 1995), (Naes 1998).

One of the simplest techniques for assessing individual performance is one-way analysis of variance (AoV) of replicate data for each panellist. The mean square error (MSE) measures their repeatability i.e. how close are the replicate data, and the probability value (p) describes their discriminative ability i.e. the probability that at least two samples are significantly different (Lea, Rodbotten et al. 1995) (Naes 1998). A plot of MSE against p-value provides an easy illustration of panelist performance.

Graphical methods are an excellent means of displaying performance data, particularly for large data sets. One popular method is the ‘egg shell plot’ (Naes 1998) in which data for each panelist is converted into cumulative rank scores. The cumulative values for each assessor are plotted against the true ranks (or panel consensus) and compared to the cumulative scores for the true ranks. Individuals not conforming to the cumulative scores for the true ranks are easily identified from the shape of their plot (shaped like the top of a broken eggshell).

Alternatively, methods have been proposed that draw analogies from the repeatability and reproducibility assessments of inter- and intra-laboratory variability in analytical measurement (Rossi 2001). Formulas for calculating repeatability (Equation 2.1) and reproducibility (Equation 2.2) are based on publications from the National Bureau of Standards (Mandel 1991).

Repeatability \( h_{ij} \) \[= \frac{S_{ij}}{S_{i}} \] (2.1)
Reproducibility \( (K_{ij}) \) = \frac{y_{ij} - x_j}{S_{ij}} \hspace{1cm} (2.2)

Where:  
\( S_{ij} \) = standard deviation of the replicate scores for each assessor
\( S_j \) = pooled standard deviation (square root of pooled variance)
\( y_{ij} \) = mean of replicates scores from each assessor for each sample
\( x_j \) = mean of individual assessor means for each sample
\( S_{yj} \) = standard deviation of the assessor means for each sample

The results can be displayed graphically, however, the plots become too numerous when the number of samples and attributes are large. In this instance displaying the raw data would be of equal value.

Graphical methods are often criticised as hard to interpret when large numbers of assessors and attributes are analysed. A simpler method, proposed by Dijksterhuis (Dijksterhuis 1995), involved calculating a consonance index for each attribute, indicating the degree of panel consensus (unidimensionality). The index value is calculated from the first eigenvalue, which is larger when the panel use an attribute in the same way. Scree plots and consonance indices are used to identify attributes with least unidimensionality. For these attributes, the loading plots of assessors will identify individuals that do not concur with the rest of the panel.

Calculating the interaction between assessor and product is another important factor in panel performance. Interaction can result from either, the assessors rating the products differently i.e. in a different order, or, not perceiving the same differences between products (Couronne 1997). Analysis of variance (AoV) of replicate data will indicate if a significant interaction exists, however, this alone will not identify which panelists are responsible. Multiple comparison tests or examination of the raw data will provide more information or, alternatively, the interaction can be examined using Principal Component Analysis (PCA). In this instance the product x assessor
matrix is centred to remove both assessor and product effects. Loading plots display only differences in assessors due to interaction. Those contributing most to the interaction lie furthest from the origin and sub groups of the panel are identified as discrete clusters. The origin of the interaction can also be determined from comparison with a non standardised PCA in which the product effect and interaction are preserved (Couronne 1997).

In a recent study, Peyvieux and Dijksterhuis (Peyvieux and Dijksterhuis 2001) proposed a method for training a time intensity panel. To monitor panel performance they used loading plots from PCA of non-centered data to illustrate the repeatability of Ti curves from individual assessors.

For the purpose of this thesis, simple techniques based on AoV were used to assess panellist and panel performance. Wherever possible, assessor precision was measured by the MSE from one-way AoV of their replicate data, whereas discriminating ability was determined from their p-value (Naes 1998), (Lea, Rodbotten et al. 1995). Interactions between assessors and products were determined from two-way AoV. Overall panel accuracy was illustrated from plots comparing the panel mean values with the stimulus concentration including error bars for standard deviation.

2.4.3.1 Assessing sweetness and mintyness in simple solutions

Results from the graphic scales were converted into scores for each sample by measuring the distance (mm) from the left-hand anchor to the vertical mark. Mean panel scores of perceived sweetness intensity compared well with the concentration of sucrose in simple solutions (Figure 2.6).
Figure 2.6: The effect of menthone on the perceived sweetness of solutions containing increasing concentrations of sucrose. Data are the mean scores of 17 assessors and error bars show the standard deviation (SD) for the panel. ν = 0ppm menthone; ν = 25ppm menthone; ν = 50ppm menthone; □ = 100ppm menthone.

The sample containing 5% sucrose (equivalent to the reference value of 50) had a mean value of 58. Relative sample scores followed the relative concentrations of sucrose e.g. mean scores for 2.5% and 1% sucrose solution were 28 and 9 respectively. Increasing concentrations of menthone in solution had no significant effect on the perceived sweetness. Standard deviation (error bars) showed a high level of variation in the panel scores particularly at the highest sucrose concentration. Standard deviation was greater for samples containing 100ppm menthone as the stronger mint aroma made it more difficult to assess the sweetness concentration.
Figure 2.7: The effect of sucrose on the perceived mint intensity of solutions containing increasing concentrations of menthone. Data are the mean scores of 17 assessors and error bars show the standard deviation (SD) for the panel. ν = 0% sucrose; ν = 1% sucrose; ν = 2.5% sucrose; □ = 5% sucrose.

Similarly, mean panel scores of perceived mint intensity compared well with the concentration of menthone in simple solutions (Figure 2.7). The sample containing 100ppm menthone (equivalent to the reference value of 50) had a mean value of 54. Relative sample scores followed the relative concentrations of menthone e.g. mean scores for 50ppm and 25ppm menthone solutions were 32 and 12 respectively. Increasing concentrations of sucrose in solution had no significant effect on the perceived mintyness. Standard deviation (error bars) showed a high level of variation in the panel scores particularly at the highest menthone concentration. A criticism of magnitude estimation is that assessors do not always produce true ratio data, particularly at high concentrations of a stimulus.
Despite the variation in individual scores, the panellists were able to distinguish between sweetness and mintyness in simple solutions irrespective of the relative concentrations of sucrose and menthone.

2.4.3.2 Flavour intensity of minty sweet gels

2.4.3.2.1 Magnitude estimation

One way AoV of assessor data showed that each panellist was able to discriminate between at least two of the gel samples (p <0.001). MSE values of replicate data ranged from 21 to 316, indicating a great degree of variability in the precision of the individual assessors (Figure 2.8).

Assessors numbered 4, 7, 8 and 11 exhibited the highest MSE value. Examination of the raw data revealed that, for 4 and 8, replicate three was very different to the other two. In this instance they may have received samples that differed from the rest of the panel or, even more likely, they may simply have had a bad day. By contrast, assessors 7 and 11 had data that varied across all sample concentrations and replicate number. They were clearly unable to consistently score relative differences in concentration. This may be due to a lack of understanding of mintyness or confusion over which parameter to rate, sweetness or mintyness. Subsequent results from these assessors were monitored closely and, if necessary, removed from the data set.
**Figure 2.8**: The precision of assessors using magnitude estimation to rate the intensity of mint in 6% gelatine gels containing 125, 250, 500, 750, 1000ppm carvone. Samples also contained 30% sucrose, 30% glucose and 1% citric acid. Numbers 1-17 relate to individual assessors; the MSE is from one factor analysis of variance (AoV) of three replicates for each concentrations of carvone.

Despite poor precision of certain assessors, the mean panel results for mintyness correlated well with concentration of the stimulus ($R^2 = 0.98$) (Figure 2.9). Overall the panel were accurate in rating the intensity of mint flavour in gelatine gels.
Figure 2.9: The relationship between perceived mint intensity and concentration of carvone in 6% gelatine gels. Data points are the mean panel score for 17 assessors.

2.4.3.2.2 Time intensity

Triplicate results for each assessor for a single concentration of carvone were analysed using PCA (Peyvieux and Dijksterhuis 2001). The data were non-centered to preserve the individual shape of each curve. Each curve translates to a single point and triplicate results from each assessor were displayed graphically as three points joined together. The closer together the three points (the smaller the triangle created) the more reproducible the results from that assessor. The closer together triangles from each assessor, the more similar the time intensity curves from those assessors.

2.4.3.3 Almond flavour and sweetness intensity in viscous solutions

One way AoV of assessor data showed that each panellist was able to discriminate between the sweetness of at least two of the liquid samples (p < 0.001). Aside from
assessor 8, the MSE values of duplicate data ranged from 5 to 44, indicating a high level precision for individual assessors (Figure 2.10).

Figure 2.10: The precision of assessors using magnitude estimation to rate the intensity of sweetness in sucrose solutions containing 1, 2, 3, 4.5, 5, 6.5, and 8% sucrose. Samples also contained 100ppm benzaldehyde and 0.6% HPMC. Numbers 1-16 relate to individual assessors; the MSE is from one factor analysis of variance (AoV) of two replicates for each concentration of sucrose.

Raw data for assessor 8 showed little consistency between duplicate scores for sweetness, particularly at the low concentrations of sucrose. It may be that this assessor was finding it more difficult to rate the sweetness and, therefore, holding the sample in the mouth for longer. This would result in large variations in sucrose concentration as saliva diluted the sample. The dilution effect would be more marked at lower sucrose concentrations.

One way AoV of almond intensity ratings showed that each panellist was able to discriminate between at least two of the liquid samples (p <0.001). MSE values
ranged from 5 to 118, indicating that individual assessors were not as precise when rating almond intensity as they had been for sweetness (Figure 2.11). Assessors 4 and 11 show very consistent results except for the highest concentration of benzaldehyde whereas, assessors 9 and 10 are generally inconsistent for all concentrations.

**Figure 2.11:** The precision of assessors using magnitude estimation to rate the intensity of almond flavour solutions containing 10, 55, 75, 100 and 200ppm benzaldehyde. Samples also contained 5% sucrose and 0.6% HPMC. Numbers 1-16 relate to individual assessors; the MSE is from one factor analysis of variance (AoV) of two replicates for each concentration of benzaldehyde.

Mean panel scores for sweetness compared well with the intensity of the stimulus (Figure 2.12). The hidden control sample (identical to the reference = 100) had a mean value of 98 and 102 for samples without and with added flavour respectively. The addition of almond flavour had no effect on perceived sweetness intensity in the thickened sucrose solutions. Mean values for each assessor showed very little variation across the panel (indicated by error bars). The highest sucrose concentration, with and without almond flavour, showed the greatest variation in
panel data. As stated previously, this methodology has been shown to deviate from true ratio scores at higher concentrations of a stimulus.

![Graph showing the relationship between perceived sweetness and sucrose concentration]

**Figure 2.12: The relationship between perceived sweetness and the concentration of sucrose in viscous solutions.** Samples also contained 0.6% HPMC and either (ν) 0, or (ν) 100ppm benzaldehyde. Data are the panel average of 16 assessors and the error bars show the standard deviation.

Mean panel results correlated well with the stimulus concentration ($R^2 = 1.00$) (Figure 2.13). Once again panel results show greatest variation at the highest concentration of benzaldehyde.
Overall, the panel showed that they could accurately rate the sweetness and almond flavour intensity in thickened solutions irrespective of the relative concentration of sucrose and benzaldehyde.

2.5 CONCLUSION

In summary, the selection and training of this new panel was a great success. In the first instance to find a group of tasters that satisfied the selection criteria was extremely fortuitous. The new panel performed very well throughout their general training with the strengths and weaknesses of each soon becoming apparent. They received feedback well and accepted comments for improvement.

In preparation for each specific experiment, the panel displayed reasonable accuracy in rating the intensity of the stimulus when compared to true concentrations,
although the precision of individual assessors varied dependent on the task. To help develop skills and provide experience all panel members were included for each experiment regardless of their performance during training, where individuals yielded inconsistent data for a particular flavour or matrix their results were discarded *a priori*. 
3.0 VOLATILE RELEASE FROM SOLUTIONS

This experiment was undertaken as part of a BBSRC LINK project with industrial partners Nestle (Lausanne and York), Mars UK Ltd and Firmenich SA, and Institute of Food Research (IFR) as one of the academic partners. One of the objectives for the project was to produce a software program, which could predict the release of any flavour molecule from a variety of matrices. Such a software package would have great benefit for the food industry with many applications, not least of which would be to reduce the lead-time for New Product Development (NPD).

The program would be based on fundamental mathematical models, which described all the factors likely to affect flavour release. They included physicochemical properties of volatiles, the effect of matrix composition on volatile release (models describing the release of volatile from different food matrices) and the anatomical and physiological interaction of human beings with food (chewing, breathing, swallowing, mouth volume, saliva production, thickness of mucous layer, dimension of respiratory tract etc.).

Whilst numerous attempts have already been made to describe, mathematically, the release of volatile compounds from solutions (see Section 1.2.4.2 for review) very little work has been done to model the transport of flavour molecules from the mouth to the nasal epithelia. One of the criticisms directed at mechanistic models is the lack of validation with experimental data and the need to estimate parameters that cannot be determined experimentally. For this project, experimental validation was an integral part of the model building process.

This chapter investigated the effect of swallowing, breathing rate and chewing on the release of volatile compounds from the simplest type of food matrix – solutions containing only volatiles and distilled water.
3.1 METHOD

3.1.1 Sample preparation
Two solutions were prepared (A & B), each containing a cocktail of four volatile compounds (see Table 3.1). The volatile compounds were dispersed in propylene glycol (Aldrich, Dorset UK) before being mixed with distilled water to achieve the final concentrations listed below. Solutions were mixed for 6 hours on an orbital shaker and then refrigerated until use (within 24 hours of preparation).

Table 3.1: Composition of solution A and B. All volatiles were supplied by Firmenich SA, Geneva, Switzerland.

<table>
<thead>
<tr>
<th>Volatile</th>
<th>Concentration (ppm)</th>
<th>Volatile</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethole</td>
<td>7.5</td>
<td>Methyl acetate</td>
<td>10</td>
</tr>
<tr>
<td>Menthofuran</td>
<td>7.5</td>
<td>Ethanol</td>
<td>75</td>
</tr>
<tr>
<td>Cymene</td>
<td>7.5</td>
<td>Ethyl butyrate</td>
<td>10</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>7.5</td>
<td>Pyrazine</td>
<td>75</td>
</tr>
</tbody>
</table>

3.1.2 Experimental design
A single assessor consumed 15ml aliquots of either solution A and B according to the following protocol. The solution was placed in the mouth after a normal inhalation. The assessor was instructed to swallow and then to exhale and continue to breathe at a rate of 1.5 breaths per minute (slow), 3 breaths per minute (medium) or 6 breaths per minute (fast) for 2 minutes. The rate of breathing was timed using a stopwatch. The assessor was given time to practice breathing at different rates without markedly affecting the tidal volume. Both solutions were tested in duplicate for each breathing rate.
In a second experiment the assessor held 15ml of solution in the mouth and simulated chewing movements during a single exhalation.

3.1.3 Instrumental analysis

Exhaled air was sampled in real time at a rate of 30ml/min using the MS Nose™ (Micromass, Manchester, UK) (Section 2.3.1.2). Volatile molecules were ionised (4kV-corona discharge) and the release profile followed by monitoring the appropriate molecular ion (MH⁺) (see Table 3.2). The dwell time was 0.05s.

Table 3.2: Molecular ion masses monitored by MS Nose™ from solutions A and B.

<table>
<thead>
<tr>
<th>Volatile compound</th>
<th>Cone voltage</th>
<th>Ion (MH⁺) mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethole</td>
<td>23</td>
<td>149.2</td>
</tr>
<tr>
<td>Menthofuran</td>
<td>20</td>
<td>151.2</td>
</tr>
<tr>
<td>Cymene</td>
<td>20</td>
<td>134.1</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>20</td>
<td>153.2</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>22</td>
<td>75.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>18</td>
<td>47.1</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>18</td>
<td>117.1</td>
</tr>
<tr>
<td>Pyrazine</td>
<td>25</td>
<td>81.1</td>
</tr>
</tbody>
</table>

3.2 RESULTS

3.2.1 The effect of volatile type and breathing rate on aroma delivery.

The highest concentration of volatile in exhaled air occurred directly after swallowing the solution, followed by an exponential decrease over subsequent exhalations. The release profiles from compounds in both solutions A and B showed marked differences (Figure 3.1). Methyl salicylate, anethole, ethanol, menthofuran and pyrazine were detected on the breath up to 2 minutes after swallowing, the latter being the most persistent. Cymene, ethyl butyrate and methyl acetate were undetectable after the first inhalation.
Figure 3.1: Release profiles for volatile compounds on the breath after swallowing. Peaks = exhalation, troughs = inhalation.
In mouth, the volatile compounds are released from the liquid phase into the gas phase and, on swallowing, the aroma-rich air is injected into the breath stream (Taylor 1996). Water-soluble components are adsorbed to a significant extent by the mucous lining the moist tissues of the mouth, nose and throat (Overbosch, Afterof et al. 1991), (Plug and Haring 1994), (Taylor 2002). Persistence of menthone and anethole due to adsorption to the mouth lining was described by Hussein et al (Hussein, Kachikian et al. 1983). Not all the flavour reached the olfactory epithelium in the same ratios as originally released. Absorption during their transport through the upper airway affects their concentration profiles in nose, such that they may be attenuated in magnitude and shifted in time (Overbosch, Afterof et al. 1991).

The persistence of volatile compounds on the breath was studied by Linforth and Taylor (Linthorft and Taylor 2000), using similar methodology to that described in this chapter. A single panellist (to remove person to person variation) consumed forty-one different volatile compounds. Persistence was calculated from the release profile as the ratio of 1st to 2nd peak heights and modelled using QSPR methodology. The validated model (correlation coefficient 0.83) contained four key parameters – hydrophobicity (log of the octanol water partition coefficient), volatility (vapour pressure), ether linkage and carbonyl count.

The rationale given by Linforth and Taylor (2000) is as follows. After swallowing, volatile-rich liquid coated the back of the throat. During the first exhalation the aroma already present in the gas phase was expelled. Compounds that were more water-soluble (low logP) or with low volatility (low vapour pressure) e.g. anethole, pyrazine and ethanol repartitioned into the mucous layer creating a reservoir of volatile for release on subsequent breaths (persistence). The same reservoir did not exist for the more hydrophobic or volatile molecules e.g. cymene, ethyl butyrate and methyl acetate.
Figure 3.2: The effect of breathing rate on the release profile of volatile compounds.
Another interesting feature of the data was the duration of the volatile signal from a single exhalation after swallowing. The more persistent compounds described above were present throughout the entire exhalation. By contrast, the least persistent compounds were released at the very beginning of the exhalation, which suggested that the volatile laden air does not mix with the exhaled air but instead moved as a plug to the nasal receptors (Linthor, Martin et al. 2001). The lack of mixing may have been due to the water vapour present in exhaled air, although hydrophobicity alone does not explain the behaviour of less persistent compounds such as ethyl butyrate, which is readily water soluble but released at the start of the first exhalation. It was more likely, assuming that mixing of air did not occur, that the continued signal during exhalation was due to the compounds with the greatest affinity for adsorption to the mucus layer, partitioning back into the stream of exhaled air as virgin air passed over the thin mucous layer.

An increase in breathing rate caused a minor increase in volatile concentration on the first exhalation (observed as an increase in peak height) (Figure 3.2). This was observed for all compounds, regardless of their subsequent persistence. Assuming that there was no mixing of air from the mouth with exhaled air from the lungs, the initial plug of air should have had the same concentration regardless of the velocity of its delivery. Furthermore, the shape and magnitude of the release profile following the first breath was unaffected by an increase in breathing rate, which suggested that the increase in 1st peak height might be an artefact.

3.2.2 The effect of chewing on aroma delivery
Simulated chewing whilst holding 15ml of sample in the mouth produced minor perturbations in the release profile of the volatile during a single exhalation (Figure 3.3). This was indicative of local turbulence producing short bursts of volatile at a higher concentration. The level of the perturbation was related to the vigour of oral movements. The chewing motion caused the mouth to function as a bellows, injecting flavour into the breath stream (Overbosch, Afterof et al. 1991), (Plug and Haring 1994), (Taylor 2002).
3.2.3 Modelling flavour release from liquids

The release data for the eight volatile compounds from the solutions was modelled by Kevin Wright (IFR – Norwich) (Wright, Hills et al. submitted). The process of flavour mass transfer was modelled using the two-film theory of interfacial mass transfer (Hills and Harrison 1995). The benefit of this approach was that it allowed the effect of breathing to be incorporated into the model. The best fit was obtained by modelling the airflow as a simple oscillatory process using a sine function. Figure 3.4 shows a typical output from the software developed at IFR.

The input boxes on the left-hand side allowed entries for different physiological and anatomical parameters. Separate pages entitled ‘flavour data’ and ‘experimental’ allowed for entry of the physicochemical properties of the molecule and the specific experimental details respectively.
Figure 3.4: Illustration of the software program designed to model flavour release from foods. This page of the program includes input boxes for anatomical and physiological parameters.

The fit of the experimental and modelled data was excellent for all compounds and all replicates but only after the first breath. It was postulated that the amount of aroma in the first breath depended mainly on oral physiology e.g. the way the mouth pumped air into the throat during swallowing. Whereas, thereafter, release was well explained by the mathematical model, which assumes release from the throat, which is coated with the aroma solution.

One of the limitations of the modelling was the lack of reliable data on factors such as air volume on each breath and the dimensions of the throat. The values in Figure 3.4 were obtained as a result of fitting experimental data to the model. However, it is now possible to measure the volume of each individual breath as well as the chewing and swallowing frequency. It would be interesting to repeat the experiments with
these additional data and determine whether this produced a better correlation between experimental and predicted release.

3.3 CONCLUSION
After swallowing, volatile compounds were released into the breath stream. The maximum concentration on the breath occurred with the 1st exhalation after swallowing. Physicochemical properties determined the persistence of the volatile during subsequent breaths. More water-soluble and less volatile compounds interacted with the mucous layer of the nose and throat creating a reservoir of volatile for release during subsequent breaths. Breathing rate did not affect the release profile of the volatile compounds, although there was some evidence to suggest that it caused an increase in the first peak height (first exhalation post swallow). Whilst this finding warranted further study it was, at the time, beyond the scope of this project.

The release data were modelled using penetration theory with a modification to account for breathing and fitting parameters to represent anatomical and physiological measurements. The experimental data were a good fit for the predicted data (apart from the first breath).
4.0 FLAVOUR RELEASE AND PERCEPTION IN GELATINE GELS

Psychophysics is the branch of experimental psychology devoted to the study of relationships between sensory stimuli and human responses (Lawless and Heymann 1998) (Meilgaard, Civille et al. 1999). Not surprisingly, one of the most important aspects of this discipline is investigating the mathematical relationship between stimulus and perception.

Historically, two forms of psychophysical function have been proposed to explain the relationship. Based on the work of Ernst Weber, Fechner’s Law utilises Just Noticeable Difference (JND) as a means of measuring the strength of sensation. The magnitude of the JND is dependent on the original concentration in the sample. Category scaling techniques, commonplace in sensory analysis, support the theory of Fechner’s Law.

By contrast Stevens Law states that the magnitude of perceived sensation grows as a power function of stimulus intensity (Stevens 1957). Stevens proposed that only ratio scales are valid for the measurement of perceived sensation (Stevens 1970). Despite considerable popularity, both methods suffer the disadvantage of ignoring the temporal dimension and, instead, relating a single stimulus to a single perceptual measurement; this takes no account of the effect of preceding stimuli on subsequent ones. In the same way, traditional sensory methods reduce the profile of perception to a single measurement, which is explained in terms of the original concentration in the sample consumed. For a comprehensive review of psychophysical theory see Lawless and Heyman (1998).

Overbosch modified psychophysical law to take account of a temporal dimension, which he proposed was due to adaptation (Overbosch 1986) (Overbosch and Dejong 1989). He concluded that, if the receptors were subjected to a constant level of stimulation then the response would decrease with time until eventually none was
perceived. The mathematical model calculated the adaptive process and subtracted it from the applied stimulus to obtain the actual signal that triggered the receptor.

The development of new techniques has allowed us to measure the changing stimulus presented to the nasal receptors during the consumption of food, rather than using the original sample concentration (see section 1.2.1). Similarly, time intensity studies have allowed us to measure the changing perception throughout the eating event. Together these techniques have provided a powerful tool for investigating and understanding the relationship between stimulus and perception.

Unfortunately, to date, the means of analysing Ti curves have focused on producing an average curve which is considered the most useful way of understanding underlying trends and causal relationships (Overbosch, Vandenenden et al. 1986) (MacFie and Liu 1992) (Liu and MacFie 1990) (Garrido, Calvino et al. 2001). However, each individual assessor will give a unique signature, which relates their perception to the stimulus delivered to their receptors. This embodies all the physiological variables that are difficult or impossible to measure. Whilst methods of averaging Ti curves are now more sophisticated than earlier attempts, in that they ascribe more weight to common curve shapes, still a great deal of information can be lost.

This study adopted a simple approach to analysing volatile release and time intensity curves for individuals and tried to explain, in terms of some extracted parameters, the differences that existed within a panel and the effect they may have on the relationship between stimulus and perception. The effect of concentration on release and perception was studied for a single volatile and the effect of volatile type was studied for five volatile compounds with very different physicochemical properties.

Gel systems were studied as they provide an excellent matrix for flavour release with the maximum signal intensity occurring anything up to two minutes after the start of eating.
4.1 METHOD

4.1.1 Volatile release and perception from mint flavoured gelatine gels

4.1.1.1 Sample preparation
A base gel mixture was prepared using 30% granulated sugar (Tate and Lyle), 33% water, 30% glucose syrup (Cerestar, UK), 6% gelatine (Type A – US mesh 20, 250 bloom – Firmenich SA, Geneva), and 1% citric acid (Aldrich, Dorset UK). All quantities were on a w/w basis. The gelatine was hydrated, dissolved at 60°C in a water bath and then added to the sugar and glucose solution (which had been previously boiled and cooled to below 100°C). The molten gel mixture was cooled to 60°C and mixed with quantities of carvone (Firmenich SA, Geneva, Switzerland), which had been dispersed in propylene glycol (Aldrich, Dorset, UK), to give final volatile concentrations of 125, 250, 500, 750 and 1000 ppm (mg/kg).

When set, the gel was cut into individual cubes weighing 6g +/- 1g. Samples were stored at 4°C but allowed to equilibrate to room temperature (18-20°C) prior to eating.

4.1.1.2 Experimental design
The five aroma concentrations were tested in duplicate, presented in a randomised complete block design. Samples were presented singularly with a break of 15 minutes between each. Plain crackers and mineral water were used as palate cleansers.

4.1.1.3 Sensory panel
A sensory panel consisting of 4 men and 10 women aged between 25 and 60 were selected on the basis of their ability to discriminate between samples and rate the intensity of mint flavour in sweet gelatine gels using magnitude estimation and time intensity (Section 2.4.3.2).
4.1.1.4 Simultaneous instrumental and sensory analysis

Panelists were instructed to place the sample in their mouth, to chew normally and record the intensity of mint flavour using the lever system (Section 2.3.1.1). During eating, panelists kept their mouth closed, swallowed at will and maintained a regular breathing pattern (monitored from acetone release). Exhaled air was sampled in real time at a rate of 30ml/min using the MS Nose™ (Micromass, Manchester, UK) (Section 2.3.1.2). Volatile molecules were ionised (4kV corona discharge, sample cone voltage 20) and the release profile followed by monitoring the appropriate molecular ion (MH⁺) (carvone – m/z 151, dwell time 0.05s).

The assessment was complete when no more mint flavour was perceived. The MS Nose™ was calibrated by direct comparison of the peak height for carvone released on each breath against the peak height for a known concentration of volatilised carvone injected directly. The concentration was expressed in parts per billion by volume (ppbv).

4.1.2 Effect of volatile type on release characteristics and flavour perception from gelatine gels

4.1.2.1 Sample preparation

Gelatine samples were prepared containing five separate volatile compounds - Hexenal, Limonene, Carvone, Isoamyl acetate and Anethole (Firmenich SA, Geneva). The volatile compounds were selected on the basis of their congruity with a sweet base gel and for their different physicochemical properties (Table 4.1). The base gel mixture was prepared from the recipe described in section 4.1.1.1. In this instance, a different volatile was added to each of the five gelatine samples - the final concentrations are listed in Table 4.1. When set, the gel was cut into individual cubes weighing 6g +/- 1g. Samples were stored at 4°C but allowed to equilibrate to room temperature (18-20°C) prior to eating.
Table 4.1: Details of volatile compounds used to flavour 6% gelatine gel samples. Concentration (ppm) does not allow for any losses during manufacture. Hydrophobicity was calculated from either UNIFAC model calculation (*) (Reid, Prausnitz et al. 1987) or Advanced Chemistry Development Inc (ACD) (**) (Espinosa Diaz, Landy et al. 1996). Description of flavour is taken from Aldrich catalogue of flavours and fragrance.

<table>
<thead>
<tr>
<th>Volatile compound</th>
<th>Concentration (ppm)</th>
<th>Hydrophobicity (logP)</th>
<th>Flavour description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexenal</td>
<td>450ppm</td>
<td>0.994*</td>
<td>apple, fruity, chemical</td>
</tr>
<tr>
<td>Limonene</td>
<td>2000ppm</td>
<td>4.60**</td>
<td>sweet, orange, lemon</td>
</tr>
<tr>
<td>Carvone</td>
<td>1000ppm</td>
<td>2.34*</td>
<td>sweet, spearmint</td>
</tr>
<tr>
<td>Anethole</td>
<td>200ppm</td>
<td>3.42*</td>
<td>aniseed, spicy</td>
</tr>
<tr>
<td>Iso amyl acetate</td>
<td>1600ppm</td>
<td>2.30**</td>
<td>sweet, fruity, banana</td>
</tr>
</tbody>
</table>

4.1.2.2 Experimental design

Each gel flavour was tested during a separate session. Samples were tested in triplicate with a break of 15 minutes between each. Plain crackers and mineral water were used as palate cleansers.

4.1.2.3 Sensory panel

A sensory panel consisting of 4 men and 10 women aged between 25 and 60 were selected on the basis of their ability to identify and describe each of the volatile flavours and their experience in producing time intensity and time release data.

4.1.2.4 Simultaneous instrumental and sensory analysis

Panelists were instructed to place the sample in their mouth, to chew normally and record the intensity of flavour using the lever system (Section 2.3.1.1). During eating, panelists kept their mouth closed, swallowed at will and maintained a regular breathing pattern (monitored from acetone release). Exhaled air was sampled in real time at a rate of 30ml/min using the MS Nose™ (Section 2.3.1.2). Volatile molecules were ionised (4kV corona discharge, sample cone voltage 20) and the release profile followed by monitoring the appropriate molecular ion (MH⁺) (hexenal – m/z 99;
limonene – m/z 137; carvone – m/z 151; anethole – m/z 149; isoamyl acetate - 131, dwell time 0.05s).

The assessment was complete when no more flavour was perceived. The MS Nose™ was calibrated by direct comparison of the peak height for volatile released on each breath against the peak height for a known concentration of each compound injected directly. The concentration was expressed in parts per billion by volume (ppbv).

4.2 RESULTS

4.2.1 Volatile release and perception from mint flavoured gelatine gels
The raw volatile release data obtained from the MS Nose™ was subjected to a low-smoothing algorithm which averaged across five successive peak heights (two preceding and two succeeding each data point). The algorithm removed the “spiky” nature of volatile release data whilst maintaining the overall shape of the profile. Time intensity data was used in its raw form and was not subjected to any smoothing. Instrumental and sensory curves were checked for consistency across the replicates. Parameters for data analysis were extracted from duplicate volatile release (ins) and time intensity (sen) curves for each assessor (Figure 4.1).
4.2.1.1 The Effect of Increasing Carvone Concentration on Volatile Release

The maximum concentration of carvone in exhaled air (Imax$_{ins}$) was calculated for each assessor and sample. The panel mean Imax$_{ins}$ correlated well with the concentration in the gel sample (Figure 4.2) ($R^2 = 0.99$). It would be reasonable to assume that increasing the concentration of volatile in the sample resulted in a proportional increase in volatile delivered to the olfactory epithelium. Non linear behaviour would occur, however, if there were changes in the way in which the volatile was distributed in the food matrix (e.g. formation of droplets at high concentrations).
Figure 4.2: The relationship between the maximum carvone concentration measured in exhaled air (Imaxint) and the concentration in 6% gelatine gel sample. Data are the mean of 14 assessors; error bars show the standard deviation from the mean.

The standard deviation (shown as error bars) highlighted a significant variation, between panelists, in the quantity of volatile delivered to the nose. Results for individual assessors showed that Carole achieved in-nose concentrations three times greater than those seen for Kay (Figure 4.3). This variation, which was independent of age and sex, may have arisen from differences in their human physiology or may have been due to the mechanics of their eating, swallowing and breathing during eating.
4.2.1.2 The relationship between stimulus and perception

The maximum perceived flavour intensity (Imax\text{sen}) was determined for each sample and assessor. Mean panel values were calculated for each concentration. Assuming that all the data were collected in the same way, pooling data from individual curves was considered to be a robust measure of sensory effects in relation to changes in stimuli (Overbosch 1986) (Overbosch, Vandenenden et al. 1986). The relationship between perceived mintyness and sample concentration was almost linear for the concentration range used (R² = 0.97) (Figure 4.4). These results were consistent with magnitude estimation data collected from the same samples (Section 2.4.3.2.1). Previous studies have shown a good correlation between single point scaling techniques and the maximum intensity (Imax\text{sen}) of Ti curves (DuBois, Crosby et al. 1977).
4.2.1.3 Comparison of assessors

In Figure 4.4 the relationship between stimulus and perception was illustrated using mean data from 14 assessors. Whilst there was a slight suggestion of a sigmoidal curve, characteristic of Stevens Power Law (Stevens 1957), the concentration range fell mainly in the linear range of the stimulus-perception curve ($R^2 = 0.97$). Despite a high correlation coefficient, the standard deviation suggested a high degree of variation between assessors. On the one hand, this may simply reflect their rating ability using time intensity or, alternatively, it may be linked to their physiology and eating habits. To investigate this further, the correlation coefficient ($R^2$) was calculated from stimulus and perception data for each assessor. As the maximum concentration of volatile delivered to the nasal receptors ($I_{\text{max}_{\text{ins}}}$) was assessor dependent (Figure 4.3), it was more appropriate to correlate the perceived intensity with the concentration of volatile in exhaled air rather than the sample. This had the added benefit of accounting for sample variation within each concentration. Results showed that assessors did not exhibit the same linear response (Figure 4.5).
Comparing two individuals, Mike achieved a higher maximum concentration in-nose ($I_{\text{max,ins}}$) and a poorer linear correlation between perception and stimulus ($R^2 = 0.81$) whereas for Sally the in-nose concentration was lower but the linear correlation was much higher ($R^2 = 0.94$).

![Figure 4.5](image)

**Figure 4.5:** The relationship between maximum perceived mint intensity ($I_{\text{max,sen}}$) and the maximum concentration of volatile in exhaled air ($I_{\text{max,ins}}$). Results are shown for two assessors Sally = ♥; Mike = λ. Each data point is the mean of duplicate results.

It was hypothesised that an individual’s ability to rate changes in stimulus concentration might be dependent on the speed of eating and/or the efficiency of volatile delivery. To investigate this further, eating speed was calculated as the time to reach maximum carvone concentration in-nose ($T_{\text{max,ins}}$). Previous work has shown that the rate of flavour release in gelatine samples is dependent on the rate of chewing (increasing surface area) and melting (Harrison and Hills 1996). It was reasonable to assume, therefore, that the longer the food remained in the mouth (slower eating) the greater the value for $T_{\text{max,ins}}$. 
Efficiency of volatile delivery was represented by the maximum concentration of carvone (Imax_ins) measured in exhaled air when consuming 1000ppm gel samples. This sample was used as it gave the greatest signal, however, this measure of efficiency could have been calculated using any of the sample concentrations.

There was no significant correlation between eating speed, or efficiency of delivery, and the ability to rate changes in stimulus concentration ($R^2$). Panelists who ate quickly or who had greater Imax_ins values performed no better or worse than the others. Furthermore, the speed of eating (Tmax_ins) and the maximum delivery of volatile to the nose (Imax_ins) were independent variables. From assessor to assessor, the maximum carvone concentration achieved in nose for the 1000-ppm gel did not increase just because the food remained in their mouth for longer.

4.2.1.4 Adaptation

A comparison of the time to maximum concentration in-nose (Tmax_ins) and the time to maximum perception (TmaxSen) (data not shown) revealed differences in the timing of the two events. The results showed that for several assessors, TmaxSen occurred before Tmax_ins. Previous studies have indicated that, for volatiles released slowly from a food system, the perceived maximum intensity occurs before the in-nose maximum due to adaptation. Conversely, volatiles released quickly from a food system tend to show a perceived maximum intensity after that measured in-nose (Linforth, Baek et al. 1999).

In this study, the gelatine concentration and volatile type remained constant, therefore differences in Tmax could not be explained in terms of a matrix effect (Baek, Linforth et al. 1999) or physicochemical properties (Linforth, Friel et al. 2000). A more likely explanation was the difference in eating habits of individual assessors.

To investigate this further a measure of adaptation was determined for each assessor, and compared with his or her eating speed (Tmax_ins) and efficiency of volatile
delivery (Imaxins for 1000ppm gel). Adaptation was calculated as the time difference between T50ins and T50sen for normalised curves; these were the times to fall to 50% of the maximum intensity for stimulus and perception respectively (Figure 4.6). T50 values from the decay slopes of both curves were used as this provided a suitable length of time for assessors to adapt to the stimulus.

![Fig 4.6](image)

**Figure 4.6:** An illustration of how to calculate adaptation from normalised curves. Adaptation is represented by the difference between T50ins and T50sen; time intensity curve = λ; volatile release curve = ν.

Results showed that the level of adaptation to the stimulus was dependent on the speed of eating (Figure 4.7). The linear relationship (R² = 0.62) indicated that, the higher the value of Tmaxins, the greater the adaptation to the stimulus. No relationship existed between adaptation and the efficiency of volatile delivery.
Flavour release and perception in gelatine gels

4.2.2 Effect of volatile type on release characteristics and flavour perception from gelatine gels

The data obtained from the MS Nose™ and time intensity was prepared as for Section 4.2.1. Parameters for data analysis were extracted from volatile release (ins) and time intensity (sen) curves for each assessor. Figure 4.8 shows an illustration of a curve, which was representative of either the instrumental volatile release or the sensory time intensity data. The illustration details the parameters extracted for further analysis.

- $I_{\text{max}}$ = the maximum concentration achieved in-nose (ppbv) ($I_{\text{maxI}}$) or the maximum perceived intensity (arbitrary units) ($I_{\text{maxS}}$).

- $T_{\max}$ = time taken to achieve maximum concentration in nose (min) ($T_{\text{maxI}}$) or maximum perceived intensity (min) ($T_{\text{maxS}}$).

Figure 4.7: The effect of eating speed on adaptation. Eating speed is represented by the time to maximum volatile concentration in exhaled air ($T_{\text{maxins}}$) and adaptation is represented by the difference between $T_{50_{\text{sen}}}$ and $T_{50_{\text{ins}}}$ as calculated in Figure 4.6.
Figure 4.8: Illustration of release curve or time intensity curve and the parameters extracted for data analysis.

- **T50 incline** = time taken to achieve 50% of the maximum concentration in nose (min) (T50 incI) or 50% of the maximum perceived intensity (min) (T50 incS)

- **T50 decay** = time taken to fall to 50% of the maximum concentration in-nose (min) (T50 decayI) or 50% of the maximum perceived intensity (min) (T50 decayS)

- **Onset rate** = the rate of flavour delivery to the nose (ppbv/min) (onsetI) or the rate of increase in perceived intensity (arb/min) (onsetS). The onset rate was calculated from:

  \[
  \text{Onset rate} = \frac{I_{\text{max}} \times 0.5}{T75 - T25}
  \]

Where:

- **T75** = time to reach 75% of the maximum
- **T25** = time to reach 25% of the maximum
- Decay rate = the rate of decrease in flavour delivery to the nose (ppbv/min) (decayI) or the rate of decrease in perceived flavour intensity (arb/min) (decayS).

The decay rate was calculated as for onset rate.

Extracted parameters from triplicate data for each assessor and volatile combination were averaged. These data are listed in Appendix 1-5. The mean panel values for each volatile are presented in Table 4.2.

Table 4.2: Extracted parameters from volatile release and time intensity curves for 6% gelatine gel samples flavoured with 5 different volatile compounds. Data are the mean of 14 assessors.

<table>
<thead>
<tr>
<th>Volatile</th>
<th>ImaxI (ppbv)</th>
<th>TmaxI (min)</th>
<th>t50incI (min)</th>
<th>T50decI (min)</th>
<th>onset rateI (ppbv/min)</th>
<th>decay rateI (ppbv/min)</th>
<th>ImaxS (arb)</th>
<th>TmaxS (min)</th>
<th>t50incS (min)</th>
<th>T50 decS (min)</th>
<th>onset rateS (arb/min)</th>
<th>decay rateS (arb/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamyl acetate</td>
<td>9548</td>
<td>0.33</td>
<td>0.09</td>
<td>1.39</td>
<td>33919</td>
<td>5119</td>
<td>8.1</td>
<td>0.62</td>
<td>0.31</td>
<td>1.25</td>
<td>16.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Anethole</td>
<td>892</td>
<td>1.15</td>
<td>0.50</td>
<td>1.95</td>
<td>821</td>
<td>645</td>
<td>7.2</td>
<td>0.89</td>
<td>0.53</td>
<td>1.53</td>
<td>10.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Carvone</td>
<td>2231</td>
<td>1.08</td>
<td>0.40</td>
<td>1.93</td>
<td>2419</td>
<td>1615</td>
<td>8.6</td>
<td>0.85</td>
<td>0.50</td>
<td>1.50</td>
<td>14.3</td>
<td>8.9</td>
</tr>
<tr>
<td>Limonene</td>
<td>14631</td>
<td>0.15</td>
<td>0.07</td>
<td>0.32</td>
<td>109926</td>
<td>55115</td>
<td>7.0</td>
<td>0.32</td>
<td>0.12</td>
<td>0.68</td>
<td>32.9</td>
<td>12.7</td>
</tr>
<tr>
<td>Hexenal</td>
<td>3505</td>
<td>0.85</td>
<td>0.44</td>
<td>1.27</td>
<td>5306</td>
<td>9238</td>
<td>6.6</td>
<td>0.98</td>
<td>0.45</td>
<td>1.59</td>
<td>7.5</td>
<td>7.6</td>
</tr>
</tbody>
</table>

4.2.2.1 The effect of hydrophobicity (logP) on volatile release

The five volatiles used in this experiment were selected on the basis of their differing affinity for water. It was assumed that volatiles with a high affinity for water (low logP) would show different release characteristics from those with a low
affinity (high logP). A comparison of volatile release curves for limonene and carvone supports this assumption (Figure 4.9). Whilst the absolute values varied for each assessor, the general trend was similar (data not shown).

The more hydrophobic limonene was released very quickly; it almost “squirted” out of the gel as soon as it was placed in the hydrophilic environment of the mouth. The maximum concentration (Imaxins) of limonene on the breath was rapidly achieved (small Tmaxins) followed by a fast decline and negligible persistence. By comparison, hydrophilic carvone was released steadily as the gel fragmented and began to melt. The maximum concentration was achieved much later (larger Tmaxins) and the release curve showed marked persistence, long after the sample had been cleared from the mouth.

When considering the results for all five volatiles, however, the relationship between Tmaxins and logP, contrary to expectation, was not inversely proportional (Figure 4.10). Iso amyl acetate and carvone had very similar logP values but a threefold
difference in \( T_{\text{max}_{\text{ins}}} \). Clearly, hydrophobicity was neither the only, nor the most important, factor in determining the release kinetics of flavour volatiles.

![Figure 4.10: The relationship between \( T_{\text{max}_{\text{ins}}} \) (time to reach maximum volatile concentration on the breath) and \( \log P \) (hydrophobicity). Data are the mean of 14 assessors. Limonene = 4.6; anethole = 3.42; isoamyl acetate = 2.3; carvone = 2.34; hexenal = 0.994.](image)

4.2.2.2 *Comparing volatile release and time intensity curves.*

As stated previously, Overbosch’s theoretical model describing the relationship between stimulus and perception over time (Overbosch 1986) (Overbosch, Vandenenden et al. 1986) took into account the possible effects of adaptation. He predicted that significant adaptation would occur in the time taken to chew and swallow food, which would cause deviations between time intensity and volatile release curves late in the eating event. The model predicted that \( T_{\text{max}_{\text{ins}}} \) and \( T_{\text{max}_{\text{sen}}} \) would be identical, such that maximum stimulus intensity would be perceived at the point of maximum stimulus delivery.

It is not surprising, therefore, that a comparison of the mean \( T_{\text{max}} \) values for each volatile showed a linear trend (Figure 4.11). However, the trend line did not pass through the origin, suggesting some deviation from the original Overbosch model.
To visualise the deviations in the data set more accurately, the ratio of $T_{\text{max}_\text{sen}}$ to $T_{\text{max}_\text{ins}}$ was calculated for each assessor and volatile, and plotted against $T_{\text{max}_\text{ins}}$ (Figure 4.12).

If the Overbosch model were correct then the ratio between the two values would always be 1.0, regardless of the properties of the volatile. However, the results showed that when the maximum concentration on the breath was achieved more quickly ($T_{\text{max}_\text{ins}} < 1.0$ min), the ratio between the sensory and instrumental data ($T_{\text{max}_\text{sen}}/T_{\text{max}_\text{ins}}$) was >1. By contrast, when the maximum concentration on the breath was achieved more slowly ($T_{\text{max}_\text{ins}} > 1.0$ min), the ratio was <1.
Figure 4.12: The effect of Tmax_{ins} on the ratio of Tmax_{sen} and Tmax_{ins}. Data are the mean of three replicates for each assessor; □ = limonene; ○ = isoamyl acetate; Δ = hexenal; σ = anethole; Ω = carvone.

When the release rate was fast and the maximum in nose concentration occurred quickly, the maximum perceived intensity lagged slightly behind the stimulus. Previous studies involving fewer volatile compounds and assessors have shown similar results (Linforth, Taylor et al. 1998) (Linforth, Baek et al. 1999) (Baek, Linforth et al. 1998). The delay in perceptual maxima was explained as temporal integration i.e. the time taken for neural processing of the signal (Berglund and Lindvall 1982).

When the release rate was slower and maximum in nose concentration occurred much later, the maximum perceived intensity preceded that of the stimulus. The rate of volatile delivery remains fairly constant up to the maximum concentration in nose. When the time course of volatile delivery is extended and the rate of delivery constant, individuals will adapt to the stimulus (Koster and Wijk 1991). The intensity of perception is more closely correlated with the rate of volatile release rather than the maximum concentration achieved (Baek, Linforth et al. 1998).
Another interesting feature of this data, and that published by Linforth et al (1998), was the variation between assessors consuming the same sample type. This was particularly noticeable for carvone and anethole for which the ratio values were >1 and <1, dependent upon the assessor. These volatiles were very persistent and remained on the breath after the food bolus was swallowed (Ingham 1996) (Linforth, Ingham et al. 1996). This may be due to the persistence of the compounds in the mouth, but will also be influenced by their adsorption to the nasal mucosa (Hornung, Mozell et al. 1980). Persistent compounds will gradually build up on the nasal mucosa, and Tmax will be dependent on the rate of release of the compound, its speed of migration to the nasal cavity and its elimination from the mouth and mucosal membranes. Consequently, the eating habits and physiology of assessors will greatly influence the temporal parameters extracted from the volatile release and time intensity curves.

As part of the BBSRC research project, for which this work was undertaken, Dr Wendy Brown (Institute of Food Research) investigated the mastication and swallowing patterns for a group of consumers and trained panellists consuming 6% gelatine samples. She identified two distinct groups of chewers (fast and slow), for whom oral breakdown and swallowing patterns were very different. The different strategies adopted by the two groups affected the way the surface area and physical state changed over time and, consequently, the rate at which flavour was released from the sample. Short-term chewers put more effort into a relatively slow chew rate to be more effective. They swallowed larger fragments (less surface area) than did the longer chewers. By contrast, longer chewers had a less aggressive approach with a faster chew rate. Food stayed in the mouth for longer achieving a higher temperature and greater surface area for flavour release. The differences were observed for the trained and consumer panels.

Given that eating habits and physiology are undeniably important factors, it is essential to examine assessor differences and, in particular, to identify if there is any
structure in the data before resorting to the more common practice of averaging time intensity curves.

4.2.2.3 Comparing differences between assessors

The curve parameters detailed in Figure 4.8 were determined for each assessor and volatile (see Appendix 1-5). They represented all the key features, from the start of eating to the end of release and perception. In trying to establish a mathematical relationship between sensation and perception these would be the important parameters for modeling.

To study the possible sources of variation between assessors, the data were analysed using Principal Components Analysis (PCA) where assessors were considered as samples and the extracted curve parameters as variables. The variables had very different units of measurement and, therefore, to remove the influence of those with larger numbers and a greater absolute range of values, the data was standardised (multiplied by 1/Standard deviation).

The results showed considerable inter-assessor variation in the extracted parameters from both the volatile release and time intensity curves. The differences between assessors were more apparent for persistent volatiles carvone and anethole (Figures 4.13 and 4.14 respectively). Results from limonene, hexenal and isoamyl acetate showed much less structure in the sample data (see Appendix 6-8). The faster release of these less persistent compounds do not provide a long enough time span to identify differences in assessor behaviour. In these cases any difference between assessors is masked by the intra assessor variation.

The loadings for parameters extracted from the carvone results showed that principal component 1 (PC1) represented 57% of the total variation within assessors. PC1 was described mainly by Tmax and T50 decay, from both volatile release and time intensity curves. Not surprisingly, the onset rate was negatively correlated with the Tmax values i.e. assessors with larger Tmax values would have a slower rate of increase in flavour delivery and perception.
Figure 4.13: Sample maps showing sample scores and variable loadings from data for carvone gels. Data are centred and standardised. Suffix ‘I’ denoted parameters extracted from volatile release curves; suffix ‘S’ denotes parameters extracted from time intensity curves.

PC1 separated the assessors into those with the smallest Tmax values and fastest onset rate (Claire, Mike and possibly Gill), the average assessors (Tracy, Diane, Carole, Sally, Kay, Sue, John and Stuart), and those representing the largest Tmax and slowest onset rate (Nina, Maureen, David and Carl). PC2 represented 17% of the total variation and was described by Decay rateI and T50 inclineS although there was no logical reason why these two parameters should be linked. There was no obvious grouping of assessors based on PC2; they were largely clustered around the origin. PC3 and 4 provided no additional information regarding assessor differences.
The loadings for parameters extracted from the anethole results showed that PC1 represented 53% of the total variation between assessors. As for carvone, PC1 was described mainly by Tmax and T50 decay, from both volatile release and time intensity curves. PC1 separated the assessors into those with the smallest Tmax values and fastest onset rate (Claire, Gill and possibly Tracy), the average assessors (Diane, Sally, Kay, Sue and Stuart), and those with the largest Tmax and slowest onset rate (Carole, John, Nina, David and Carl). PC2 represented 23% of the variation and was described by the decay rate from both volatile release and time intensity curves.
intensity data. In contrast to the carvone data, the assessors were separated into those with slow, average and fast decay rates.

Kay stood out from the rest of the panel with a particularly fast decay rate. This applied to both her instrumental and sensory data and, therefore, would most likely be explained in terms of her eating habits or physiology. It was, however, unusual to find no similar structure in the carvone results. Like anethole, carvone is a persistent compound and the average panel data showed almost identical Tmax\textsubscript{ins} results. Despite this, the sample map for carvone shows Kay with an average decay rate.

Looking at other individual assessors, Claire and Gill were identified as having the shortest Tmax\textsubscript{I} and fastest onset rate\textsubscript{I} for both carvone and anethole whilst Nina David and Carl were at the other extreme with the longest Tmax\textsubscript{I} and slowest onset rate\textsubscript{I} for both volatiles. Results for limonene, hexenal and isoamyl acetate (See Appendix 6-8) showed that, generally, Claire and Gill were fast eaters, achieving the maximum breath concentration very quickly followed by a rapid rate of decay. By contrast only David was consistently slow to achieve Tmax for the other volatile compounds.

Whilst physiology and eating habits provided a source of variation among assessors, it was also evident that their relative groupings were dependent on volatile type. This suggested an interaction between assessor and volatile such that some individuals changed their eating habits for different flavoured gels e.g. a subjective response whereby more ‘pleasant’ samples were eaten slowly and ‘unpleasant’ samples cleared as quickly as possible.

The loading plots for anethole and carvone showed a correlation between some of the parameters extracted from the volatile release curves and their sensory counterparts e.g. T50 decline, Tmax and decay rate. However, there were instances where a parameter associated with a principal component was not correlated with its sensory or instrumental counterpart e.g. isoamyl acetate – decay rate described PC2.
but was not correlated with decay rate; limonene – TmaxS described PC1 but was not correlated with TmaxI. A lack of correlation between these key parameters would make it very difficult to model the perceptual data, or to validate a model built from mean panel data.

When developing models to describe flavour perception, it is important to identify subgroups of consumers with different eating strategies for that particular foodstuff. Eating strategies and physiology are important factors in determining aroma delivery and, therefore, models should include terms that reflect this source of consumer variation.

4.3 CONCLUSION

The investigation into the effect of increasing the carvone concentration on the perception of minty flavor in a 6% gelatine gel revealed that, on average, the relationship between stimulus and perception was linear for the system used. Some individual assessors showed more linearity in this relationship than others. Further analysis of the data revealed that the speed of eating and the maximum quantity of volatile delivered to the nose had no effect on the linear correlation. However, there was evidence to suggest that the speed of eating affected the level of adaptation to the stimulus.

Investigating the effect of volatile type revealed a marked difference in temporal parameters extracted from instrumental and sensory curves from five different volatiles. Assessor differences were more apparent for hydrophilic compounds.

Differences in the release characteristics from assessors consuming the same sample type highlighted the importance of eating habits and physiology on delivery of the volatile stimulus.
5.0 INVESTIGATING THE EFFECT OF VISCOSITY ON THE PERCEPTION OF FLAVOUR

Hydrocolloid thickeners are common ingredients in many food products. Utilised for their thickening properties at low concentration, they have a profound effect on both food texture and flavour. Reformulation of food flavour using empirical, trial and error methodology can be commercially inefficient. A fundamental understanding of how changes in matrix influence flavour release would be of great benefit to the food industry. Furthermore, understanding the relative contribution of hydrocolloid, non-volatile and volatile components to flavour perception could allow changes in perception to be predicted for a modified recipe.

This chapter investigates the effect of HPMC concentration on the volatile release from viscous solutions and the perceived intensity of flavour and taste. In addition, it attempts to use low order polynomial models to explain the perceptual responses in terms of HPMC, flavour and sugar composition of the samples.

5.1 METHOD

5.1.1 Effect of viscosity on release and perception of strawberry flavour.

5.1.1.1 Sample preparation

Liquid samples were prepared containing Hydroxy Propyl Methylcellulose (HPMC) (Methocel, DOW Germany) in distilled water at concentrations of 0.0625, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0% (w/w). Each sample contained 2% (w/w) sucrose (Tate and Lyle) and 200ppm of a strawberry flavour (Firmenich SA, Geneva, Switzerland).

To prepare the samples, appropriate quantities of distilled water and sucrose were weighed into a beaker and heated to 55-60°C. The hot sugar solution was stirred, without turbulence, using a motorised paddle and the HPMC powder carefully added.
to the side of the vortex. The solution was then cooled, with continual stirring, to 4°C. A flavour concentrate was prepared by mixing 800μl of strawberry flavour with 200μl of carmoisine food colour in a 10ml volumetric flask and making up to volume with 100% absolute ethanol. The flavour concentrate was added to a pre-weighed quantity of the cooled viscous solution such that the final concentration was 200ppm. This was mixed using a roller bed (SRT2 - Stuart Scientific, Redhill, U.K.) for 6-10 hours, prior to ingestion by the panel. The carmoisine acted as a marker for complete mixing.

5.1.1.2 Experimental design
Samples were presented in a randomised complete block design. Each assessor consumed all eight samples in duplicate. The presentation order was randomised, using simple random number generation, to reduce sample order effects and presented as groups of three to minimise sensory fatigue.

5.1.1.3 Sensory panel training
A group of 13 trained assessors were selected on the basis of their sensory acuity, in particular their ability to distinguish between concentrations of the same stimulus and their ability to perform magnitude estimation. In magnitude estimation the intensity of a stimulus is determined relative to the intensity of a fixed reference stimulus, often called a fixed modulus, or relative to the intensity of the preceding sample (Stevens 1957) (Moskowitz 1977).

5.1.1.4 Sensory evaluation
A trained sensory panel used magnitude estimation with a fixed modulus to rate the intensity of sweetness and strawberry flavour for each of the prepared samples. The modulus, which contained 0.25% (w/w) HPMC, 2% (w/w) sugar and 200ppm strawberry flavour, was assigned an arbitrary score of 100. This concentration was selected as it fell within the HPMC concentration range used for the experiment. The sweetness and strawberry flavour intensities of each sample were rated relative to the perceived intensity of the modulus. Assessments were carried out in individual
booths designed to international standards (ISO 8589 – Design of Sensory Test Facilities) with Northern Hemisphere daylight lighting at 750 –1070 lux.

Samples were presented at room temperature (18-23°C) in sealed containers. Assessors were instructed to place a level dessert spoonful (10ml) into the mouth, to allow the liquid to pass over the tongue and to swallow. They were advised not to hold the sample in the mouth for longer than a few seconds as it would become diluted with saliva and make rating difficult. A break of 15 minutes was given between each set of three samples to prevent fatigue. Plain crackers and still mineral water were used as palate cleansers between each sample.

5.1.1.5 Instrumental analysis - volatile release during consumption.

As the samples were consumed, the release of ethyl butyrate onto the breath was measured using the MS Nose™ interface fitted to a platform LCZ mass spectrometer (Micromass, Manchester, UK). Ethyl butyrate was selected as a marker for the strawberry flavour, which contained several fruit esters with similar release profiles. Each assessor consumed all eight samples in a single session with a break of at least 15 min between each sample. Plain crackers and water were used as palate cleansers. The method of consumption was standardised; assessors were asked to take a normal breath in, place 10ml of sample in their mouth and close, place their nose over the sampling tube, swallow the liquid and exhale normally - thereafter continuing to breath regularly and normally into the tube. The sampling tube, which was attached to the MS Nose transfer line, allowed exhaled air to be sampled in real time at a rate of 30ml/min. Volatile molecules were ionised (4kV corona discharge, sample cone voltage 18V) and the volatile release followed by monitoring the appropriate molecular ion (MH+) (ethyl butyrate – m/z 117, dwell time 0.05s). The concentration of ethyl butyrate on the first and second breath was determined against the signal from an ethyl butyrate standard (in hexane) (Taylor, Linforth et al. 2000).
5.1.1.6 Rheological studies
Seventeen samples of HPMC in distilled water were prepared at concentrations 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 15.0, and 20.0g/kg. They were prepared using the method detailed in section 5.1.1 but for simplicity, without the addition of sucrose and flavour. The concentrations of sucrose and flavour used in this experiment would not affect the rheological properties of the solutions. The viscosity of each solution were determined using a CS10 Controlled Stress Rheometer (Bohlin Instruments, Lund, Sweden) at 25°C, for a range of shear rates (5 – 100s\(^{-1}\)). Double gap geometry was used for low concentrations of HPMC, whereas cone and plate geometry was used for the higher concentrations.

5.1.2 Effect of viscosity on release and perception of almond flavour

5.1.2.1 Experimental design
A three factorial response surface design (Design Expert 5.0 Statease, Minneapolis USA) was used to investigate the effect of HPMC, sugar and volatile concentration on the perception of sweetness and almond flavour, and the release of benzaldehyde on the breath (Figure 5.1).
Figure 5.1: Diagrammatic representation of the full factorial response surface design to study the effect of varying sucrose, thickener and volatile concentration on volatile release and the perception of flavour and sweetness.

Within the experimental design, samples containing 0, 0.3, 0.9 and 1.2% (w/w) HPMC were duplicated, samples containing 0.6% (w/w) were replicated 4 times and the centre point (0.6% (w/w) HPMC, 5% (w/w) sucrose, 55ppm benzaldehyde) was replicated an additional 24 times. This resulted in a grand total of 132 samples presented overall. The design was split into 12 blocks, each containing 11 samples. Of these 11 samples, 9 were of different composition and 2 were replicate samples of the centre point. The samples selected for any single block were orthogonal, therefore creating a design in which the variables were not correlated with each other or with the blocks. This is important as it allows the results to be modelled using independently assessed design variables. Each block represented the set of samples presented to any one assessor. The orthogonality and blocking structure allowed any variation in results due to assessors to be separated from the main effects and residual error when analysing the data. All 11 samples in a block were prepared separately, including the two centre point replicates. Any variation in the centre point values provided a measure of pure error for the experiment.
5.1.2.1 Sample preparation

Samples were prepared containing HPMC at concentrations of 0, 0.3, 0.6, 0.9, and 1.2% (w/w). At each concentration of HPMC, samples were prepared containing 2, 5 and 8% (w/w) sucrose. For each combination of HPMC and sucrose, samples were prepared containing 10, 55 and 100ppm benzaldehyde (Firmenich SA, Geneva, Switzerland). This produced a total of 45 samples (Figure 5.1).

Low, medium and high intensity flavour concentrates were prepared by mixing 40, 220 and 400µl of benzaldehyde with 200µl of carmoisine in a 10ml volumetric flask and making up to volume with 100% absolute ethanol. The appropriate flavour concentrate was added to a pre-weighed quantity of the cooled viscous solution such that the final concentration was 10, 55 or 100ppm. Samples were mixed on a roller bed for 6-10 hours, prior to ingestion by the panel.

5.1.2.3 Sensory panel training

Due to the complexity of this experiment, the panel was given additional training in magnitude estimation of sweet and almond flavour solutions. This involved familiarising the panel with sucrose solutions of differing concentrations (1, 2, 3, 4.5, 5, 6.5 and 8% (w/v)) and then, in a further exercise, asking individuals to score their perceived intensity of sweetness against a modulus, given an arbitrary score of 100. The samples were presented randomly, in triplicate and included internal references. This exercise was repeated using solutions containing a fixed concentration of sucrose (2% (w/v)) but differing concentrations of benzaldehyde (10, 55, 75, 100, and 200ppm), with assessors asked to score sweetness and almond flavour (results shown in section 2.4.3.3).

5.1.2.4 Sensory evaluation

The panel used magnitude estimation with a fixed modulus to rate the intensity of sweetness and almond flavour for each sample within their block. The modulus, which contained 0.6% (w/w) HPMC, 5% (w/w) sugar and 55ppm benzaldehyde, was
assigned an arbitrary score of 100. The sweetness and almond flavour intensities of each sample were rated relative to the perceived intensity of the modulus. The tasting protocol was as described in section 5.1.1.4.

5.1.2.5 Static equilibrium headspace

The concentration of benzaldehyde in the headspace at static equilibrium was determined for the 45 almond flavour samples. Approximately 100ml of each sample were placed in a 250ml bottle (Schott bottle; Fisher Scientific, Loughborough, UK). Samples were allowed to equilibrate for 60 minutes at room temperature (20-22°C), after which the headspace was sampled using the MS Nose™ fitted to a platform LCZ mass spectrometer (Micromass, Manchester, UK). The headspace was sampled at a rate of 10ml/min. Compounds present in the gas phase were ionised (4kV corona discharge, sample cone voltage 18V) and the resulting molecular ion (MH+) was monitored (benzaldehyde – m/z 107; dwell time 0.05s). Headspace concentrations were calibrated against a signal from a benzaldehyde standard in hexane (100ppbv) (Taylor, Linforth et al. 2000).

5.1.2.6 Instrumental analysis - volatile release during consumption.

As the samples were consumed, the release of benzaldehyde onto the breath was measured using the MS Nose™, as detailed in section 5.1.1.5. Each assessor consumed all 11 samples in a single session with a break of at least 15min between each sample. Volatile molecules released on the breath were ionised (4kV corona discharge, sample cone voltage 18V) and the volatile release followed by monitoring the appropriate molecular ion (MH+) (benzaldehyde – m/z 107, dwell time 0.05s). The concentration of benzaldehyde on the first breath was determined against benzaldehyde standard (in hexane) (Taylor, Linforth et al. 2000).
5.2 RESULTS

5.2.1 Determination of c* for HPMC
The viscosity, determined using the Bohlin CS-10 Rheometer (see section 5.1.1.6), was plotted as a function of the shear rate to produce typical flow curves for each HPMC solution. The flat plateau of each flow curve indicated that, for these samples, the viscosity was independent of shear rate, with very little shear thinning even at high shear rates (Figure 5.2). Flow curves were fitted to the Cross-equation and the viscosity at zero shear ($\eta_0$) was determined (Cross 1965; Baines and Morris 1987). These data were used to calculate the reduced and inherent viscosity of each HPMC sample, described by equation 5.1 and 5.2 respectively.

Reduced viscosity \( (\eta_{\text{red}}) = \frac{\eta_{\text{rel}} - 1}{C} \) (5.1)

Inherent viscosity \( (\eta_{\text{inh}}) = \frac{\ln(\eta_{\text{rel}})}{C} \) (5.2)

Where: 
- \( C \) = concentration of thickener (g/l)
- \( \eta_{\text{rel}} \) = relative viscosity \( (\eta_{\text{rel}} = \eta / \eta_s \) a dimensionless ratio)
- \( \eta \) = the solution viscosity (cps)
- \( \eta_s \) = the solvent viscosity (cps)

When the concentration of thickener tends towards zero, the reduced and inherent viscosity terms describe the intrinsic viscosity of the hydrocolloid \([\eta]\).

Plotted graphically as a function of the concentration, the reduced viscosity (Huggins plot) and inherent viscosity (Kraemer plot) show a common intercept at \( C = 0 \) (y axis) which represents the intrinsic viscosity \([\eta]\) (Figure 5.3). Extrapolation to \( C=0 \) eliminates intermolecular interactions. The intrinsic viscosity of the HPMC was
determined to be 0.60 l/g. The units of intrinsic viscosity are the reciprocal of the concentration units. In this instance the concentration units are g/l, therefore the intrinsic viscosity units are l/g.

Figure 5.2: Flow curves showing the change in viscosity with shear rate between 5 – 100 s\(^{-1}\) for a range of solutions containing 0.25 - 20.0 g/kg HPMC at 25°C.
The value of $c^*$ (the concentration at which hydrocolloid molecules begin to interpenetrate and overlap) was then determined from a plot of log (specific viscosity) versus log($C [\eta]$) (Figure 5.4). Specific viscosity ($\eta_{sp}$) is the viscosity increase due to all polymer molecules and is described by equation 5.3.

$$\eta_{sp} = \eta_{rel} - 1$$  \hspace{1cm} (5.3)

The characteristic shape of this plot (Figure 5.4) shows two distinct straight-line sections representing different rheological scenarios. The shallow slope represents an increase in viscosity relative to the increase in molecule number per unit volume, below the point at which space becomes limited. The steeper part of the graph represents the point beyond which the viscosity rises steeply with increasing...
molecular number as space is limiting and the molecules are forced together. The point of inflection between these two slopes represents $c^*$, the onset of coil overlap.

From Figure 5.4 this is equivalent to $\log (C[\eta]) = 0.54$. From Figure 5.3 $[\eta] = 0.60$, therefore $c^*$ (the point of random coil overlap) occurred at a concentration $0.57g/100g$.

![Figure 5.4: The calculation of critical coil overlap ($c^*$) from determination of intrinsic viscosity in HPMC solutions of varying concentration. Double gap geometry ($\lambda$ and $\mu$); cone and plate geometry ($\sigma$).](image)

5.2.2 The effect of viscosity on the perception of sweetness and strawberry flavour intensity.

Analysis of variance (two factor, repeated measures, with interaction) showed a significant difference in perceived strawberry flavour intensity and perceived sweetness intensity between samples containing increasing concentrations of HPMC ($P<0.001$). Fisher’s LSD ($P=0.05$) showed that, for strawberry intensity, samples
containing >0.5% (w/w) HPMC were significantly different to all others, whereas lower concentrations were not significantly different (Table 5.1).

**Table 5.1: Significant differences in perceived strawberry flavour.** Flavour intensity scores are from the average of 13 assessors tasting duplicate samples. Significance is denoted by the use of different letters - A, B, C etc. If samples do not share the same letter they are significantly different.

<table>
<thead>
<tr>
<th>HPMC % (w/w)</th>
<th>Flavour Intensity (panel average)</th>
<th>Significance (P=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>54.17</td>
<td>A</td>
</tr>
<tr>
<td>1.5</td>
<td>68.75</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>82.71</td>
<td>C</td>
</tr>
<tr>
<td>0.75</td>
<td>95.00</td>
<td>D</td>
</tr>
<tr>
<td>0.5</td>
<td>108.33</td>
<td>E</td>
</tr>
<tr>
<td>0.25</td>
<td>107.29</td>
<td>E</td>
</tr>
<tr>
<td>0.125</td>
<td>108.75</td>
<td>E</td>
</tr>
<tr>
<td>0.0625</td>
<td>108.33</td>
<td>E</td>
</tr>
</tbody>
</table>

Similarly, for sweetness intensity, many significant differences were evident between samples containing increasing concentrations of HPMC. Generally, the higher the thickener concentration the more differences were observed (Table 5.2).

**Table 5.2: Significant differences in perceived sweetness.** Sweetness intensity scores are from the average of 13 assessors tasting duplicate samples. Significance is denoted by the use of different letters - A, B, C etc. If samples do not share the same letter they are significantly different.

<table>
<thead>
<tr>
<th>HPMC % (w/w)</th>
<th>Sweetness Intensity (panel average)</th>
<th>Significance (P=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>53.08</td>
<td>A</td>
</tr>
<tr>
<td>1.5</td>
<td>59.23</td>
<td>A B</td>
</tr>
<tr>
<td>1</td>
<td>78.85</td>
<td>B</td>
</tr>
<tr>
<td>0.5</td>
<td>95.58</td>
<td>C</td>
</tr>
<tr>
<td>0.75</td>
<td>98.85</td>
<td>C</td>
</tr>
<tr>
<td>0.25</td>
<td>104.23</td>
<td>C D</td>
</tr>
<tr>
<td>0.0625</td>
<td>111.15</td>
<td>D E</td>
</tr>
<tr>
<td>0.125</td>
<td>118.65</td>
<td>E</td>
</tr>
</tbody>
</table>
Results also showed a significant difference between assessors (P<0.001) and a significant interaction between samples and assessors (P<0.001) for flavour and sweetness. Despite the use of a fixed modulus, individuals used a varying range of scale values to score the flavour properties. These differences show a lack of consistency across the panel and may be due to a poor understanding of ‘strawberry flavour’ and ‘sweetness’, or confusion associated with experiencing different viscosities in mouth.

The results for strawberry flavour and sweetness intensity (arbitrary units) were averaged for the panel and plotted against HPMC concentration (%). Initially, perception of strawberry flavour was constant below a HPMC concentration of approx. 0.5% (w/w), after which point, the perception of flavour intensity decreased steadily with increasing HPMC concentration (Figure 5.5). The minimum concentration of HPMC at which flavour perception was reduced was consistent with the value of c*, determined to be 0.57% (w/w).

![Figure 5.5: The effect of HPMC concentration on perceived intensity of strawberry flavour.](image)

Solutions contained 2% (w/w) sucrose and 200ppm strawberry flavour.
The results for sweetness intensity (Figure 5.6) showed a similar reduction with increased HPMC concentration, however, the intensity tends to decrease steadily rather than show a sharp decline at the concentration corresponding to c*.

![Figure 5.6: The effect of HPMC concentration on perceived sweetness intensity. Solutions contained 2% (w/w) sucrose and 200ppm strawberry flavour.](image)

This pattern of results is similar to those obtained by Baines and Morris (1987). To explain the decrease in perception they hypothesised that, at c*, the hydrocolloid molecules begin to overlap and entangle, resulting in inefficient mixing of solution and poor replenishment of volatile at the surface of the liquid.

As detailed in the introduction, many studies have reported a decrease in perceived intensity of volatile and non-volatile flavour or a decrease in release rate of volatile under dynamic conditions. This is commonly attributed to a decrease in aroma release due to the increased viscosity affecting movement of the volatile through the liquid. Theoretical models derived to describe aroma release from liquid assume that
the mass transfer coefficient \( k \) is dependent on the diffusion coefficient \( D \) and, therefore, viscosity. For two film theory, this is a first order relationship where \( k \) is directly proportional to \( D \), whereas for penetration theory, \( k \) is proportional to \( \sqrt{D} \).

Investigations into the dynamic release of volatiles from thickened solutions in simulated mouth conditions have used theories of interfacial mass transfer to model the experimental data that, in each case, showed a decrease in release rate of volatile with increasing concentrations of thickener (Nahon, Harrison et al. 2000) (Darling, Williams et al. 1986) (Bakker, Boudaud et al. 1998). If these models were an accurate reflection of aroma release in vivo, we would expect to see a decrease in concentration of volatile on the breath.

5.2.3 The effect of viscosity on volatile release from liquids during consumption.

The concentration of ethyl butyrate (mg/m\(^3\)) in the first and second breath was calculated from the MS-Nose™ data, for each sample consumed by each assessor. Analysis of variance (two factor, repeated measures, without interaction) showed no significant effect of HPMC concentration on the release of ethyl butyrate onto the breath \( (p=0.18) \). This was true for both the first and second breaths. Large differences were seen between assessors, reflected in a significant difference in their results \( (P<0.001) \) (Figure 5.7). These are a consequence of differing physiology e.g. size and shape of buccal cavity, size and movement of tongue, diameter of airway, size of nasal cavity, and are a common feature of flavour release studies involving human subjects.

Contrary to previous thinking, in this study the increase in viscosity had no effect on the release kinetics of ethyl butyrate from solutions containing increasing concentrations of HPMC.
Figure 5.7: The effect of HPMC concentration on the release of ethyl butyrate on the breath. These are the results for a sample of 4 out of 13 assessors. Claire = υ; Stuart = λ; Mike = ν; Gill = σ|. Solutions contained 200ppm strawberry flavour and 2g/100g sugar.

Whilst previous studies have shown a decrease in volatile release rate with increasing viscosity, these were conducted under simulated in-mouth conditions and assume that air flow over the liquid represents the movement of aroma rich air to the nasal receptors. Buettner and co-workers (Buettner and Schieberle 2000) showed that, when consuming liquids, it is possible to prevent the passage of aroma to the nasal receptors whilst the liquid is in the mouth. The first wave of aroma delivered to the nasal receptors is in the first pulse of respiration after swallowing, the so-called ‘swallow breath’. When swallowing a liquid, therefore, flavour is released into the gas stream from (a) the volatile rich air in the mouth and (b) a thin film of liquid coating the pharynx after swallowing.

If the conditions in mouth prior to swallowing were in equilibrium, then the concentration of volatile in the exhaled air will be independent of HPMC concentration (assuming no interaction between ethyl butyrate and HPMC). If, however, the conditions were not in equilibrium, volatile release becomes dependent
Effect of viscosity on the perception of flavour

on theory of mass transfer, which would suggest that the increase in viscosity decreases the volatile release rate due to its effect on the diffusion coefficient D.

In studies investigating the effect of viscosity on diffusion coefficient, the addition of polymer molecules had no effect in unstirred systems (Darling, Williams et al. 1986) (Voilley and Bettenfeld 1985). In these studies hydrocolloids were used to create structure for solutions thickened with smaller sugar molecules. When a macromolecular thickener is present in low concentrations, it provides a structure to the solution, rather like a net, leaving large pores through which small volatile molecules can easily diffuse. The rate of diffusion would be affected, however, by increasing viscosity with smaller molecules, such as sucrose. In this case the microenvironment of the solution is changed where the smaller molecules do impede the diffusion of aroma compounds. Studies by Menting (Menting 1970) investigated the relationship between logD and water content. Large changes in logD only occurred when the water content was reduced to around 25%.

If the ‘net theory’ is correct, how do we explain the wealth of experimental evidence showing a viscosity dependent decrease in volatile release rate? As stated previously, flavour release studies tend to involve model mouth system with some sort of paddle or stirrer to mimic the movement of the tongue. In some instances the viscosity is changed by increasing concentration of sugars, in which case the viscosity will extend to the microenvironment of the solution (Roberts, Elmore et al. 1996) (Nahon, Harrison et al. 2000). In other studies the viscosity effect is confounded by the interaction of volatile and thickener (Roberts, Elmore et al. 1996) (Bakker, Boudaud et al. 1998). Given that the release rate is dependent on the concentration of unbound volatile, it is difficult to determine what contribution viscosity or interaction makes to the rate decrease. There are, however, investigations where equilibrium studies have shown no interaction between volatile and thickener (deRoos 1997). In this instance, the viscosity dependent decrease in volatile release rate may be due to an increase in the effective thickness of the liquid interfacial layer (Taylor 2002). From two-film theory, the mass transfer coefficient
is inversely proportional to the thickness of the interfacial layer. Alternatively, the increase in viscosity may reduce the effectiveness of stirring and inhibit the regeneration of flavour at the interface (Darling, Williams et al. 1986). Penetration theory dictates that mass transfer is proportional to the stirring rate. Bakker et al (1998) showed that the release rate of diacetyl increased with stirring rate in solutions of equal viscosity.

For this experimental data, it might seem reasonable to conclude that the conditions in mouth are at equilibrium, therefore accounting for the independence of breath concentration relative to viscosity. However, studies by Linforth et al (2002) showed that in mouth breath concentrations are markedly lower than headspace concentrations at equilibrium, for a range of volatiles with different physicochemical properties. It is, therefore, unlikely that equilibrium is reached in mouth before swallowing the samples.

A more likely explanation for these results is that the rate of ethyl butyrate release is governed solely by its partition coefficient as the closed in-mouth system tries to achieve equilibrium. The diffusion coefficient is independent of viscosity, as the HPMC creates a net through which the aroma molecule can easily diffuse. The conditions in mouth do not reflect the highly stirred model systems, in which we have previously seen a reduction in release rate due to viscosity, but are rather more static where the solution is allowed to flow over the tongue before swallowing.

An increase in solution viscosity may result in a thicker mouth coating of sample after swallowing. This might provide a reservoir of volatile flavour for release in subsequent breaths (Harrison 1998). The persistence of ethyl butyrate was calculated from a ratio of 1st and 2nd breath concentration (mg m\(^{-3}\)). There was no significant effect of increasing HPMC concentration on persistence. This is consistent with previous studies, which have shown no effect of HPMC on the persistence of several volatile compounds regardless of physicochemical properties. (Linforth and Taylor 2000).
5.2.4 The effect of viscosity on flavour release and perception in almond flavour liquids during consumption.

5.2.4.1 Static Equilibrium Headspace

Static equilibrium headspace concentrations of benzaldehyde were calculated for each sample. There was no significant effect of HPMC or sugar concentration on the headspace concentration of benzaldehyde. As expected, there was a significant effect of volatile concentration on the headspace values (P<0.001). For illustration (Figure 5.8), headspace concentrations (mg/m³) were averaged across the different sugar concentrations to give a mean result for each volatile level in 0, 0.6 and 1.2% (w/w) HPMC. The lack of an effect due to HPMC concentration suggested that no binding or chemical interactions occur between the hydrocolloid and volatile molecule.

![Figure 5.8: The effect of HPMC concentration on the static equilibrium headspace concentration of benzaldehyde at 10, 55 and 100ppm of volatile. Each result is the mean of three samples. Error bars show the standard deviation from the mean.](image-url)
5.2.4.2 Sensory Perception and In-nose Volatile Release

The results for perceived almond intensity, sweetness intensity and benzaldehyde release showed the same relationship with HPMC concentration as the previous study (section 5.2.1). The data were analysed using multiple linear regression (Design Expert 6.0). Low order polynomial models were derived to explain the variation in the data and to predict volatile release (equation 5.4), sweetness intensity (equation 5.5) and almond flavour intensity (equation 5.6) in terms of sample composition.

5.2.4.2.1 Modelling volatile release from viscous solutions

\[ \sqrt{BENZ_{\text{nose}}} = 0.23 + 0.02[BENZ_{\text{samp}}] - 7.33 \times 10^{-5}[BENZ_{\text{samp}}]^2 \]  \hspace{1cm} (5.4)

Where \( BENZ_{\text{nose}} \) = benzaldehyde concentration in-nose (mg m\(^{-3}\))
\( BENZ_{\text{samp}} \) = benzaldehyde concentration in the sample (ppm)

The model describing the release of benzaldehyde on the breath only included terms relating to the volatile concentration used in the sample. This was in agreement with static equilibrium headspace results, which showed no evidence of molecular binding or interaction; and with in-nose volatile release which also showed no effect of thickener. As determined in section 5.2.3, there was a significant variation in the data due to assessors (P<0.01). The flavour release model was highly significant (P<0.0001) with adjusted R\(^2\) and predicted R\(^2\) values of 0.78 and 0.73 respectively, and an ‘adequate precision’ (signal to noise ratio) of 30.22. The predicted R\(^2\) indicates how precise the model is at predicting the results from the samples tested, whereas the adjusted R\(^2\) indicates how well the model would describe variation outside the samples range. The closer these two values are, the more robust the model and the better it describes the variation across the design space. This was further illustrated when the experimental values were plotted against values predicted from the model (Figure 5.9).
5.2.4.2.2 Modelling perceived sweetness in viscous solutions

\[
\sqrt{SWEET} = 0.87 - 1.21[HPMC_{samp}] + 2.43[SUCROSE_{samp}] - 2.01[HPMC_{samp}]^2 - 0.09[SUCROSE_{samp}]^2 + 0.19(SUCROSE_{samp}[HPMC_{samp}])
\]  
(5.5)

Where:  
\(SWEET\) = The perceived sweetness intensity  
\(HPMC_{samp}\) = The concentration of HPMC in the sample (%)  
\(SUCROSE_{samp}\) = The concentration of sucrose in the sample (%)

The model for perceived sweetness included linear and quadratic terms for sugar and HPMC concentration, and an interaction term for thickener and sugar. It was highly significant (\(P<0.0001\)) with adjusted \(R^2\) and predicted \(R^2\) values of 0.97 and an ‘adequate precision’ of 57.42 suggesting that the model was robust and well
described the variation in the data. Predicted values from the model plotted against the experimental values are shown in Figure 5.10. The interaction term indicates that the relationship between sweetness and sugar concentration is dependent on HPMC and, conversely, that the relationship between sweetness and HPMC concentration is also dependent on sugar level.

![Graph showing R² = 0.97]

**Figure 5.10**: Perceived sweetness in viscous solutions: A comparison of predicted values from the model (equation 5.5) and experimental results.

A further illustration of the model for perceived sweetness intensity is shown in Figure 5.11. This graph represents a slice through a 3 dimensional model at ‘benzaldehyde = 55ppm’. Each contour represents a sweetness value (60, 80, 100, 120 etc). As would be expected, the contour ‘sweetness =100’ passes through the point 0.6% (w/w) HPMC, 5% (w/w) sugar and 55ppm benzaldehyde (the concentration of the modulus). The ability of the assessors to rate a blind coded sample identical to the modulus as ‘100’, gives a good indication of their consistency. The shapes of the contours indicate that, for any given sweetness intensity, the concentration of sugar must be increased to compensate for an increase in thickener.
5.2.4.2.3 Modelling perceived almond flavour in viscous solutions

\[
\sqrt{ALMOND} = -1.63 + 1.01[HPMC_{samp}] + 1.72[SUCROSE_{samp}] + 0.14[BENZ_{samp}]
- 2.30[HPMC_{samp}]^2 - 0.15[SUCROSE_{samp}]^2
- 7.68 \times 10^{-4}[BENZ_{samp}]^2
+ 6.25 \times 10^{-3} ([SUCROSE_{samp}] [BENZ_{samp}])
\]

Where:
- \(ALMOND\) = The perceived almond flavour intensity
- \(HPMC_{samp}\) = The concentration of HPMC in the sample (%)
- \(SUCROSE_{samp}\) = The concentration of sugar in the sample (%)
- \(BENZ_{samp}\) = The concentration of benzaldehyde in the sample (ppm)
The model describing perceived flavour intensity was, once again, highly significant (P=<0.0001). It included terms for HPMC, sugar and benzaldehyde concentration with quadratic terms for each variable and an interaction between sugar and benzaldehyde. All terms included had a significant effect on the model. The adjusted and predicted R² values for the model were 0.89 and 0.85 respectively and the ‘adequate precision’ was 27.85, suggesting that the model was robust and well described the variation in the results. The predicted values from the model plotted against the experimental values are shown in Figure 5.12.

![Graph showing predicted vs experimental results with R² = 0.89](image)

**Figure 5.12: Perceived almond flavour in viscous solutions: A comparison of predicted values from the model (equation 5.6) and experimental results.**

The inclusion of the interaction term suggests that, for any given level of HPMC, the relationship between perceived almond intensity and volatile concentration is dependent on sucrose level. A further illustration of the model is shown in Figure 5.13. Each contour represents almond flavour intensity; the contour shape illustrates the effect of HPMC and sucrose concentration at 55ppm benzaldehyde.
Effect of viscosity on the perception of flavour

### Figure 5.13: Two-dimensional contour plot derived from the model for perceived flavour intensity

Each contour represents a perceived almond flavour intensity whilst its shape illustrates how flavour is affected by relative concentrations (%) of sucrose and HPMC.

For HPMC values greater than 0.5% (w/w) and for any given almond flavour intensity, the sugar level can be increased to maintain perceived flavour. This holds true until a level of 6-6.5% (w/w) sucrose, after which point an increase in sucrose results in a decrease in flavour perception. This effect is most dramatic at low levels of HPMC and may, in part, be due to the intense sweetness masking the almond flavour.

As discussed previously, interactions between volatile and non-volatile stimuli are well documented (Noble 1996). The degree of interaction (synthesis of volatile and non-volatile into a flavour) has been shown to depend largely on the congruency of
the two stimuli. In other words, the likelihood that individuals have previously experienced them together or that aromas described as sweet would be paired with sweet tastants (Frank and Byram 1988) (Stevenson, Prescott et al. 1999) (Frank, Wessel et al. 1990). Stevenson et al (Stevenson, Boakes et al. 1998) were able to manipulate the interaction by exposing the panel to otherwise incongruent or unfamiliar pairings of taste and aroma.

In this study the pairing of sucrose with benzaldehyde was highly congruent, with the aroma of benzaldehyde being described as sweet, cherry and almond. Recent work combining saccharin and benzaldehyde at sub threshold concentrations (Dalton, Doolittle et al. 2000), showed a positive interaction when, presented together at 63% of their individual detection thresholds, both were identified. The data suggest direct integration of the two modalities at a neural level rather than the cognitive mechanisms suggested from studies at supra-threshold levels.

The magnitude of the interaction effect is not necessarily independent of the testing methodology. Overall intensity ratings have provided evidence of additivity (Murphy and Cain 1980) (Murphy, Cain et al. 1977), suggesting that flavour is an analytic sensation made up of its component parts (taste and aroma). Evidence of interaction exists where research has focused on specific flavour attributes, such as sweetness or specific fruity flavours, although in these instances the possibility of a ‘dumping’ effect warrants consideration. Allied to this, studies where all flavour attributes are rated show no evidence of interaction compared to those rating a single attribute (Frank and van der Klaauw 1993) (Clark and Lawless 1994). In the present study, it is important to consider the impact of perceived ‘thickness’ on flavour intensity and to ask, in the absence of a thickness rating, was this stimulus ‘dumped’ into that for sweetness and almond intensity?

For this study an interaction was evident despite the fact that flavour release was independent of HPMC concentration. It follows, therefore, that the decrease in flavour perception observed from these results may be due to the effect of HPMC on
stimulation of taste receptors by sugar molecules rather than volatile stimulation of nasal receptors.

One possible hypothesis may be the effect of HPMC on the mobility of free water in solution (particularly at concentrations above c*). Studies carried out by Mathlouthi (Mathlouthi 1984) (Mathlouthi, Cholli et al. 1986) (Mathlouthi and Seuvre 1988) have shown that sweetness increases as water mobility increases. Conformational changes in sucrose molecules in solution enhance sweetness intensity. Furthermore, disassociation of free water molecules arranged around the periphery of the sugar molecule produce a high membrane potential across the taste cell, thereby enhancing sweetness perception.

Alternatively, the perception of viscosity itself may affect overall flavour perception. Interactions may occur at a neurological level where gustatory and trigeminal inputs converge or even at a perceptual level where previous dietary experiences could influence taste judgements in thick and thin solutions (Christensen 1980).

5.3 CONCLUSION

The perception of flavour and sweetness is greatly reduced when HPMC is added to sugar/flavour solutions at concentrations above c*. However, the concentration of volatile released onto the breath is not affected by the increase in viscosity. Significant statistical models were derived to describe the results and to predict the intensity of perceived flavour, sweetness and the release of volatile from the thickened liquids.

Possible explanations for the decrease in perception may be the effect of increasing HPMC on the free water available in solution resulting in a decrease in sweetness intensity and therefore, a decrease in flavour intensity. Investigation of this would require NMR studies into the mobility of water and conformation of sweeteners in thickened solutions.
Alternatively, the perception of a thickened solution in the mouth may have an impact on the perception of tastants and, consequently, overall flavour.
6.0 GENERAL DISCUSSION

As stated at the outset, one of my principal aims was to recruit and train a sensory panel. To the uninitiated this may seem like a fairly straightforward task but in my previous experience developing a new panel can be very demanding. Under normal circumstances this can take up to twelve months but in my case, realistically I had no more than six months, from writing the advert to generating my first data for the LINK project.

I believe that the success of this phase is evident from the quality of the data presented within this thesis, but what is not so obvious from each chapter is the relationship that evolved between the panel and myself over the three years of this project. Working with people in this context is not the same as using analytical instruments to generate data. The initial phase of training was as much about building rapport and gaining their trust, as it was about instruction in sensory techniques.

Anyone who works in sensory will understand why I am very proud that none of the panel left during the three years of my project, especially considering the unusual nature of MS Nose™, the complexity of time intensity and the completely bizarre ‘food’ samples we tested. I am often asked about my methods for panel motivation and really they come down to good common sense. Irrespective of the reasons for getting involved with sensory testing, whether they are financial, social or achievement based, people want to feel respected, valued and to know that they are doing a good job. I think that this is best achieved by spending time with people and providing feedback, with particular emphasis on the positive.

Of course the challenge did not stop with simply recruiting this group of individuals but continued with the necessity of designing a training programme for Time Intensity. In many respects the freedom to play with different methodologies was quite liberating, but the lack of formal guidelines meant that it was very difficult to
judge when the training was complete. Whilst the historical review papers gave me some good tips on ways to introduce the method, it still came down to common sense in terms of designing the training exercises. In the end I decided to play it safe - training was complete when the panellist could provide reasonably reproducible curves for the same sample over subsequent sessions. Two years into the project Peyvieux and Dijksterhuis 2001 published their very useful paper detailing a way to visualise the reproducibility of time intensity curves using PCA.

The Ti and volatile release data collected from the gelatine gel samples was originally intended for the LINK project, however, it soon became apparent that \textit{in vivo} volatile release from this solid system was too complicated to model. At the time this was a big disappointment as the large data set had taken three months of twelve-hour days to gather (not least because, using MS Nose™, I could only work with one panellist at a time). At least the traditional scaffolding technique used to extract curve parameters was sufficient to determine that; the affinity of the aroma compound for water (hydrophobicity) was an important factor in influencing the temporal characteristics of the instrumental and sensory data. It also allowed me to investigate differences between individuals and to examine which curve parameters varied most amongst the panel. Unfortunately the statistical techniques available to me could not do justice to the vast amount of information inherent in this data, but luckily it is now being used as part of another BBSRC project for which we are working with the statistics department of University College London. The purpose of the project is to investigate the mathematical relationship between stimulus and perception and as a starting point we are using the data to validate existing models published in the literature.

When we discovered that aroma release from the gel system was too complex to model, I took a large step back and started investigating aroma release from aqueous solutions of volatile (with no sensory evaluation). This was the simplest system possible and ironically it took just a few days to collect the necessary data. The simplicity of the sample meant that the fundamental model did not need to include
terms for the breakdown and flavour release from solid samples. My academic partners were successful in modelling this data using penetration theory. With the exception of the first exhalation after swallowing, the model described well the release of volatile on the breath during subsequent exhalations. Coincidentally (or maybe not) the raw traces showed another interesting feature relating to this parameter; the concentration of volatile in the first exhalation increased with increasing breathing rate despite the fact that the concentration in subsequent breaths was unaffected. Mathematically it was not possible to include this first peak in the model and logically, assuming no mixing of volatile with exhaled air, there was no apparent explanation of this phenomenon. As with much of the research involving in vivo release of aroma compounds, it raised questions about the physiological aspects of eating. We are reminded that very little is known about the differences in anatomy and physiology and the effect on aroma release. To investigate this further the university is currently supporting a PhD studying the impact of breathing rate, swallowing and mastication on the release of volatiles from different food matrices.

Until this point, my PhD research was running in parallel with the BBSRC LINK project, the main difference being that, in addition to aroma release, I was interested in the perceptual dimension. My work investigating the effect of viscosity on aroma release and perception was somewhat of a departure from the original plan. This strand of the research was driven solely by my own interest. Of course it was important that it fitted with my previous work and in fact it created a good link between these investigations. This time I used the much more structured response surface design to investigate potential taste/aroma interactions and the effect of viscosity. In this instance it was the sample preparation that presented the biggest challenge. Using HPMC meant that all viscous solutions needed to be prepared at least nine hours in advance so that the solution had time to cool and the flavour mixed heterogeneously before tasting. Unfortunately the concentration of flavour was not stable over time so the samples had to be prepared exactly the same number of hours before tasting regardless of whether this was 3am or 3pm.
For this reason it was some weeks before I had chance to examine the data and comprehend the importance of my results. Prior to this, researchers had assumed that the decrease in flavour perception in thickened solutions was in some way linked to either binding or decrease in rate of diffusion. I had certainly assumed that my aroma release data would confirm this theory. However, to show so conclusively that this was not the case - that there was no effect of viscosity on aroma release was unequivocally the most exciting point of my PhD research. The fact that this excitement spread to colleagues and peers also took me a little by surprise.

Naturally the next question had to be – Why was perception effected so dramatically? Aside from proposing some possible theories there was no time left to investigate further. I did attempt some preliminary experiments using NMR to study the effect of viscosity on the mobility of water around the sugar molecules but the results were too ‘noisy’ to present in this thesis and no conclusions could be drawn. This responsibility of answering this important question fell to another colleague who’s PhD was to start where my investigation ends. A further three years research should also allow us to determine if savoury systems behave in the same way as sweet and if other thickeners provide similar results.

On reflection, I am certainly very happy with the path my research has taken, particularly now that I can see all of my efforts documented. It is satisfying to know that, whilst my research has fundamental scientific value, it also has application. More remarkable is the way in which sensory science has become an integral part of research at Nottingham University. The panel continues to be a valuable resource and at the time of writing are contributing to six postgraduate and two postdoctoral research projects.
7.0 PUBLICATIONS


8.0 BIBLIOGRAPHY


Brauss, M. S., R. S. T. Linforth, et al. (1999). The effect of changing the fat content on flavour release in a simple food system. COST96, Athens, Greece, EU.


Bibliography


Bibliography


APPENDICES
Appendix 1: Extracted parameters from instrumental (volatile release) and sensory (Time intensity) curves for isoamyl acetate in 6% gelatine gels.

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Appendix 2: Extracted parameters from instrumental (volatile release) and sensory (Time intensity) curves for anethole in 6% gelatine gels.

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Appendix 6: Sample maps showing sample scores and variable loadings from data for isoamyl acetate. Data are centred and standardised. Suffix ‘I’ denoted parameters extracted from volatile release curves; suffix ‘S’ denotes parameters extracted from time intensity curves.
Appendix 7: Sample maps showing sample scores and variable loadings from data for limonene gels. Data are centred and standardised. Suffix ‘I’ denoted parameters extracted from volatile release curves; suffix ‘S’ denotes parameters extracted from time intensity curves.
Appendix 8: Sample maps showing sample scores and variable loadings from data for hexenal gels. Data are centred and standardised. Suffix ‘I’ denoted parameters extracted from volatile release curves; suffix ‘S’ denotes parameters extracted from time intensity curves.