Creating Innovative Flavour and Texture Experiences

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Abstract

The work presented in this thesis describes the use of scientific research in the development of novel texture and flavour experiences and their potential for use in fine gastronomy. In order to create an interesting textural experience, modified celluloses were investigated. Their unique property is that they have the ability to gel at high temperatures, but return to the solution state upon cooling. This phenomena was used to test the hypothesis that hot gels made from these materials could melt at temperatures greater than mouth temperature, providing a melt-in-the-mouth sensation on consumption in a fashion analogous to gelatine. Melting temperatures of these hot gels were calculated using the techniques of rheology and differential scanning calorimetry, as well as more empirical methods, and results showed that gels made from a number of different hydroxypropylmethylcelluloses (HPMCs) displayed melting temperatures above 37°C. In order to predict their flavour release properties, the mixing efficiency of the solutions were investigated and results showed that solutions made from the lower molecular weight HPMCs showed more desirable mixing behaviour, suggesting better flavour release than those made from high molecular weight HPMCs. Furthermore, these solutions also had more desirable mouth-feel attributes, as determined by sensory analysis, yet their inherent flavour attributes were less pleasant. Therefore, modified celluloses show potential use in producing hot gels that melt in the mouth.

The second part of this thesis was focussed on developing novel flavour experiences, and this was done by creating drinks that changed their flavour as they were consumed. The development of a tomato flavoured drink, whose flavour changed due to the sequential heightening of its tastant profile, is described here, and it was found that the presence of each tastant at a volume needed to invoke swallowing was more important than the order in which the tastes were administered in terms of ensuring that each different flavour was perceived. In addition, a banana flavoured drink whose flavour changed on consumption to mimic a ripening banana was also created, and this work showed the importance of the matrix on flavour perception at both the physical and psychological level, which has important consequences in general in the development of flavoured products.

The work described in this thesis therefore shows the potential use that scientific research can play in aiding the creation of novel concepts applicable to the world of fine gastronomy.

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Overall introduction

The use of scientific equipment, ingredients and methods, as well as knowledge and research, have all been extended in recent years to the restaurant setting, to develop and create novel texture and flavour experiences for the diner. This concept was originally termed Molecular Gastronomy in the 1980s by Nicholas Kurti, a professor of physics at Oxford University, and the French chemist, Hervé This. Having realised that there was a growing gap between food science and home cooking, Kurti said 'I think it is a sad reflection on our civilisation that while we can and do measure the temperature in the atmosphere of Venus, we do not know what goes on in our soufflés' (Royal Institution, 1969). Since then the scientific field of Food Science has become of increasing interest to chefs, providing information relating to food and cooking processes that give insights into how to improve and optimise certain techniques, while the equipment and ingredients offer the possibility of creating new textures and concepts. The Fat Duck is an example of such a restaurant. Owned and run by Chef Heston Blumenthal, the restaurant is renowned world-wide for its creative and scientific approach to cooking. The restaurant now even has its own "developmental" laboratory next door, where a team of scientific researchers and chefs spend their days perfecting current dishes, and inventing new ones, with the aid of scientific expertise.

In order to further expand his research, Blumenthal formed a collaboration with the University of Nottingham to sponsor a BBSRC Case Award Project on the application of science to restaurant cooking. The Division of Food Sciences at the University of Nottingham is one of the largest research groups in the UK, and is divided into two main groups – the Food Structure Group, led by Prof John Mitchell; and the Food Flavour Group, led by Prof Andy Taylor.

The Food Structure Group carry out extensive research into the structure of food stuffs, and how the addition of hydrocolloids in particular can affect this. This particular area of knowledge and expertise was harnessed for the first part of this PhD (part A), which focused on the processes involved in developing a hot gel that melts in the mouth. It was thought that this may provide a novel textural experience for the diner.

The Food Flavour Group carry out research into how different factors affect flavour release, and how this impacts on subsequent flavour perception – this was intended to inform the second part of this thesis (part B), which focussed on the use of scientific research to create innovative flavour experiences. The emphasis was on investigating foods that changed flavour during consumption (inspired by an idea conceived by Roald Dahl in his book Charlie and the Chocolate Factory) in order to create an unusual, almost magical experience, to excite and surprise the diner.

The work described here shows how carefully controlled scientific research can help in the creation and development of concepts that could serve as bases for gastronomic dishes. As a result, throughout the PhD, efforts will be made to ensure that the final concepts that are developed, and the methods that are used to create and test them, are all practical and possible for future use in the busy environment of a fully functioning restaurant.

Part A. Novel texture experiences (an alternative to gelatine)

General Introduction

Gelatine has been used by chefs and home cooks to make gels for many years. However, recent concern with Bovine Spongiform Encephalopathy (BSE), as well as the increasing demand for vegetarian options in restaurants, has driven chefs to start considering other gelling agents. Recently, a number of novel hydrocolloids that can act as gelling agents (including agar, carrageenan, and gellan gum), previously only used in the food industry, have become available to everyone. However, despite their emergence, gelatine continues to be extensively used. The temperature at which the helical associations between gelatine molecules break up on heating is human body temperature (McGee 2004). Therefore, when placed in the mouth, a gelatine gel melts back effortlessly to a fluid, producing a desirable "melt-in-the-mouth" sensation, and this is thought to be responsible in part for its popularity. Szczesniak (1975) reports that solid foods exhibiting significant textural changes with temperature during consumption (e.g. ice cream, gelatin gels, chocolate) provide a pleasant textural contrast which is a valued consumer attribute (Szczesniak 1975). Gels made from the more novel hydrocolloids, however, melt at much higher temperatures. Although these gels therefore have the advantage of stability on a hot plate, and inclusion into hot dishes without melting, it also means that they do not display this desirable "melt-in-the-mouth" sensation.

A few years ago, modified celluloses have become available on the market. Solutions made from modified celluloses display many of the properties provided by other hydrocolloids. Some of these properties, such as film forming and making mousses, have been of benefit to the Fat Duck restaurant. For example, edible sweet wrappers for tangerine sweets served in the restaurant were successfully prepared by drying out modified cellulose solutions, and adding small quantities of certain modified celluloses was found to help reduce syneresis and improve stability of xanthan based foams. As well as these more common properties, solutions of modified celluloses also display the interesting property of gelling on heating, a feature not possessed by many of the other commonly used food additives. Although the concept of heat induced gelation is not completely new (eggs set on cooking due to heat induced

gelation), the reversibility of this process *is* unique. If hot gels made from these modified celluloses melted at temperatures greater than 37°C, they could be placed in the mouth hot, and would melt as the mouth cooled them, thus potentially producing this "melt-in-the-mouth" sensation presently only offered by gelatine. Therefore a main focus of this study was to measure the melting temperatures of these gels, to see how these temperatures compare to mouth temperature. This will be the major focus of chapter 1.

As well as its ability to melt at mouth temperature, there are other factors that are thought to contribute to the continued use and success of gelatine. Gelatine gels are known to have pleasant melting patterns (specifically, they have been reported to melt quickly and evenly (Szczesniak 1975)), and it is generally agreed that the solutions that gelatine gels melt back to display good flavour release, and have a pleasant mouth-feel. Therefore, in order for these modified cellulose gels to act realistically as gelatine replacements, they need to show pleasant melting patterns, and the solutions to which they melt back should display good flavour release and pleasant mouth-feel properties. This, therefore, will be the focus of chapter 2.

The application of this research to a restaurant situation was to identify a suitable hydroxypropylmethylcellulose (HPMC) to use to prepare a hot ravioli gel that melts in the mouth on consumption. The hot gel would be prepared in two layers, which would be wrapped around a filling to make a ravioli, which when placed in the mouth melts, releasing the contents. It was thought that such a product could be served as part of the tasting menu at the Fat Duck, providing an enjoyable and unique eating experience.

1. Melting temperatures of the gels

1.1. Aim

To measure the melting temperatures of hot gels made from the modified celluloses, in order to assess their potential to melt-in-the-mouth.

1.2. Introduction

Cellulose, in its natural form, is a very hydrophilic polymer. Since cellulose molecules form strong intermolecular hydrogen bonds with each other, unmodified cellulose is insoluble in water. When some of the hydroxyl groups in the cellulose are substituted by hydrophobic groups, such as methyl or hydroxypropyl groups, the number of intermolecular hydrogen bonds is reduced, and the molecule becomes water-soluble (Xu, Li et al. 2004). Methylcellulose (MC) is the name given to cellulose substituted with methyl groups only. Hydroxypropylmethylcellulose (HPMC) is the name given to cellulose substituted with both methyl and hydroxypropyl groups. Structures of such molecules are shown in Figure 1-1.

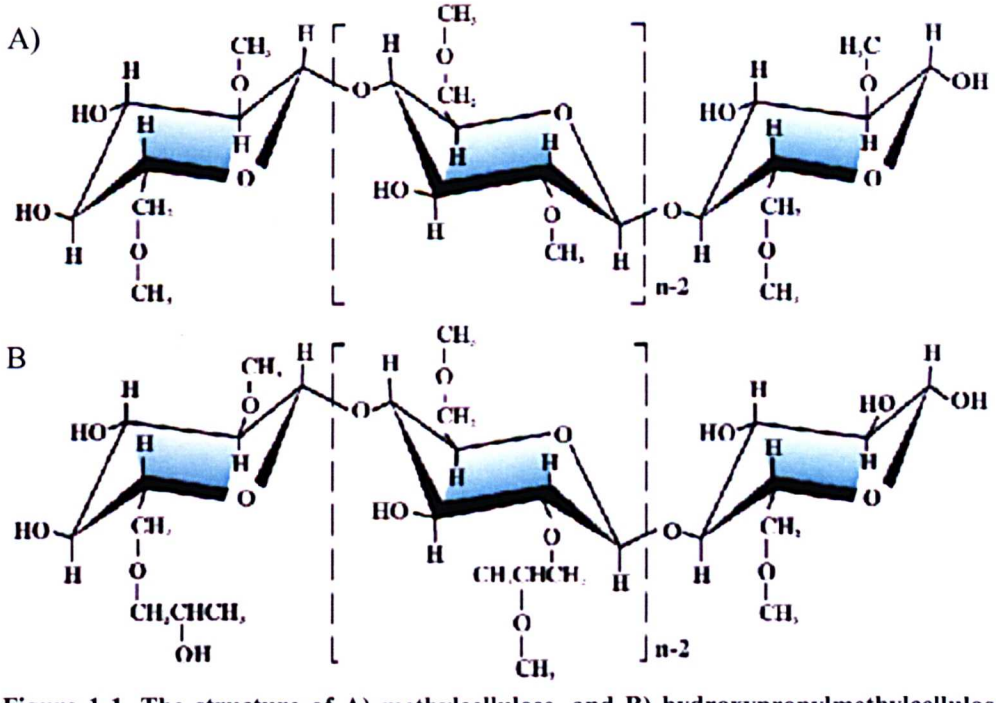

Figure 1-1. The structure of A) methylcellulose, and B) hydroxypropylmethylcellulose. Taken from www.dowchemicals.com.

In their solution state, there is very little interaction between the modified cellulose polymer chains, and they exist as aggregated "bundles", held together by packing of unsubstituted/sparingly substituted regions and by hydrophobic clustering of methyl groups in more densely substituted regions. In the early stages of heating, there is a partial dissociation, or swelling, of the clusters, followed by the separation of strands at the ends of the bundles, exposing methyl groups to the aqueous environment, which form structured water cages. At higher temperatures, these cages are disrupted and the polymer chains gradually lose water, which is followed by the formation of the final gel network by hydrophobic association of strands radiating from the different bundles (Sarkar 1979). The temperature at which this transformation occurs in known as the gelling temperature. A schematic representation of this process is shown in Figure 1-2.

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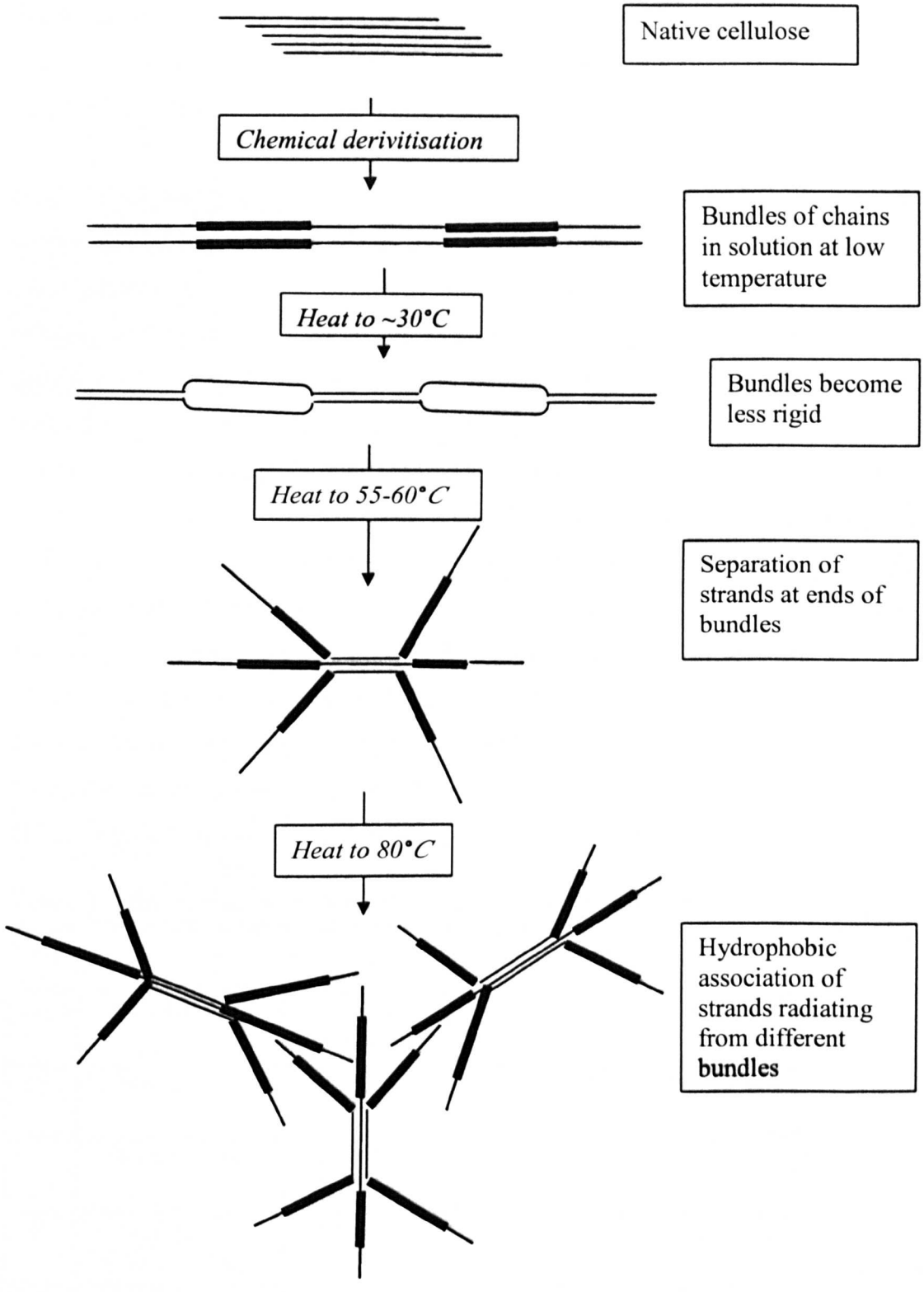


Figure 1-2. Schematic illustration of the postulated structures and processes involved in the thermogelation of Methocel. (Adapted from (Haque and Morris 1993)).

When removed from the heat source, the gel will start to melt and eventually become a clear liquid again. The temperature at which this occurs is known as the "melt-back" temperature.

Dow Chemicals Ltd, a major supplier of the modified celluloses (tradename Methocel*) offer five different types for commercial purchase, and each differs in their relative methyl and hydroxypropyl substitutions (see Table 1-1). The substitution type affects the exact temperature at which a modified cellulose solution gels, the strength of the resulting gel, as well as the temperature at which this gel melts back to a liquid. Within each type, molecules of different molecular weights are available. According to Sarkar (1979), it is convenient to describe molecular weight in terms of the viscosity it imparts to a solution, since the two are related, so different molecular weight molecules are often referred to by Dow according to their viscosity at 2% at 20°C. Low viscosity grades have lower molecular weights than high viscosity grades. The lowest viscosity grade imparts virtually no viscosity to a product, whereas the high viscosity grades impart high viscosities at relatively low concentrations (www.dow.com/methocel/food/prodline.htm - 04/05/2005). Molecular weight, however, unlike substitution type, has been reported to have little effect on gelling temperature (www.dow-answer.custhelp.com, 04/05/2005).

Table 1-1. Table showing the substitutions, the gelling and melt back temperatures, and the gel texture, for the five different modified cellulose groups. Trademark of the Dow Chemical Company

Type	Substitution	Gelling temperature	Melt-back temperature	Gel texture
SG	Methyl only	38-44°C	15°C	Very strong
A	Methyl only	50-55°C	25°C	Strong
E	Methyl and Hydroxypropyl	58-64°C	~50°C	Semi-firm
F	Methyl and Hydroxypropyl	62-68°C	~35°C	Semi-firm
K	Methyl and Hydroxypropyl	79-90°C	~50°C	Soft/ mushy

1.2.1. Preliminary work

Preliminary experiments focussed on evaluating the temperatures at which sol-gel transitions for each of the five types occur, as well as the texture of the gels formed. Solutions of similar viscosity grades were prepared at 1-2% for each of the five different substitution types. Solutions were gelled at 60°C for the SG and A types, and 85°C for the E-F- and K-types, since preliminary experiments showed these temperatures produced well formed gels that did not display syneresis (syneresis is defined as the contraction of a gel accompanied by the separating out of liquid). Results on melt-back temperatures and gel textures were in agreement with the above table (unpublished data). Since gels made from the SG- or A-type methylcelluloses needed to be cooled down to 25°C or below in order to melt, these gels would stay in their gelled state, and therefore not melt, at mouth temperatures. Gels made from the K-type hydroxypropylmethylcelluloses had a very weak mushy structure that made them unmanageable to work with, so were not considered for the melt-in-the-mouth application here. Therefore for the purposes of this investigation, only the E-type and the F-type HPMCs will be considered, since gels made from these groups had both melting temperatures and textures that were suitable. Although the guidelines from the above table suggest that the E-type HPMCs may have more appropriate melting temperatures, the guidelines given for melting temperatures are not very precise.

For both the E-type and the F-type classes, Dow Chemicals Ltd offers four different viscosity grades (see Table 1-2), so eight different modified celluloses were considered for this application. The molecular weights of each viscosity grade were recently determined by Keary (Keary 2001) for many of the Dow products commercially available (see Table 1-2), however the molecular weight of a particular viscosity grade has been reported to have a moderately wide distribution (Linda Bellekom-Allen, personal communication).

Although the modified celluloses are commonly used in the food industry, they are always added with other ingredients to *improve* heat stability. They are rarely used in isolation to make gels. Therefore, information on their minimum gelling concentrations was not available. It has been reported that different viscosity grades are required at different concentrations to form gels. According to the manufacturers, low viscosity varieties require higher concentrations to gel to ensure sufficient

intermolecular associations (www.dow-answer.custhelp.com, 04/05/2005). Therefore, time was spent calculating the minimum gelling concentrations for each of the eight different modified celluloses. Details of the methods used can be found in the Materials and Methods section, but the ranges of concentrations at which the different celluloses could form manageable gels are outlined in Table 1-2. For simplicity, solutions containing these HPMCs at their minimum gelling concentration will be referred to by their DOW trade name e.g. "E5" refers to a sample containing the Dow product E5 at its minimum gelling concentration.

Table 1-2. Viscosity range available from Dow Chemicals for the E- and F-type HPMCs. All data listed is according to the product specification sheet.

Dow Methocel* Trade Name	Viscosity of 2% aqueous solution (mPas)	Average molecular weight according to Conklin publication (Da)	% methyl substitutions	% hydroxyl-propyl substitutions	Range for minimum gelling concentration (as calculated by preliminary work)
E5	4-6	28,700	28-30	7-12	8.5-9.5%
E15	12-18	60,300	28-30	7-12	7-8%
E50	40-60	86,700	28-30	7-12	4.5-5.5%
E4M	3,000-5,600	323,000	28-30	7-12	2.5-3.5%
F50	40-60	89,400	27-30	4-7.5	5.5-6.5%
F450	400-630	-	27-30	4-7.5	2.75-3.25%
F4M	3,000-5,600	341,500	27-30	4-7.5	~3%
F220M	5,500-8,800	-	27-30	4-7.5	1.5-2%

As expected, the higher the molecular weight or "viscosity grade" of the molecule, the lower the concentration needed to form a gel. For low molecular weight molecules, a larger number of molecules, or higher concentration, is needed to ensure that there are enough hydrophobic groups present, and in adequate proximity, to allow gelation.

In order to display melt-in-the-mouth behaviour, the gels formed from these E and F-type HPMCs need to melt at temperatures *above* mouth temperature. Therefore, initially an investigation into mouth temperature was carried out.

1.2.2. Investigation into mouth temperature

Although it is universally accepted that human body temperature, and therefore mouth temperature, is 37°C, it was important to see to what extent, if at all, this temperature varied between different people. Many different people eat at any given restaurant, and for this melt-in-the-mouth concept to work, the gel would need to melt in the mouth of each and every diner. Therefore, mouth temperatures of a range of people, modelled to mimic a selection of potential diners, were taken. Furthermore, because this gel would be eaten as part of a meal, most probably the tasting menu, it was important to ensure that mouth temperature did not change by a large amount after eating something very hot or very cold, and if it did, how long it took to return afterwards to its original temperature. This, therefore, was also investigated.

1.2.3. Investigation into the melting temperature of the gels

Events that occur during gelation and melting of the modified celluloses can be monitored using a number of techniques. The most commonly used techniques are rheological, microcalorimic, and turbidimetric (Sarkar 1979; Haque and Morris 1993; Haque, Richardson et al. 1993; Sarkar 1995; Sarkar and Walker 1995; Nishinari, Hofmann et al. 1997; Hussain 2002; Xu, Wang et al. 2004; Zheng, Li et al. 2004; Sanz, Fernandez et al. 2005).

1.2.3.1. Turbidimetric techniques

The gelation of modified cellulose solutions is associated with hazing of the solution just prior to gelation, and as the gel melts again, this hazing is lost. Turbidity changes in a solution can be quantified by reduction or augmentation in the amount of light transmitted through a sample (Haque and Morris 1993). The temperature at which light transmission is reduced to 50% on heating is known as the cloud point, and this has been used by authors as an indication of gelling temperature (Sarkar and Walker 1995; Zheng, Li et al. 2004; Sarkar 1979). The point at which the light transmission reaches 50% on cooling (or the return cloud point) has similarly been used to indicate melting temperature (Li, Shan et al. 2002; Zheng, Li et al. 2004). However, this method could not be used for the investigation here because a heat controlled calorimeter was not available.

1.2.3.2. Rheological techniques

Rheology is the study of the deformation and flow of matter. The term was coined by Eugene C. Bingham in the early 20th century, and is derived from the Greek expression "panta rhei", meaning everything flows. According to Haque and Morris (1993), the first systematic study into the thermogelation of hydrophobically substituted materials used measurements of solution viscosity to monitor the onset of gel formation, and was carried out by Heymann in 1935. More recently, Sarkar (1979) used similar methods to monitor the thermogelation of cellulose derivatives – as the temperature of modified cellulose solutions is raised, the viscosity of solution decreases until the temperature reaches the incipient gelation temperature, at which point there is a sharp rise in viscosity, indicating gelation. However, other authors criticise this method of determining gelation, arguing that gelation should be considered in terms of the viscoelastic properties of the system, rather than the viscosity alone (Hussain 2002). Furthermore, "large-deformation" viscosity measurements may disrupt delicate networks as they form. Therefore, the reversible thermogelation of the solutions under study here will be followed using non-destructive oscillatory measurements, as has previously been done (Haque and Morris 1993).

Rheometers measure the mechanical properties of viscous liquids, elastic solids, and substances with both elastic and viscous properties (termed viscoelastic). Deformation is principally observed in solids, and flow in liquids, and therefore measurement of how a sample deforms or flows will indicate the contribution of liquid-like and solid-like properties the sample has. During oscillatory tests, samples are subjected to an oscillatory shear. A shear stress refers to a force that is applied in the direction of a tangent to the surface of a body. When stress is applied to a material, the material deforms or flows. Strain represents the relative deformation of matter. For an elastic body, an applied stress will produce deformation. For a viscous liquid, a deformation will occur with a time delay. Solid and liquid response can therefore be characterised independently by resolving the overall resistance into components "in-phase" and "out-of-phase" with the imposed deformation, quantified as the "storage" and "loss" moduli (Haque and Morris 1993). The loss modulus, also known as the viscous modulus (G''), represents the liquid-like components of a

sample. The storage modulus, also known as the elastic modulus (G'), represents the solid contributions.

The stress and deformation cycle of a sample is shown in Figure 1-3.

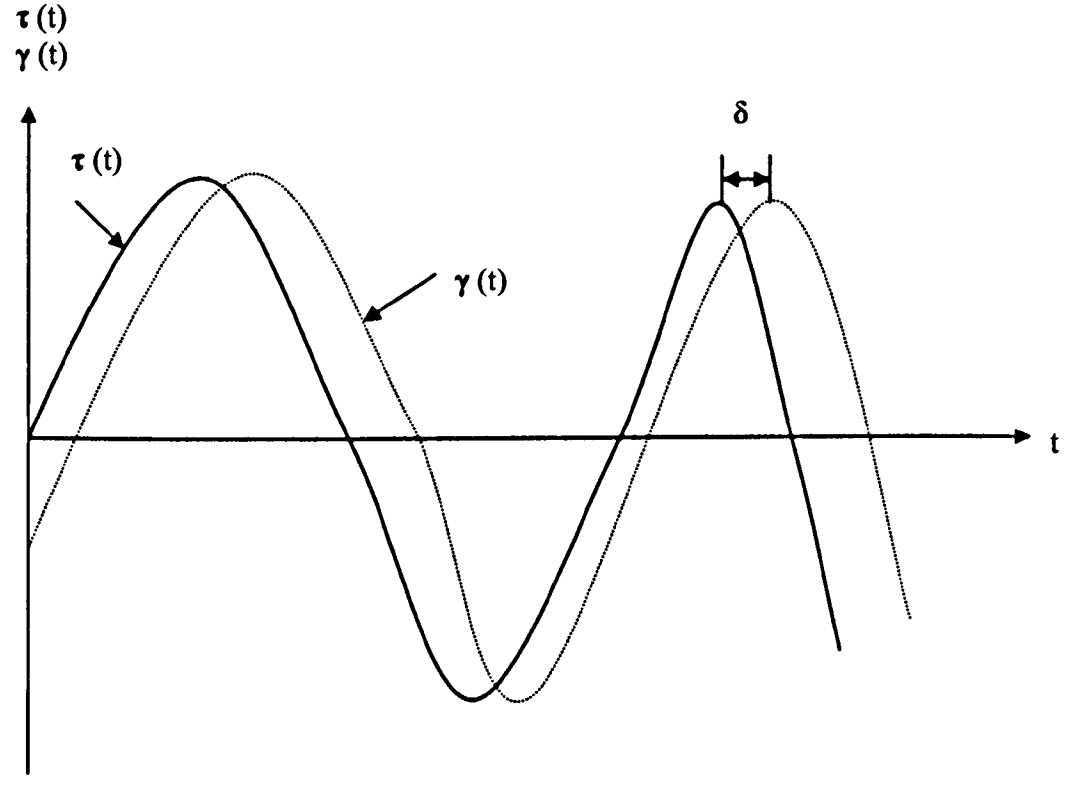


Figure 1-3. The relationship between stress and strain in the oscillation test. τ=shear stress, γ=shear strain, δ=phase angle, t=time.

As this diagram shows, δ therefore measures the shift in phase of the deformation (strain) to the applied stress, and ranges from 0 to 90°. If there is no phase shift (i.e. δ = 0°), G'' will be zero, hence the sample will only have elastic properties and can be seen as an elastic body (i.e. a solid). When phase shift is maximal (i.e. δ = 90°) G' will be zero, hence the sample will only contain viscous components, and can be seen as a viscous body.

Oscillatory tests therefore allow the determination of the viscous and elastic contributions in a sample independently, and are therefore the most common method of studying the viscoelastic behaviour of a food, since foods in general contain both elastic and viscous components. Since gelation involves a transition from a predominately liquid substance to a gel (predominately solid), measuring changes in

the two moduli with temperature is ideal for monitoring temperature driven sol-gel transitions. In solution states, the viscous modulus (G'') values tend to be considerably larger than elastic modulus (G') values. As a solution passes from a fluid to a fully gelled state, which occurs on heating for the modified celluloses, a gradual transition occurs in the elastic modulus from a value initially lower than G'' to a final value that is higher than G'', indicating that a gelled network has formed (Sarkar 1995). Tan δ measures the ratio of the contribution of elastic and viscous modulus (tan δ = G''/G'). A large G' value in comparison with G'' indicates pronounced elastic (gel) properties of the product being analysed, and therefore the value for tan δ will be low. Conversely, a large G'' value in comparison with G' indicates pronounced viscous (liquid) properties of the product, and consequently tan δ will be high. On heating modified cellulose solutions, tan δ therefore decreases from an initially high value to a low value, upon formation of the gel. A decrease in tan δ on heating, and a subsequent increase on cooling with evident thermal hysteresis, has previously been noted for the reversible thermal gelation of both methylcelluloses and hydroxymethylcelluloses (Haque and Morris 1993; Haque, Richardson et al. 1993). The point at which G' and G'' are equal (or tan δ equals 1) may therefore be used to indicate gelling and melt back temperatures, since this is the point at which elastic (or solid) properties and viscous (or liquid) properties contribute equally. This method was first used by Tung and Dynes (1982) to measure the gel point of cross-linking polymers.

1.2.3.3. Differential scanning calorimetry (DSC)

The formation and dissociation of ordered structures (i.e. gels) that occurs on changing temperature are normally accompanied by enthalpy changes. These can be detected by sensitive calorimetric measurements, and enable the user to monitor the transitions that are occurring in the sample, and measure the temperature at which they occur. DSC has been used by a number of authors to characterise gelation and melting processes of methylcelluloses (Nishinari, Hofmann et al. 1993; Li, Shan et al. 2002).

According to Haque and Morris (1993), it is generally agreed that the driving force to gelation of hydrophobic polymers at elevated temperatures is their disruptive effect

on the structure of water. As in any chemical process, the relative stabilities of the solution and gel states are determined by the difference in free energy (ΔG) between them, which in turn is determined by the temperature and the differences in enthalpy and entropy (ΔH and ΔS, respectively):

$$\Delta G = \Delta H - T\Delta S$$

Conversion from one state to the other will only occur when ΔG is negative (i.e. if the overall free energy of the system is decreased), which requires a negative value of ΔH and/or a positive value of ΔS.

<u>During melting of normal polysaccharide gels:</u>

In thermal melting of normal polysaccharide gels, ΔS is positive, because of the increase in chain mobility, but ΔH is also positive, because the system must absorb heat to disrupt the bonding within the intermolecular "junction zones". The two effects balance exactly at the transition-midpoint temperature ™, where ΔG is zero:

$$\Delta H = Tm\ \Delta S.$$

At higher temperatures, $T\Delta S > \Delta H$, giving $\Delta G < 0$, so that the system shifts to the solution state. At lower temperatures, $T\Delta S < \Delta H$ and $\Delta G > 0$, favouring the gel state.

<u>Solutions containing polymers with hydrophobic substituents:</u>

Solutions containing polymers with hydrophobic substituents are in the low-entropy, low-enthalpy state (the exact reverse of the situation in conventional polysaccharide gels). The low enthalpy values are thought to be due to the hydrocarbon-water interactions being more favourable than hydrocarbon-hydrocarbon interactions, while the low entropy is normally interpreted as distortion of the hydrogen bonding between water molecules to give highly constrained "cage-like" structures around the foreign species (if these themselves form hydrogen bonds with water). Raising the temperature increases the relative importance of the entropy term, and therefore promotes gelation. Hydrophobic gelation could to some extent be seen as a "melting" process (disruption of the water cages).

For the purposes of this investigation, both rheological and calorimetric methods will be used to characterise the changes occurring to the eight modified cellulose solutions on heating and cooling, in order to determine their melting temperatures. Although these fundamental methods can provide a lot of interesting information, analysis of the data obtained is complex, and furthermore these are not methods that can be used by the restaurant to characterise samples in the future. Therefore, a more

empirical method was also sought that could provide similar information to these more advanced methods of measuring melting temperature, but which could be carried out in the developmental kitchen of the restaurant.

1.2.3.4. The falling ball technique

This technique is an empirical method that involves heating a gel slowly until a ball bearing placed on the top of it starts to fall (Taylor, Besnard et al. 2001). The temperature at which this occurs is taken as the gel melting temperature. This technique was modified slightly to measure the melting temperature of hot HPMC gels as they cooled. However, preliminary work showed that this technique was not appropriate for the following reasons:

- The slightly uneven way in which the HPMC gels melted sometimes allowed the ball bearing to "fall" before it had fully melted. Furthermore, some gels displayed syneresis - this allowed the ball bearing to pass through the water layer surrounding the gel and "fall" prematurely.
- For samples containing the higher viscosity grades, even when the gel *had* fully melted the ball bearing could not move easily through the liquid. Furthermore, sometimes a skin remained on the surface of the melted solution, preventing the ball from "falling" at all.

To overcome some of these problems, a modification was made whereby the ball bearing was attached to an electrical circuit, which was only completed when the ball bearing had completely fallen, rather than just started to fall (see Figure 1-4).

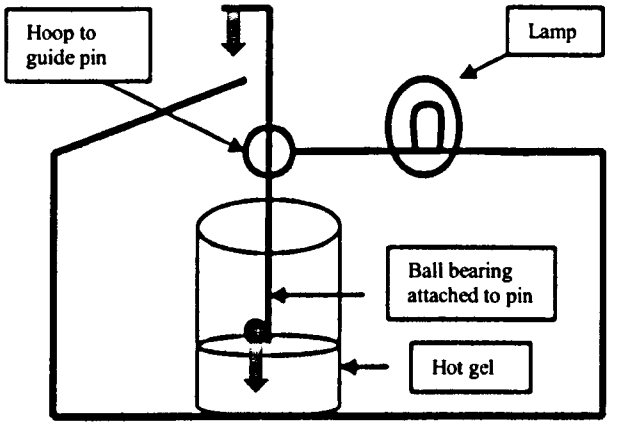


Figure 1-4. Principle of the "ball bearing attached to electrical circuit" technique

A prototype was made based on this principle; however there were still several practical limitations with this technique:

- Friction at the area where the pin passed through the hoop sometimes prevented the ball from falling
- The additional weight now attached to the ball bearing put extra pressure on the gel, sometimes forcing the ball through the gel even when it was still gelled. This was especially a problem for the weaker gels.

Therefore, the ball bearing was substituted with a disk of polytetrafluoroethylene (PTFE), a non-wetting material with low surface tension. It was thought that this disk would float on the gels surface without placing any force on the gel, and only start to move once the gel had melted. However, when the prototype was tested, the disk did not "fall" down immediately upon gel melting, and was greatly slowed down by the viscosity of the solution. Therefore, results obtained from this method were largely affected by solution viscosity (however repeating this test with solutions prior to gelation would allow the contribution of viscosity to be eliminated).

Other methods were therefore devised for measuring melting temperature and were tested, including:

- Placing a dyed gel in a beaker of water at 37°C and measuring the temperature of the gel at the point where colour started to move into the water (i.e. the gel started to melt).
- Monitoring the appearance of a "cross", marked on the bottom of the beaker, as the gel melted and lost its turbidity.
- Monitoring rate of the flow of the solution through a Buchner funnel as the gel melted.

However, all the above techniques had significant limitations that made them inappropriate for use.

1.2.3.5. Visual observations

Takahashi and Shimazaki (2001) simply used visual inspection as a technique to measure gelling and melting temperatures of various methylcellulose solutions, since the changes associated with the gelation process, namely hazing and a solidification, are easily visualised. A technique based on this, called the "tip-test", was thus

devised, and subsequently used in this investigation as the empirical method to determine the melting temperatures of the eight hot HPMC gels.

1.2.4. Factors affecting melting temperature

Various different factors have been reported to affect the gelling and melting temperatures of modified cellulose gels. According to the manufacturers, as the concentration of a modified cellulose is increased, the gelation temperature is lowered (www.dow-answer.custhelp.com, 04/05/2005). Furthermore, it has been reported that both a high gelation temperature and a long gelation time lower the subsequent melt-back temperature of a methylcellulose gel (Kato 1978). Since the melt back temperature of the gel is of key importance for this melt-in-the-mouth concept to work, it is crucial to understand the effect of factors such as gelling temperature, time and concentration on subsequent melt-back temperature, since these will have important implications for the restaurant in terms of the flexibility allowed during the preparation of these gels. It will indicate the temperature and time frame during which these gels could be prepared without affecting subsequent melting temperature. This was investigated using both the empirical and fundamental techniques described above. Only one sample was investigated for this part of the analysis.

1.2.5. Investigation into gel temperature on consumption

For the application under consideration here, this gel would be consumed hot. It was therefore important to know at what temperature this hot gel could be served without burning the mouth. Therefore, investigations were carried out into measuring the exact temperature of these hot gels after preparation, as well as their changes in temperature with time, and how this related to the temperature at which it is acceptable to consume a hot product. Gels were therefore prepared, and the temperature at their core was measured with a thermometer every minute for 10 minutes. All eight HPMC samples were analysed, because it was thought that the different HPMCs may have different heat capacities, which may affect their temperature on gelation and their rate of temperature decrease on removal from the heat source.

1.3. Objectives

- To calculate average mouth temperature, and investigate how the melting temperature of the eight gels (as measured by both fundamental and empirical methods) compares to this temperature, to ascertain whether the gels would melt at mouth temperature.
- To understand further what factors affect the melting temperature of these gels, in order to understand further the flexibility allowed in the preparation process.
- To understand at which temperature these gels could be served for the concept to work, without causing any discomfort to the consumer.

1.4. Materials and Methods

1.4.1. Investigation into mouth temperature

1.4.1.1. Natural variation in mouth temperature between individuals

Temperature of the middle of the mouth was measured using an infrared thermometer (UltimaxJR portable infrared thermometer, Ircon Inc, California). Eight subjects (3 male) were tested, selected from the Division of Food Sciences, University of Nottingham based on their willingness to participate. Triplicate measurements were made, so that the natural variation in temperature in a single mouth could also be monitored.

Temperature accuracy of the gun was checked by comparing temperature readings of a water bath set to a particular temperature to those given by both a temperature probe (TinyTag, Chichester, UK) and a normal mercury thermometer. According to a one factor ANOVA test, temperatures from the three devices were not found to differ significantly ($p=0.122$), indicating that the three different measuring devices are measuring similar, and therefore it is thought accurate, temperatures.

1.4.1.2. Variation in mouth temperature after consuming very hot, or very cold, products

Only one subject (male) was used for this study. Initially, triplicate measurements of the subject's mouth temperature were taken to obtain an ambient temperature value. Temperatures were then retaken immediately after consuming ice-cream or drinking tea. Measurements were taken every 5 seconds, in order to calculate how long the temperature took to plateau out to near the original value.

1.4.2. Sample Preparation

1.4.2.1. Solution preparation

The HPMC powder (Dow Chemicals Ltd) was dispersed in hot distilled water using a high speed stirrer (Yellowline, Fisher Scientific, Loughborough, UK) at 700 rpm for 10 minutes. The mixture was then transferred to a refrigerated room

(approximately 6°C) and stirred further for at least two hours at 200 rpm to obtain a uniform and transparent solution. Solutions were covered and refrigerated until needed. Solutions that were to be used for rheological and calorimetric studies were prepared with sodium azide (Fisher Scientific, Loughborough, UK) (0.054% ± 0.008% w/w) to prevent degradation of the solutions over the time course of the investigation.

1.4.2.2. HPMC concentration

Preliminary work – calculation of minimum gelling concentration:

HPMC solutions were initially prepared at concentrations inversely proportional to viscosity grade, and small beakers containing approximately 20g of solution were gelled in a water bath at 85°C for one hour. Beakers were then removed and assessed visually for gelation. If solutions did not gel, they were re-made at a higher concentration. Solutions that did gel were subsequently prepared at a slightly lower concentration, in order for the approximate minimum gelling concentration range of each solution to be calculated, as shown in Table 1-2.

Concentrations used for the investigation:

Solutions were prepared at the concentrations outlined in Table 1-3 for the rest of the investigation, since these concentrations fell within the ranges for gelling concentration calculated from preliminary investigations.

Table 1-3. Concentrations of the eight HPMC solutions, given as both a percentage (w/w), as well as in molar, as calculated using approximate molecular weights given by Keary (Keary 2001). For F450 and F220M estimates of molecular weight are made (at 150,000 and 1,000,000 Da respectively) since the molecular weights of these viscosity grades had not previously been determined.

Solution name	Actual concentration (in % w/w)	Concentration (in nM)
E5	8.47	2.95
E15	6.99	1.16
E50	5.05	0.58
E4M	3.04	0.09
F50	5.64	0.63
F450	2.94	*~ 0.20*
F4M	2.97	0.09
F220M	1.50	*~ 0.02*

1.4.3. Measurement of melting temperature

1.4.3.1. By the tip-test

Small 100ml beakers (Schott) containing 20g (± 0.1g) of solution were covered in foil and placed in an oven set at 85°C (actual temperature over the time course of the experiment was 82.1 ± 1.8°C) for an hour. Preliminary experiments had shown that at 85°C the HPMC solutions formed manageable gels (see section 1.2.1), and that after an hour at this temperature the core of the gel had reached a maximum and constant temperature (data not shown). Beakers were placed in the oven at staggered time intervals, to limit the numbers of samples that needed to be assessed subsequently at a single time point. The effect of opening the oven door at various different time intervals (including once an hour, once every half hour, once every 15 minutes and once every 10 minutes) on overall oven temperature was also investigated. On average, it took just under 2.5 minutes for the oven temperature to return to ambient after opening the door. The more frequently the oven door was opened, the quicker the temperature returned to ambient – it took just over 2 minutes to return to ambient temperature after opening the door every 10 minutes (data not shown), which was seen as reasonable, so this time interval was used. After an hour

in the 85°C oven, beakers were removed and placed in ovens or incubators set to temperatures ranging from 30°C to 65°C, which covered the temperature range over which these solutions were expected to melt. After an hour at this lower temperature, solutions were removed from the oven/incubator and immediately assessed for gelation. Preliminary experiments had shown that one hour at this reduced temperature was sufficient for the inside of the gel/solution to reach oven/incubator temperature (data not shown). Incubator or oven temperature was constantly monitored during the experiment and the *true* temperature is quoted each time.

Criteria used to assess gelation:

The gelation/melting state needed to be assessed as quickly as possible, so only criteria that could be assessed while the sample was still in its beaker were used.

- "Is the gel cloudy (i.e. gelled) or clear (i.e. melted)?" (called the "Cloud-Test")
- "Does it stay in a set position when the beaker is tipped (i.e. gelled) or does it flow freely (i.e. melted)?" (called the "Tip-Test")

Gels made from all eight samples passed the criteria for gelation. After melting fully, all eight solutions were clear and, with the exception of F220M (the highest viscosity grade), moved freely when tipped. This empirical method was therefore not suitable for characterising sample F220M, so was not used for this sample.

1.4.3.2. By rheology

In general, rheological methods were conducted on a dynamic shear stress rheometer in controlled strain (Bohlin CVOR), fitted with a cone and plate geometry (angle 4°, diameter 40mm). Approximately 2g of sample was transferred to the rheometer from cold before measurements.

Oscillatory measurements

To measure melting temperature, temperature ramps were performed at a rate of 2°C per minute from 5 and 85°C. 85°C was chosen to be consistent with the tip-test. A rate of 2°C per minute had previously been used by other authors (Hussain 2002). To prevent dehydration during rheological measurements, a thin layer of low viscosity

oil was placed on the peripheral surface of the sample held between the plates. Triplicate measurements were taken for each sample.

The sample was heated by a Peltier element attached to the rheometer, to make the heating process as efficient as possible. The dynamic viscoelastic functions of shear storage modulus (G') and loss modulus (G'') were measured as a function of time and temperature. Haque and Morris had previously used a frequency of 10 rad s^{-1} and a shear strain of 1% to characterise HPMC samples (Haque, Richardson et al. 1993) and amplitude sweeps were also carried out over a range of strain to ensure that at 1% strain samples were within the linear viscoelastic region. For all samples except sample E5 and to some extent sample E50 in the gelled state (where values of G' and G'' had started to decrease at 1% strain), 1% strain was well within the linear viscoelastic region for all other samples (see Figure 1-5 and Figure 1-6).

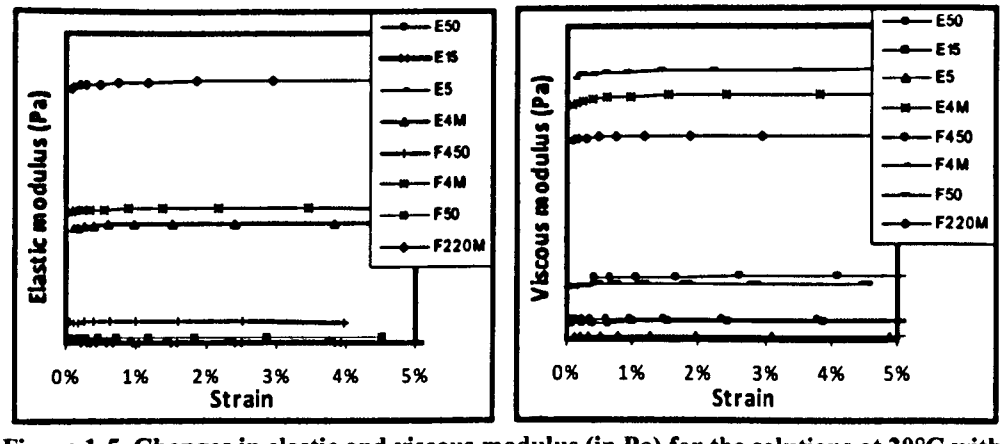


Figure 1-5. Changes in elastic and viscous modulus (in Pa) for the solutions at 20°C with varying strain.

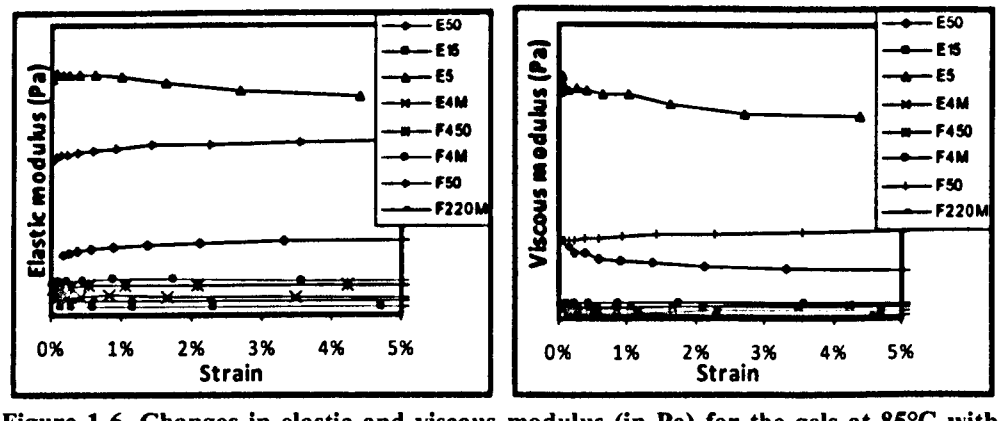


Figure 1-6. Changes in elastic and viscous modulus (in Pa) for the gels at 85°C with varying strain.

For all the eight solutions tested, values of tan δ decreased on heating and increased on cooling, indicating that gelation is indeed occurring (see Figure 1-7). An evident hysteresis was observed, as has previously been reported.

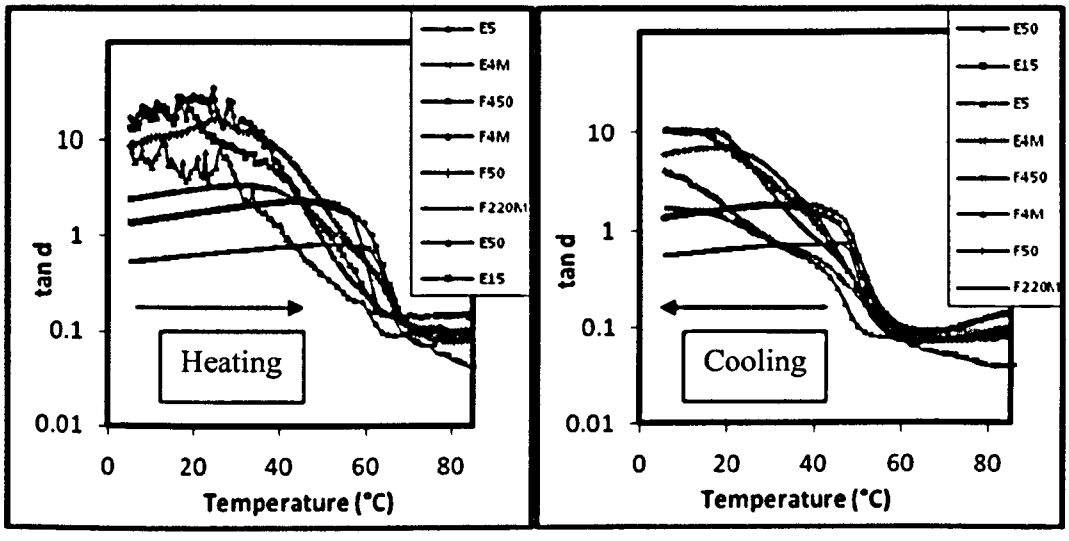


Figure 1-7. The changes in tan δ on heating and cooling HPMC samples at 2°C per minute, with an angular frequency of 10 rad s^{-1} and a shear strain of 1%. For simplicity, only one repeat for each sample is shown.

For some of the oscillatory measurements, although the value for tan δ decreased on heating as expected, it did not increase on subsequent cooling, or if it did, it did not increase as it had decreased on heating (unpublished data). This indicates that true reversible thermogelation was not occurring in these measurements, and this data was therefore *not* included in the analysis. For these samples, measurements were repeated in order that three values of G'/G'' crossover was obtained for each sample.

1.4.3.3. By microcalorimetry

The calorimetric experiments are carried out using a Micro DSC Calorimeter from Setaram (Caluire, France) equipped with batch cells (made from Hastalloy) containing approximately 0.8ml of solution. Distilled water was used as the reference. Weights in the two cells of the calorimeter were adjusted so that the overall heat capacity of each was the same, using Equation 1-1:

Reference cell: **Sample cell:**

Cp ref x mass of ref = (Cp cellulose x conc cellulose) + (Cp water x conc water)

Where ref = reference (in this case water), Cp water = 4.2, Cp cellulose = 2

Equation 1-1. Calculation used to adjust the heat capacity in the reference and sample cell.

Although weights in the two cells of the calorimeter were adjusted, the overall heat capacities of the two cells were not always exactly equal. Net heat therefore had to go into either the sample vessel or the reference vessel, which resulted in shifting the endotherm either up or down relative to the exotherm, as shown in Figure 1-8.

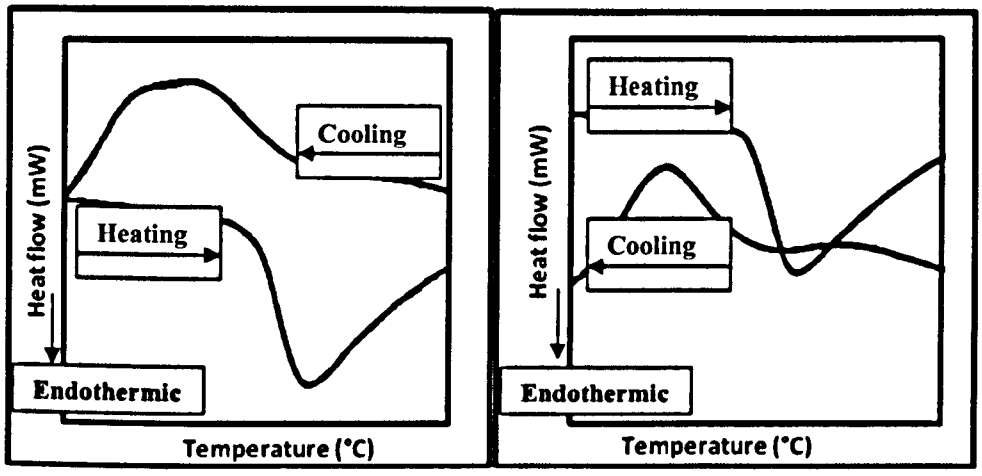


Figure 1-8. An example of the shifting of the endotherms and exotherms due to imbalanced heat capacities of sample and reference vessels

Initially temperature was ramped from 5°C to 85°C in order to be consistent with rheological measurements, but thermograms showed that processes were still occurring at 85°C. Higher temperatures (95°C) were needed to ensure that the heat flow returned to baseline values (as shown in Figure 1-9).

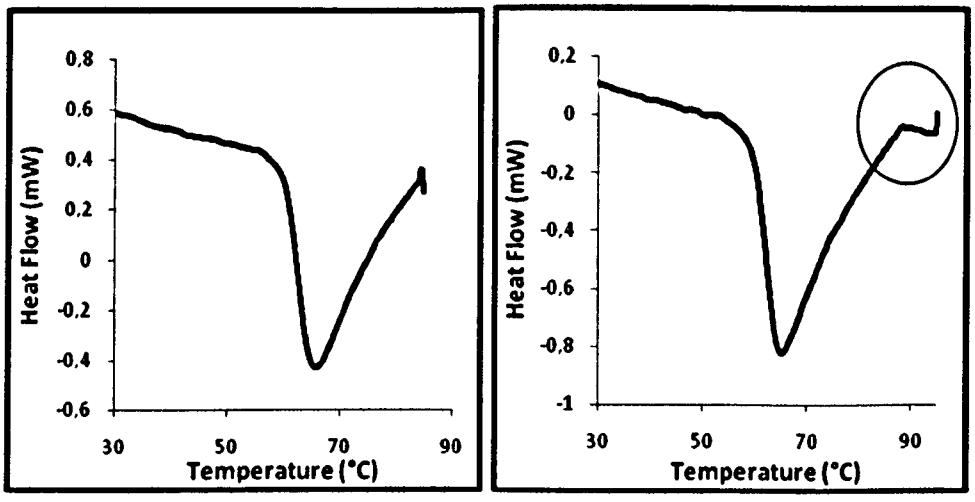


Figure 1-9. Effect of heating temperature on the return of heat flow to baseline values. The graphs show the absence of a return of the baseline when samples are only heated to 85°C.

Furthermore, a heating rate of 2°C per minute could not be used on the DSC (the maximum scanning rate is 1.25°C min^{-1}), so the lower rate of 1°C was used. This rate

had previously been used by Li, Shan et al (Li, Shan et al. 2002) to monitor enthalpic changes in modified cellulose samples.

The solution was initially left to equilibrate at 20°C before starting the cycle, to allow the heat flow value to stabilise. The following temperature profile was used:

- Cool the solution to 10°C
- Leave to equilibrate for 300s at this temperature.
- Heat from 10°C to 95°C at 1°C min^{-1}
- Cool down immediately at the same rate.
- Hold for a further 300s at 10°C before repeating the heat-cool cycle.

Calorimetric measurements were started at 10°C, rather than 5°C (which would have been consistent with rheological data) because the machine struggled to get to these low temperatures. Although slowing scanning rate may have improved this, because we were not interested in changes that occurred at these low temperatures, it was thought that a starting temperature of 10°C was adequate.

Vary cooling rate:

For some experimental runs, cooling rate was varied, to see how this affected subsequent melting events. For these runs, the standard cycle described above was followed by five successive heat-cool cycles, where cooling rates were set at 1.25°C min^{-1}, 0.1°C min^{-1}, 0.25°C min^{-1}, 0.5°C min^{-1}, and 0.75°C min^{-1} respectively. Heating rates and isotherms were unchanged.

Calculation of enthalpy values:

Enthalpy values were calculated using the software (Seteram, France) and visually choosing a baseline that extended from that observed at low temperatures.

1.4.4. Factors affecting melting temperature

In order to investigate the effect of concentration on melting temperature, a 4% solution of F450 was prepared, as according to section 1.4.2. A 5% sample was also prepared, but was shown to be too thick to be characterised using the tip test, and therefore was not included in this study. Concentrations below 3% were not investigated since the HPMC would be present at below its minimum gelling concentration, and would therefore not form a gel that could be analysed using the tip test.

1.4.4.1. Tip-test

20g (±0.1g) of 3% or 4% F450 was gelled in an oven either at 75°C or 95°C, for 40 or 80 minutes. Since the tip test required a gel to be formed in order to determine melting temperature, gels were prepared using both the mildest gelling conditions (i.e. 75°C for 40 minutes at 3%), and the harshest gelling conditions (i.e. 95°C for 80 minutes at 4%) to check that over the whole range of gelling conditions a solid gel could be formed. This ensured that the melting temperature could be characterised using this method.

1.4.4.2. By calorimetric and rheological techniques

Samples of both 3% and 4% F450 were characterised using rheology and calorimetry according to the methods described above (see sections 1.4.3.2 and 1.4.3.3), except that maximum gelling temperature was set to either 75 or 85°C for calorimetric measurements, and 75 or 95°C for rheological measurements, however preliminary experiments showed that the Peltier element struggled to get the rheometer to this higher temperature, presumably due to significant water loss, so only the lower temperature of 75°C was used. Some samples were held isothermally at each temperature for 40 minutes to investigate the effect of holding on subsequent melting events. Unfortunately, the calorimeter has a built in security system to avoid damage to the thermostat that does not allow isotherms to be run at elevated temperatures. Temperatures could therefore only be held at 95°C for a shorter time period for 3% F450 (14 or 28 minutes respectively), and could not be held at all for 4% F450. The reproducibility in traces after holding gels at high temperatures on the rheometer was also poor. For some experimental runs, the phase angle did not increase on cooling as it should after the sample had been held at high temperatures, suggesting that the gel had formed irreversibly. Furthermore the error message "overload" appeared during these runs, suggesting that the force was too great for the transducer at these elevated temperatures. This was especially a problem when solutions of 4% F450 were tested.

1.4.5. Measurements on the temperature of the gel on consumption

Small 100ml beakers containing 20g (± 0.1g) of solution were gelled at 85°C (actual oven temperature was subsequently shown to be 81°C) for one hour. Gels were

placed in the oven at 15 minute intervals, and removed after one hour. The temperature at their core was immediately recorded using an infrared thermometer (UltimaxJR). Temperature readings of the core of the gel were taken every minute for the next 10 minutes, using both the infrared thermometer and a standard thermometer, as the gel cooled.

1.4.6. Calculate acceptable temperature at which to consume a product

Subjects were supplied with a hot liquid and asked to try and drink it. When it reached a temperature at which they

a) could
b) were happy to

consume it, they were asked to measure its temperature using a standard mercury thermometer. This gave an indication of the maximum temperature at which something can be consumed. Although the application studied here involves a solid, not a liquid, it was thought that the maximum temperature at which a hot gel could be consumed would not differ from that of a hot liquid.

1.4.7. Analysis of data

Data was analysed using both Design Expert (Version 6.0.2, Statease, Minneapolis USA) and Statistical Package for the Social Sciences (SPSS). Design-Expert is a program primarily used to design experiments, but can also be used to analyze data, and graphically display the results. Design-Expert software offers an array of design options and provides the flexibility to handle categorical factors and combine them with mixture and/or process variables. SPSS is a programme used for statistical analysis of data – it can carry out descriptive statistics, bivariate statistics, predictions for numerical outcomes, and predictions for identifying groups. In addition to statistical analysis, it also offers data management and data documentation. One factor ANOVA tests were carried out on data in order to see if temperature values for different samples differed significantly from each other. Where significance existed, Tukey tests (Mahony 1986) were used to work out which samples differed significantly from each other.

1.5. Results and discussion

1.5.1. Investigation of mouth temperature

The average mouth temperature recorded across all eight subjects was 36.3°C, and values ranged from 34.5°C to 38.1°C. A one-factor ANOVA test showed that mouth temperature differed significantly between subjects – subjects were separated out into two subgroups according to their mouth temperature. The largest variation in triplicate measurements for one person was 1.7°C. It is thought that the slightly different temperatures obtained on successive measurements for the same person could be due to the temperature being measured from slightly different parts of the mouth.

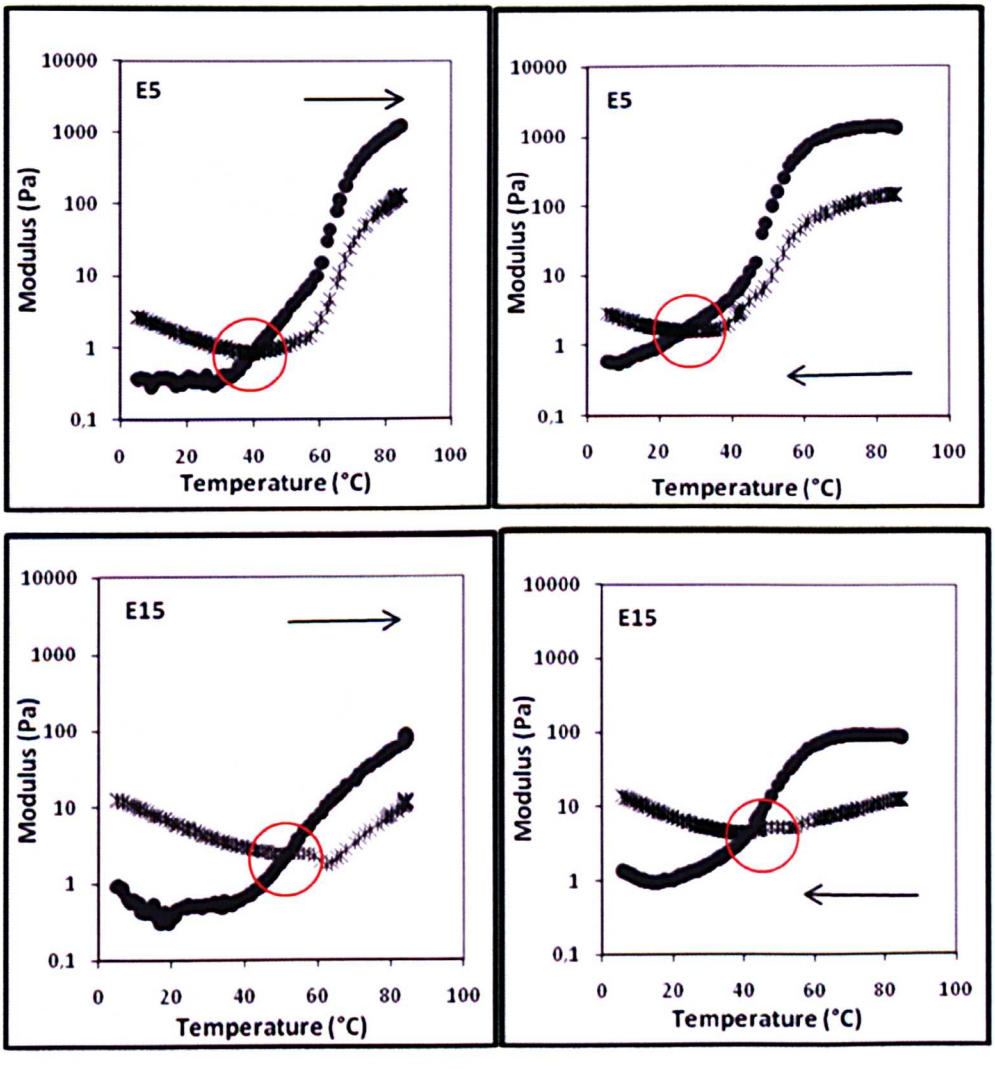
After consuming ice-cream, the subject's mouth temperature was immediately reduced by approximately 7.5°C, and took only about a minute to return to the near initial value. After consuming tea, mouth temperature rose slightly by approximately 6.5°C, and took about 2 minutes to return to near initial value. The mouth is surrounded by a good blood supply, which could explain the efficiency with which temperature returns to ambient after consuming either hot or cold products.

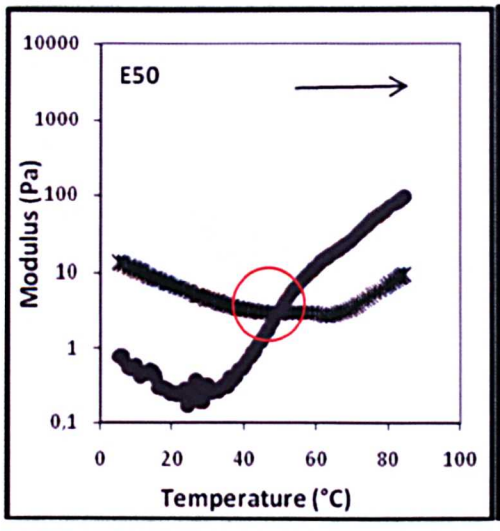
In the Fat Duck restaurant, dishes on the tasting menu are served approximately every 15 minutes. Therefore, there is ample time for mouth temperature to return to normal after consuming a hot or cold dish before the next dish is served. However, if the hot gel itself is to be served with something very hot, the impact of consuming both the hot accompaniment and hot gel itself will raise mouth temperature, so the melt-in-the-mouth gel would need to melt at temperatures higher than 37°C to ensure that it will still melt in the mouth, regardless of what is served with it. However, serving the hot gel with something cold would actually lower the mouth temperature temporarily and therefore, if anything, speed up subsequent melting of the gel.

1.5.2. Calculating the melting temperatures of the gels

1.5.2.1. The use of rheological methods to measure gelling and melting temperatures

Figure 1-10 below shows the changes in both elastic and viscous modulus on heating and cooling the eight HPMC samples. Values of elastic and viscous modulus, with the exception of sample F220M where values for the two moduli approach each other but never cross, cross once and only once during each process. The temperature at which the crossover occurs therefore could be a good indicator of gelling and melting temperatures.

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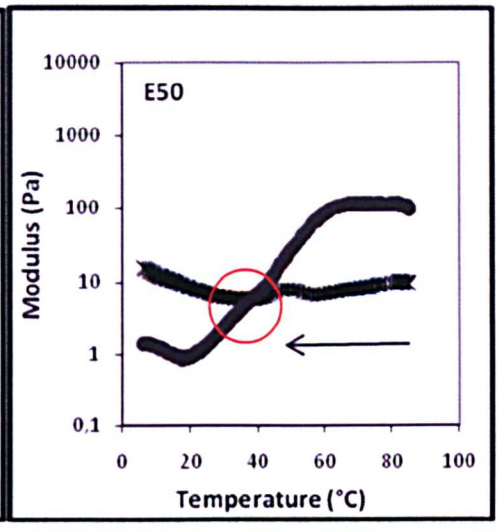
Temperature (°C)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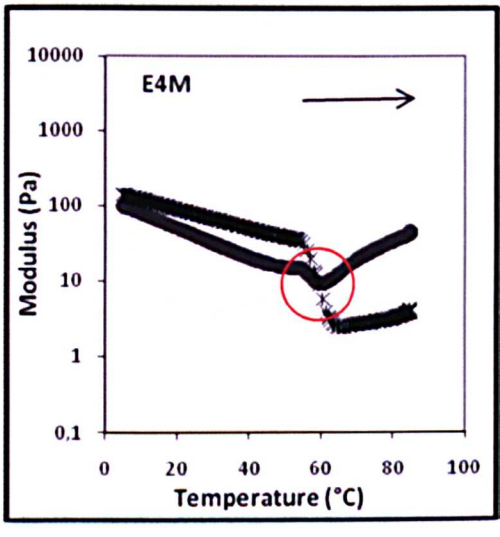
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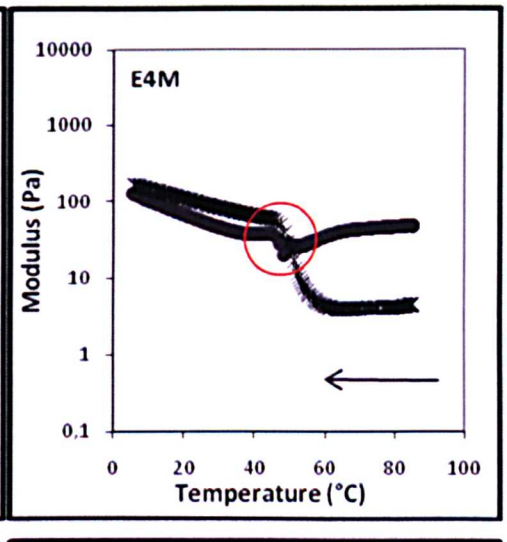
Temperature (°C)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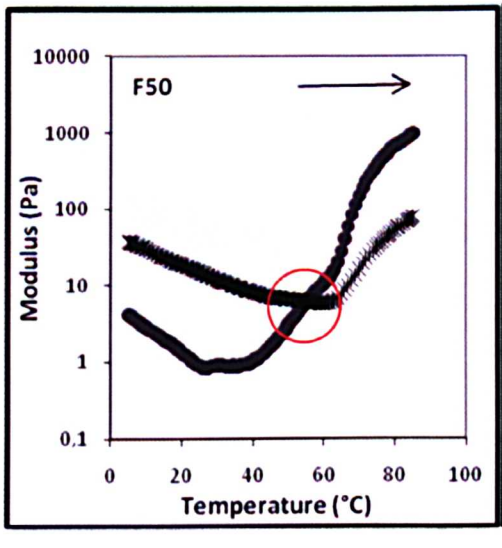
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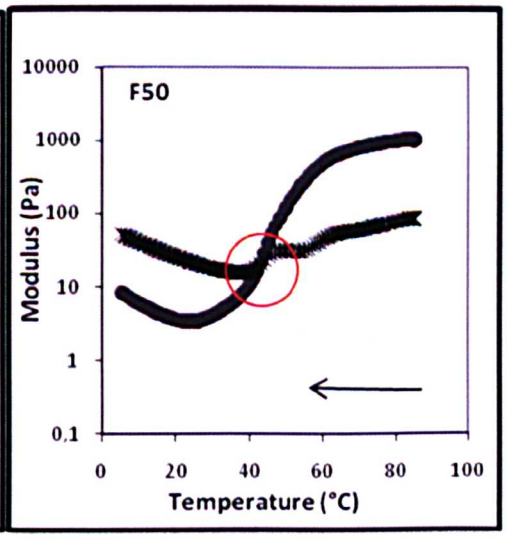
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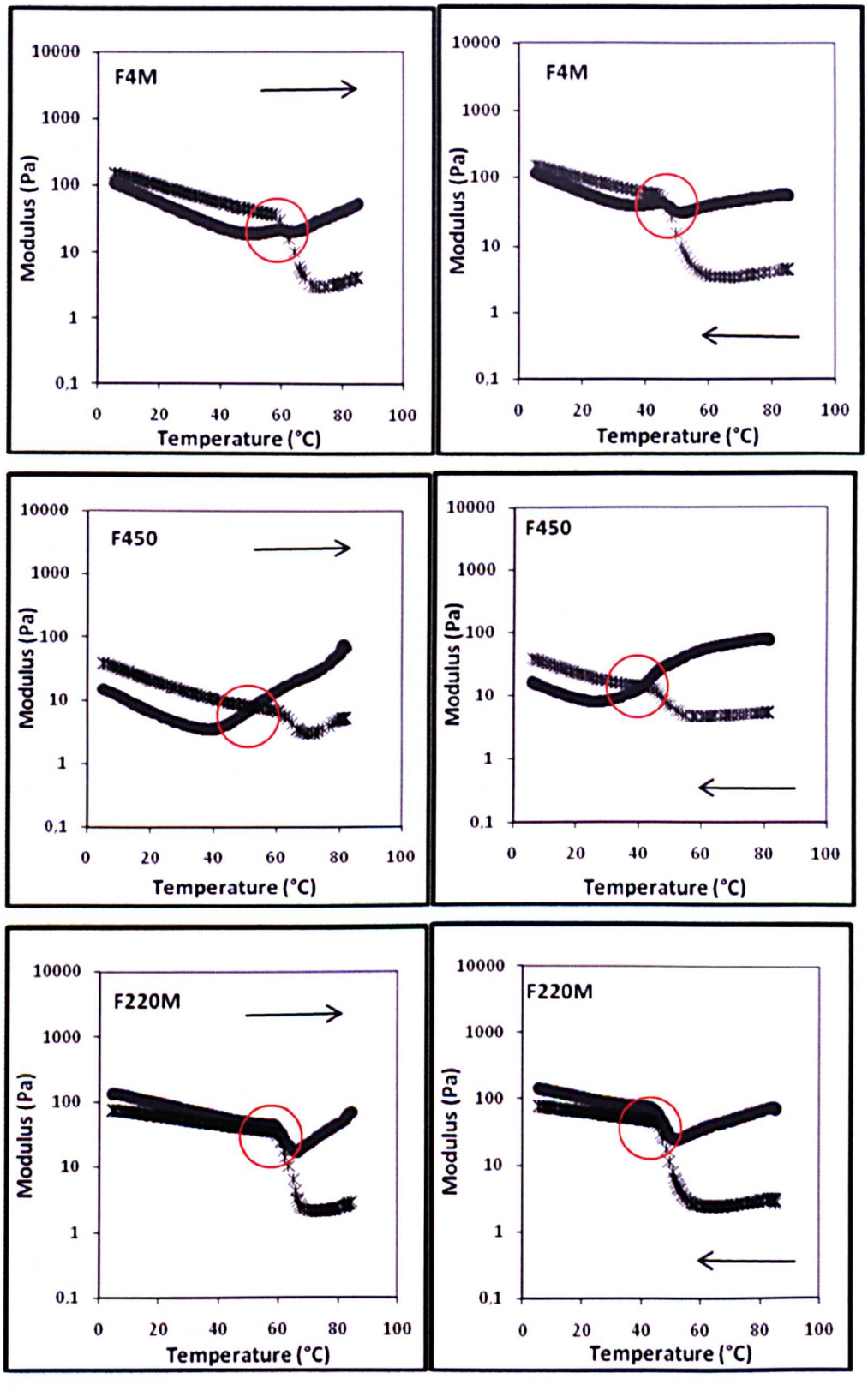


Figure 1-10. Graphs showing changes in G' and G'' values with temperature. Black crosses (x) respresent values for elastic modulus. Black circles (●) represent values for viscous modulus. The red circles indicate where G' and G'' values cross. For sample F220M, the temperature at which the values are closest is circled, since the two values never cross. Only one of the repeated measurements are shown for each sample. Temperature was ramped at 2°C per minute, with a 1% strain and a frequency of 10 rad s^{-1}. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

Values for gelling and melting temperature as determined by the G'G'' crossover are shown in Figure 1-11 below:

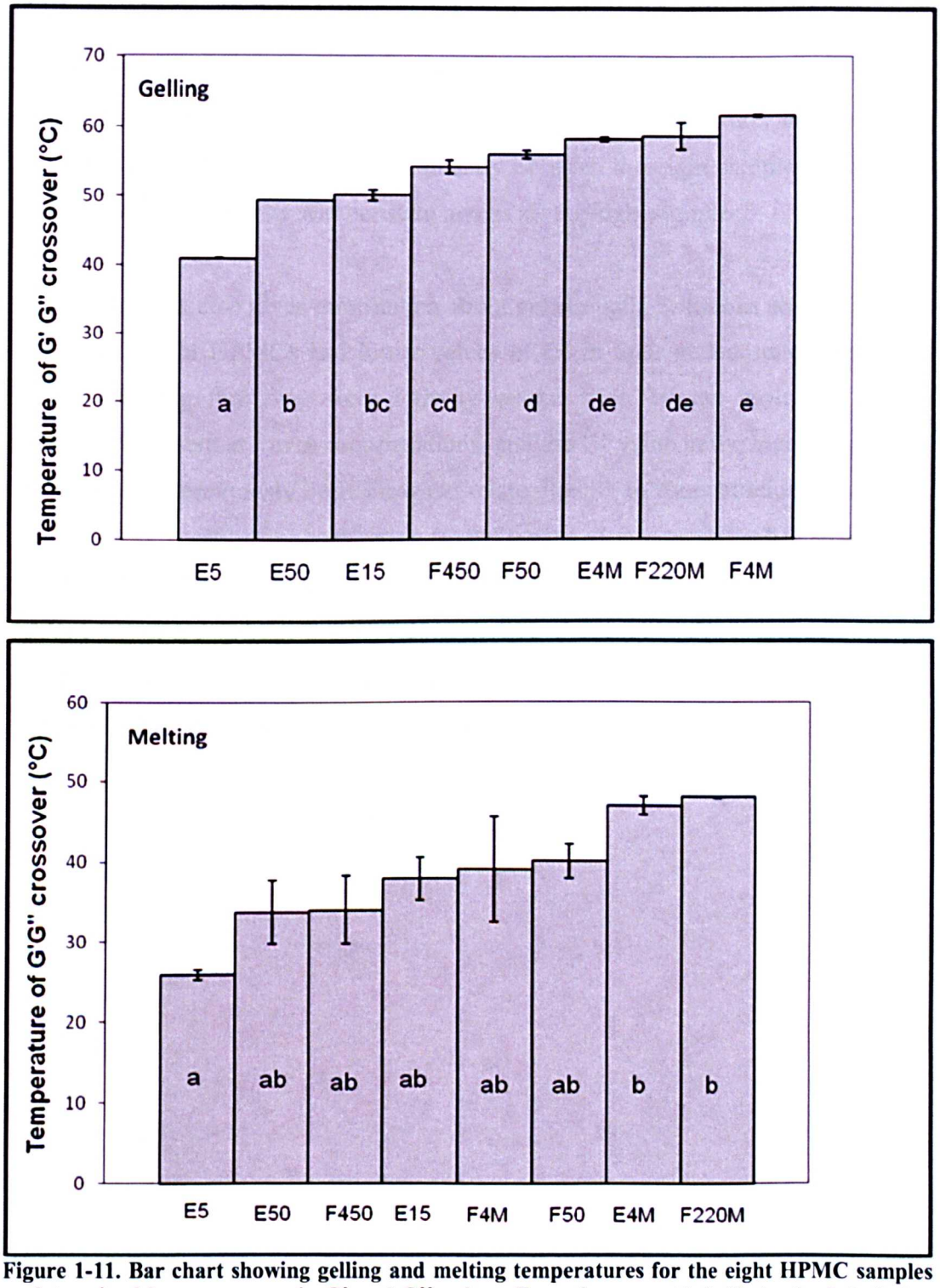


Figure 1-11. Bar chart showing gelling and melting temperatures for the eight HPMC samples as determined by a crossover in G' and G'' values. Error bars are +/- half standard deviation (s.d) of the triplicate measurements of each sample. Different letters indicate statistically significant subgroups according to a Tukey test. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

One factor ANOVA tests carried out on the data showed that there are significant differences between samples both in their gelling temperatures ($p<0.01$) and melting temperatures ($p<0.02$). It appears that in general samples containing low molecular weight HPMCs have lower gelling and melting temperatures than those containing high molecular weight HPMCs. Furthermore, all samples display hysteresis, since melting temperature is consistently lower than gelling temperature; however their values for Tg-Tm did not differ significantly between the eight samples, suggesting that the level of hysteresis was constant across all the eight samples.

Rheological data also gives information about gel strength. Solutions containing high molecular weight HPMCs had lower values of G' in their gelled state (see Figure 1-12), indicating that they were forming weaker gels. Higher molecular weight HPMCs are present at lower concentrations, and the G' value in the high temperature gelled state has previously been shown to relate directly to concentration (Haque and Morris 1993). A plot of concentration (in %) versus elastic modulus has an R^2 value of 0.5, suggesting some sort of relationship, but that there is a high level of noise. Sarkar also showed that gel strength increased as molecular weight increased (Sarkar 1979) – this may explain why the larger molecular weight molecule F4M, present at a similar % concentration to F450, had a larger value for elastic modulus.

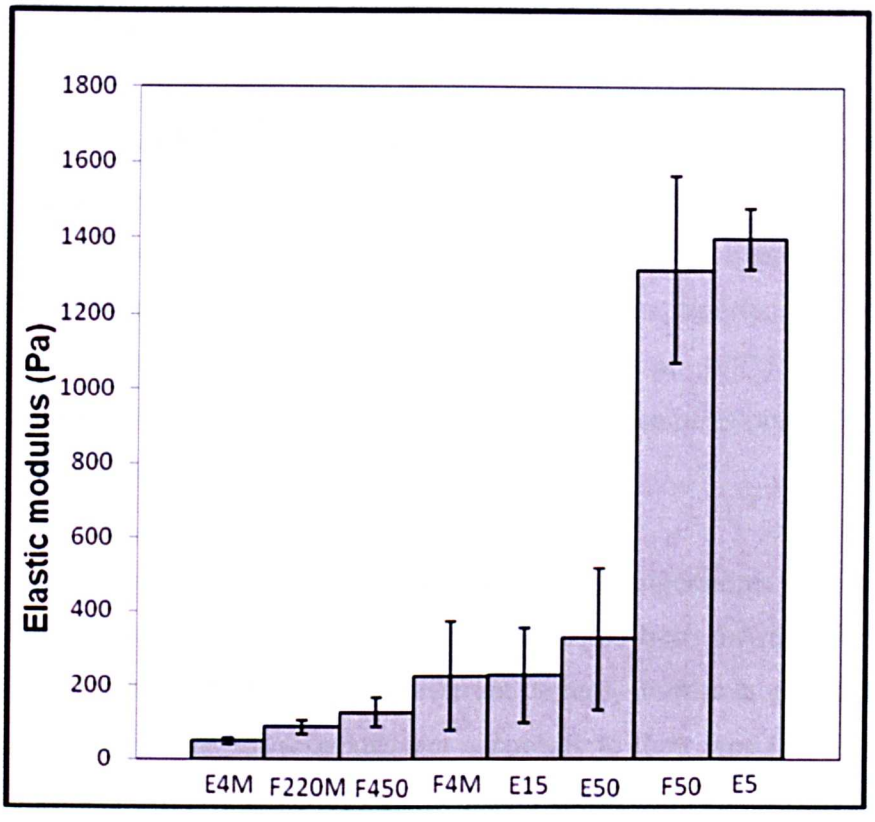


Figure 1-12. A comparison of the values of elastic moduli for samples in their gelled state. The average value across the triplicate measurements are shown. Error bars are given as ± half s.d. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

It is thought that high concentrations of low molecular weight molecules allow a larger number of, or stronger, intermolecular associations (and this causes increased gel strength). This could also explain why they have lower gelling and melting temepratures – high HPMC concentrations could increase the ease at which the gels can form (hence lower gelling temperatures), and stronger gels may take longer to melt (hence lower melting temperatures).

Error bars for melting temperatures however are very large, indicating high variability in repeated measurements. During the chemical derivitisation of cellulose, it has been suggested that substitution groups are not evenly distributed along the structure (Haque, Richardson et al. 1993). Samples containing HPMCs where the substitution is dense will gel differently to samples where the substitution is less dense. This may cause a large degree of heterogeneity of gelation within samples, which may explain the variation seen on subsequent melting. Additionally, examination of the raw data for time temperature curves showed that for some experimental runs, the rheometer seemed to struggle at high temperatures, which

sometimes caused the sample to be held at lower temperatures for up to 10 minutes before the subsequent cooling began (unpublished data), which may contribute to the natural variation. Furthermore, despite the presence of silicone oil, significant dehydration may have been occurring during the gel-melt cycles, causing shrinkage of the sample and therefore slippage of the geometry. Evaporation of water despite the presence of silicon oil has previously been reported during the gelation of methylcellulose samples (Nishinari, Hofmann et al. 1997). All these factors could contribute to the large variation in melting temperatures observed with the repeated measurements.

Furthermore, the range of gelling and melting temperatures obtained here across the eight samples is much larger than that expected from the manufacturer's guidelines (see Table 1-2) and covers different values. Furthermore, melting and gelling temperatures are not separated out according to their type (i.e. E-type or F-type) as the manufacturers suggest, but seemed to be grouped according to their molecular weight, which is contrary to what is expected (see section 1.2)

A closer look at the actual transitions of elastic and viscous moduli with heating and cooling (see Figure 1-10) shows that the samples actually show quite different patterns of changes in moduli, and this appeared to be related to the molecular weight. Samples containing the lower molecular weight molecules E5, E15, E50 and F50, showed a short initial decrease in elastic modulus, followed by an increase at approximately 30-40°C. Furthermore, samples E5 and F50, which represent the lowest viscosity grades of each of the two types, actually show a second wave of increase after this increase, at approximately 60°C. The high molecular weight samples E4M, F4M and F220M, however, show less prominent changes. On heating, these samples show a long and shallow initial decrease in elastic modulus followed by a gradual increase at a much higher temperature of 60-65°C (similar to the temperature of the second increase for samples E5 and F50). The same patterns are observed on cooling. This would suggest that changes in structure are more prominent at lower temperatures for the lower molecular weight samples compared to the higher molecular weight ones. This was somewhat of a surprise since HPMCs with similar substitution types (i.e. E-types or F-types) would be expected to behave more similarly.

Both of these two different patterns of behaviour in elastic modulus on heating (i.e. that displayed by the lower molecular weight samples, and that displayed by the higher molecular weight samples) have previously been observed for solutions of modified celluloses. Haque and Morris (1993) reported similar patterns of changes in elastic modulus on heating, and at similar temperatures, to those that were observed here for sample E5 and F50, for both 1% A4M (MC) and 1% K4M (HPMC) (Haque and Morris 1993; Haque, Richardson et al. 1993). They propose that the partial dissociation, or swelling, of the clusters in the early stages of heating, as outlined in Figure 1-2, could explain the initial reduction in G'. The first increase in G' may then arise from separation of strands at the ends of the bundles, and the exposure of methyl groups and the formation of structured water cages, which would massively increase hydrodynamic volume. At higher temperatures, the disruption of the cages and formation of the final gel network would explain the second increase in G'. It is interesting that only two of the eight samples studied here show this second increase, since all samples are expected to gel in this way (i.e. an initial swelling, followed by water cage formation, and culminating in the formation of the gel network), and therefore all samples should show these three separate changes in G'.

On the other hand, transitions similar to those observed here for the higher molecular weight HPMCs have previously been observed for K4M and E4M at higher concentrations (Haque, Richardson et al. 1993; Hussain 2002). When Haque and Morris increased the concentration of A4M, which at 1% showed behaviour similar to E5 and F50, by one percent, they observed that the initial decrease in G' was obscured, and that the first increase in G' became far less evident (Haque and Morris 1993). Furthermore, increasing K4M concentration from 1% to 6% caused a transition in elastic modulus behaviour from one that was quite dramatic (as displayed here by the lower molecular weight samples), to one that was fairly uneventful (similar to those observed here for the higher molecular weight molecules) (Haque, Richardson et al. 1993). These changes in moduli on increasing concentration have been attributed by these authors to the contribution of entanglement coupling. This suggests that for our samples, low concentrations of high molecular weight HPMCs are displaying more entanglement coupling than more concentrated solutions of low molecular weight HPMCs. A significant

contribution of entanglement coupling may explain the high values for elastic modulus observed for these high molecular weight samples in their solution state, since it may allow for some sort of network to form even without heating. Since values in the gelled state are also low (postulated to be due to the low concentration of these samples), this could explain the lack of significant changes observed in general on heating these samples. In contrast, the characteristic double wave of increase in elastic modulus for samples E5 and F50, which contain the lowest molecular weight E-type and F-type HPMCs respectively, could be because only for these small molecules is entanglement sufficiently insignificant that the second wave of increase can be observed.

Changes in viscous modulus during the heating and cooling also separates the HPMCs into two groups based on molecular weight. For solutions containing the large molecular weight molecules (F450, E4M, F4M and F220M), viscous modulus remains fairly constant during the early stages of heating, and then drops suddenly at approximately 60°C (approximately the same temperature at which the elastic modulus started to rise) before rising again. The same was seen on cooling, but changes occurred at a lower temperature. Such transitions in viscous modulus on heating have previously been observed for high concentrations of K4M and E4M (Hussain, Keary et al. 2002; Haque, Richardson et al. 1993). The viscous modulus for samples E50, E15 and F50, however, initially decreased to a plateau as the sample was heated to approximately 60°C, before it increased, whereas on cooling values remained fairly constant over the temperature range. Such transitions have previously been observed for lower concentrations of A4M on both heating and cooling (Hussain 2002). Sample E5, however, showed more of a unique behaviour – modulus remained constant at low temperatures, but rapidly increased at approximately 50°C, which was offset to 40°C on cooling. Haque and Morris (1993) observed that the plateau in G'' (which we observed at approximately 40-60 °C for the low molecular samples) was replaced by significant reduction in samples where entanglement coupling was causing the hiding and suppression of certain transitions. Therefore, the high gelling and melting temperatures obtained for high molecular weight samples from rheological methods, compared to those obtained for the lower molecular weight samples, could be an artefact due to entanglement coupling of the

large HPMC molecules hiding the low temperature transitions, rather than because they actually gel at higher temperatures.

Although other authors have proposed different criteria to determine gelling and melting temperatures (such as minimum in G' over a temperature gradient (Sarkar 1995)), any criteria used to obtain values for gelling and melting temperatures from this rheological data would still be greatly affected by the masking of changes due to entanglement coupling. Furthermore, it has been reported that changing the frequency of the measurements affects the transitions observed, so using different frequencies may have given us different results. It therefore seems that because of this, rheological techniques may not be the best way to measure the melting temperatures of the samples under investigation here.

1.5.2.2. The use of calorimetric methods to measure gelling and melting temperatures

Changes in the heat flow of the eight samples during heating and cooling was monitored by DSC. All samples gave a single endotherm on heating, which is consistent with the notion that the system requires energy to form a gel. The endotherm begins at approximately 50-60°C, and ends near 90°C for all samples (see Figure 1-13), consistent with what has previously been reported for E4M and F4M (Haque, Richardson et al. 1993; Sarkar and Walker 1995). It is assumed that the energy absorbed is needed to break the water cages surrounding the HPMC molecules, allowing the exposed hydrophobic groups to interact and form a gel.

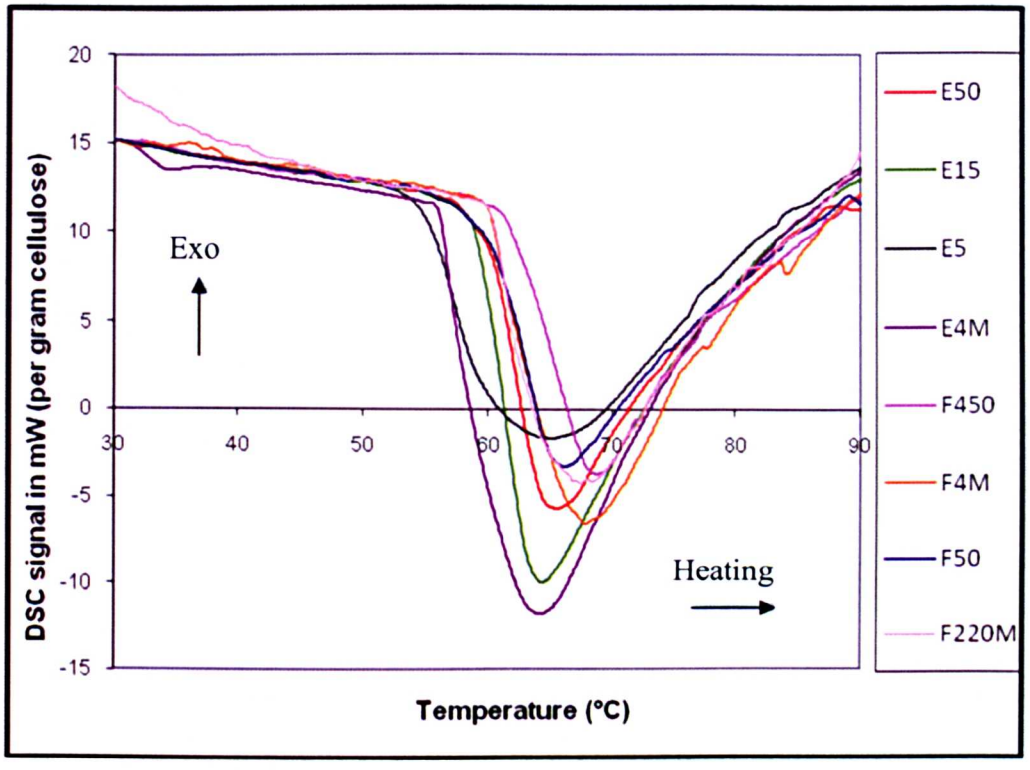


Figure 1-13. Normalised endothermic transitions observed on heating HPMC samples. Samples were heated at 1 °C per minute. For ease of comparison, all of the transitions have been vertically shifted. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

Although transitions occur over similar temperature ranges, a one way ANOVA showed that there *is* a significant difference between the samples in peak temperature. The temperature at which sol-gel transitions occur for MC solutions has previously been taken as peak temperature on the DSC (Xu, Wang et al. 2004).

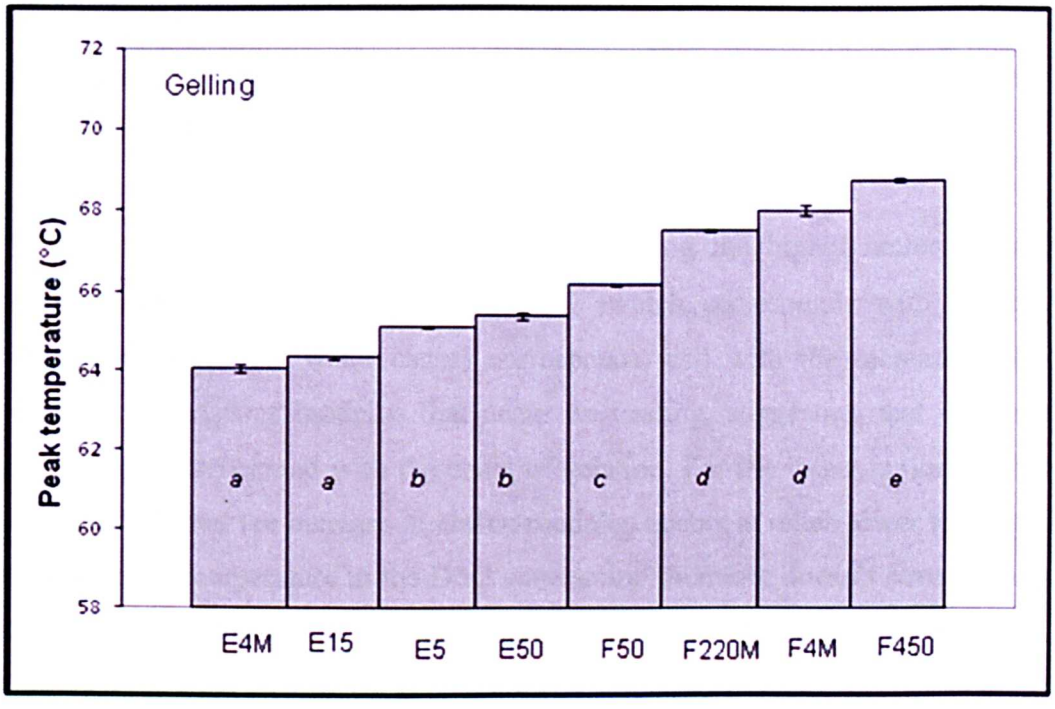


Figure 1-14. Peak temperatures on heating the HPMC samples at 1°C per minute on the DSC. Different letters indicate statistically significant subgroups according to a Tukey Test.

Inspection of the subgroups separated out according to the statistical analysis (see Figure 1-14) indicates that samples appear grouped for gelling temperature according to their substitution type. The E-type HPMCs displayed peaks at lower gelling temperatures than the F-type HPMCs, suggesting that they are undergoing gelation at lower temperatures, which is consistent with the manufacturers guidelines. Sarkar and Walker (1995) also observed the endothermic peak at a lower temperature for E4M than for F4M, although other authors did not (Haque, Richardson et al. 1993). The inclusion of small amounts of hydroxypropyl substituents is known to inhibit hydrophobic gelation (Haque, Richardson et al. 1993), which is why the A-type MCs are reported to gel at much lower temperatures than the E- or F-type (see the manufacturers guidelines, Table 1-1). Although both the E-type and the F-type HPMCs have similar proportions of methyl substitutions, according to the specification sheets from the manufacturers the F-type HPMCs under investigation here have less hydroxypropyl substitutions, so one would actually expect them to gel at lower temperatures (contrary to what we observed), since the hydroxypropyl group is big and causes steric interference. However, only ranges for the specific substitutions of the HPMCs are given, so if the E-types actually contained methyl substitutions towards the higher end of the scale (i.e. 30%), they would gel more readily. Despite Nishinari, Hofmann et al (1997) observing that the endothermic peak

temperature was shifted to lower temperatures with both increasing concentration and increasing molecular weight of methylcellulose, molecular weight and concentration do not appear to be affecting gelling temperature here.

As shown in Figure 1-15, for solutions containing the higher molecular weight molecules, peak temperature on the DSC (which corresponds with the energy required to break the water cages) corresponds well with the increase in elastic modulus and viscous modulus that occur on heating, suggesting that increases in these moduli correspond with the onset of gelation. For the lower molecular weight samples however, the increase in elastic modulus occurs at much lower temperatures than the peak temperature in the DSC curves, and therefore doesn't correspond with the breaking of water cages and gelation. Haque and Morris (1993) suggest that the first increase in G' that occurs for the lower molecular weight samples is probably due to the swelling of the cellulose bundles, and although bundles of the high molecular weight HPMCs will also swell, this initial increase in G' as a result of this swelling will be masked by the contribution of entanglement coupling. The temperature at which elastic modulus begins its second wave of increase for samples E5 and F50, and the temperature at which viscous modulus starts to increase for samples E50, E15 and F50, occur at temperatures corresponding to this maximum heat flow (and therefore the breaking of the water cages and gelation), indicating that for different molecular weight samples, the onset of gelation (as defined by the energy required to break the water cages) is determined by different rheological criteria.

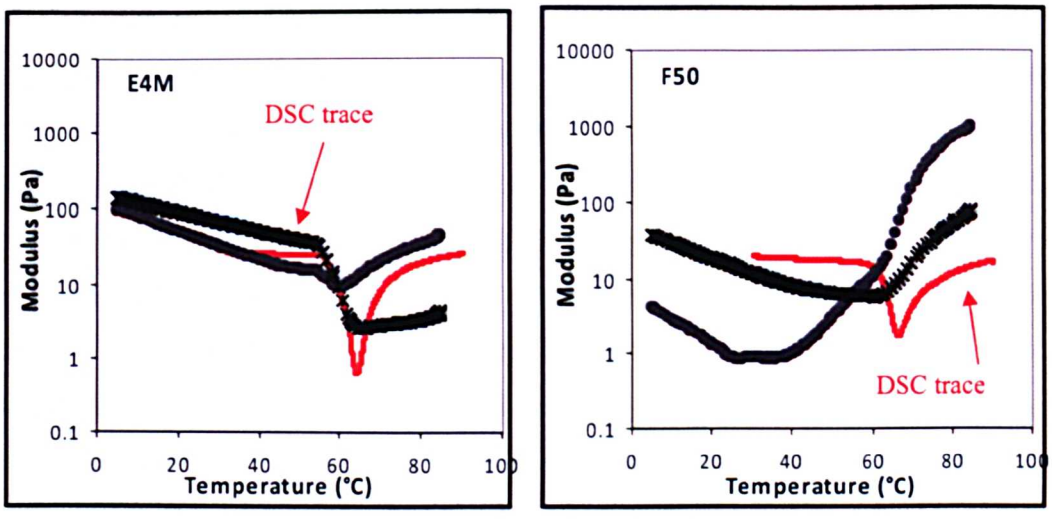


Figure 1-15. Graphs showing changes in G' and G'' values of E4M (3.0%) and F50 (5.6%) with temperature. Only one of the repeated measurements are shown for each sample. Temperature was increased at 2°C per minute, with a 1% strain and a frequency of 10 rad s^{-1}. DSC traces are shown in red.

The software on the calorimeter was used to estimate enthalpy values from the areas under the curves for the eight samples, based on an extrapolation of the low temperature baseline, as shown in Table 1-4.

Table 1-4. Enthalpy values for the eight HPMC samples, estimated from the extrapolation of the low temperature baseline.

Sample	Enthalpy value (J/gram cellulose)
E5	1509
E15	1964
E50	1930
E4M	2435
F450	1380
F4M	1712
F50	1526
F220M	1593

It was thought that since rheological measurements indicated that sample E5 formed the strongest gel, it was thought that enthalpy values for the sol-gel transition would also be greatest for sample E5, however this was not the case. This is probably because the techniques of rheology and calorimetry are actually measuring slightly

different properties of a gel network. Rheology measures the strength of the gel network due to its rigidity, and therefore it is thought the number and strength of the hydrophobic interactions (which was highest for E5 due to its elevated concentration). Calorimetry, however, measures the energy change needed to break the water cages, which occurs prior to the formation of hydrophobic linkages. Since sample E4M has the largest enthalpy value, it suggests that a large number of water cages form around this HPMC molecule during the early stages of heating (perhaps due to its large size) and therefore a large number of water cages need to be broken to allow subsequent hydrophobic gelation to occur, thus producing this large enthalpy change. The E-type HPMCs in general have higher enthalpy values compared to the F-type HPMCs, which could be because F-type HPMCs contain a smaller percentage of hydroxypropyl substituents compared to their E-type equivalents, and due to their less hydrophilic nature, water cage formation is less pronounced, and therefore less energy is required to break these water cages, hence lower enthalpy values. However, since the baseline used to calculate these enthalpy values was estimated visually, this may have led to inaccuracies in these values, so this data should be interpreted with caution.

Endotherms for the eight samples also appear to differ in their height and their width. Nishinari et al found the endothermic peak to become sharper both with increasing concentration and with increasing molecular weight (Nishinari, Hofmann et al. 1997). However differences in width and height do not appear related to sample structure, since the endotherms for the two very similar samples E5 and E15 (in terms of substitution type, molecular weight, and concentration) differ greatly in broadness and height.

On cooling the samples, exothermic transitions are observed, attributed to gel dissociation (see Figure 1-16) as has previously been observed for HPMC samples (Haque, Richardson et al. 1993). The main changes in heat flow on cooling occur between 40 and 60°C, so at lower temperatures than they do on heating – such hysteresis was also observed with the rheological data. Like for endothermic transitions, exothermic transitions for all eight samples occur at similar temperatures, however based on the manufacturers guidelines (see Table 1-1) we should expect to see a difference of ~ 15°C between samples containing E-type and F-type HPMCs.

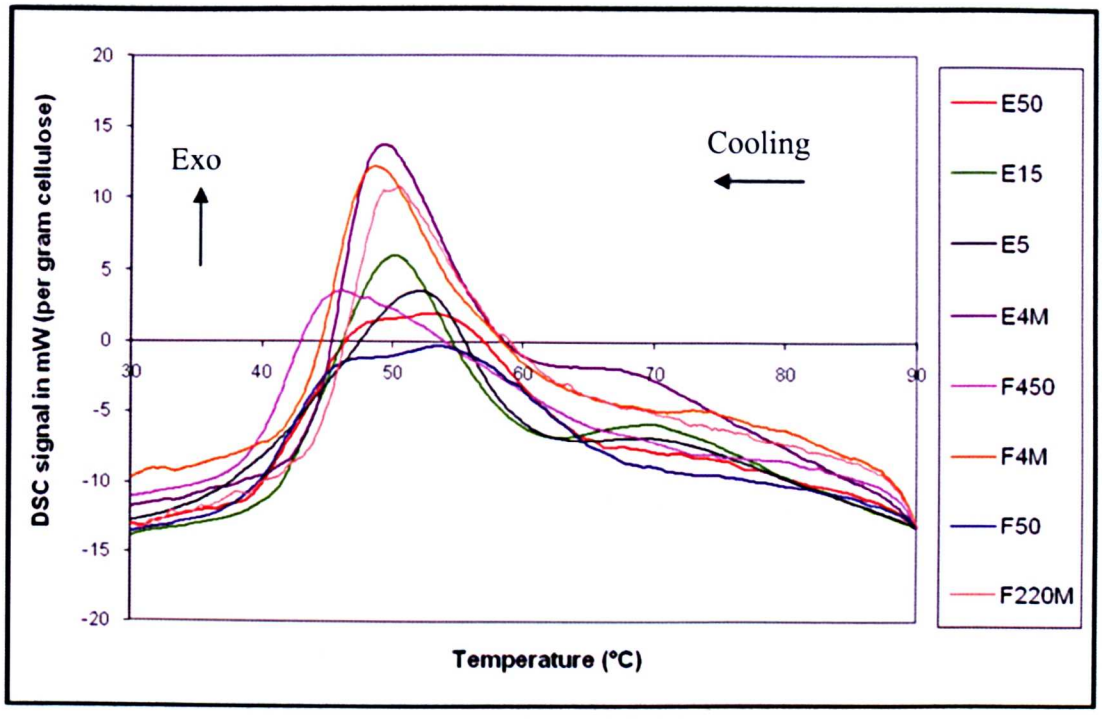


Figure 1-16. Exothermic transitions observed on cooling HPMC samples at 1°C per minute. For ease of comparison, all of the transitions have been vertically shifted. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

Although the changes in heat flow occur over a similar temperature range, a one way ANOVA showed that samples differ significantly in their peak temperature (see Figure 1-17), however the subsets in which these samples were placed appeared completely random, and does not appear to correlate to molecular weight, as Nishinari, Hofmann et al (1997) found (where the exothermic peak was shifted to lower temperatures with increasing molecular weight), or by substitution type, as we might have expected from the manufacturers guidelines, and as we found for gelling temperature. A lack of difference between the temperature at which the exothermic peak was at a maximum for the E-type and F-type HPMCs was also observed by Haque and Morris (1993), who found both E4M and F4M to show a peak maximum at ~ 45°C.

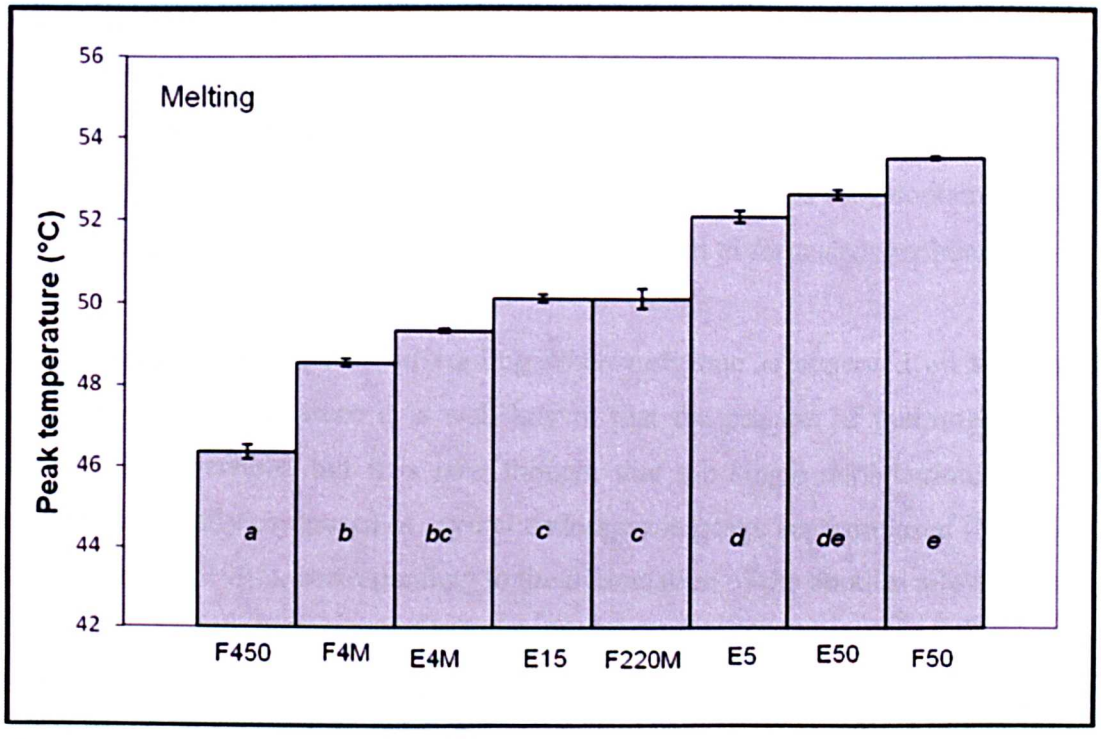


Figure 1-17. Peak temperatures on cooling the HPMC samples at 1 °C per minute on the DSC. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

This apparent discrepancy between the results obtained here and what we might have expected from the manufacturers guidelines may be due to the fact that for some of the samples studied here, a secondary peak is also apparent (see samples E4M, E15, E5, F50 and E50 in Figure 1-16). While the appearance of a secondary peak on cooling has been observed by several authors for the *methylcelluloses* (Haque, Richardson et al. 1993; Desbrieres 1998; Li, Shan et al. 2002), Haque, Richardson et al (1993), who also studied the HPMCs, failed to observe this second peak. The first exotherm observed upon cooling methylcelluloses has been proposed by Haque, Richardson et al (1993) to be due to the reforming of the water cages and the dissociation of hydrophobic "bonds", and the lower temperature peak is due to the subsequent reforming of cellulosic strands in solution, causing the gel to fall apart. Since Haque, Richardson et al. (1993) only observed one peak for the HPMCs, they suggested that the presence of hydroxypropyl substituents were causing a major reduction in the enthalpic stability of the bundles, thus moving the two peaks closer together and making them superimpose, producing only one peak. The second peak obtained here for those of the E-type HPMCs that displayed one (i.e. E4M, E15, E5 and E50) is separated much further from the other peak than when it occurs in F-type HPMC F50, where the two peaks appear much closer together. The E-type HPMCs,

due to their higher proportion of hydroxpropyl substituents, would be expected to form bundles of weaker enthalpic stability than the F-types, and therefore produce peaks even closer together – however we did not observe this, but it has previously been suggested from the results in this thesis that the E-types may contain a higher substitution of methyl groups which would allow them to form stronger bundles.

The presence of two peaks on cooling when only one is observed on heating is somewhat surprising, since it is well known that the process of thermogelation is completely reversible, but it is now thought that the single endothermic peak on heating is actually composed of several endothermic peaks superimposed (Nishinari, Hofmann et al. 1997), corresponding to the dissociation of the bundles allowing them to separate and the heat required to break the cages, which is consistent with a multi-step process of gelation. The merging of several peaks into one on heating may explain the variation in broadness of the peaks – samples where the different gelation steps occur over a similar temperature range would produce endotherms showing narrower peaks, whereas a broad peak would suggest a temperature dependent separation of these processes. Samples E4M, F4M and F220M all show fairly narrow tall peaks, suggesting that perhaps for high molecular weight molecules the dissociation of bundles and breaking of water cages occurs over a slightly shorter temperature range.

For some samples, it is hard to see if two superimposed peaks, instead of one, exist on cooling, so in order to increase resolution the experiment was repeated using a slower scan rate, since increasing scan rate has been reported to decrease resolution (Gabbott 2007) (see Figure 1-18).

Contrary to expectation, slowing scan rate actually appeared to decrease resolution - the possible presence of a second peak observed when cooling sample F50 at a scan rate of 1°C per minute was less obvious at the slow rate of 0.1°C per minute, where it appeared more as just a shoulder, rather than a more prominent second peak. However, it is possible that the different scan rates used here are so similar that a pronounced difference in sensitivity would not be observed. Furthermore, enthalpy values (based on visual assessment) and peak temperatures increased at lower scan rates, however a one way ANOVA test showed that the difference in temperatures on

varying scan rate was not significant (p=1.00). This finding is in agreement with Li, Shan et al. (2002) who observed that the temperature of the main exotherm on cooling remained almost the same when the cooling rate was varied. The slight difference in peak temperature and enthalpy values is probably an artefact due to temperature lag, and because samples were held for longer times at higher temperatures with a slower scanning rate, allowing more water cages to be formed/destroyed, and thus more energy to be released.

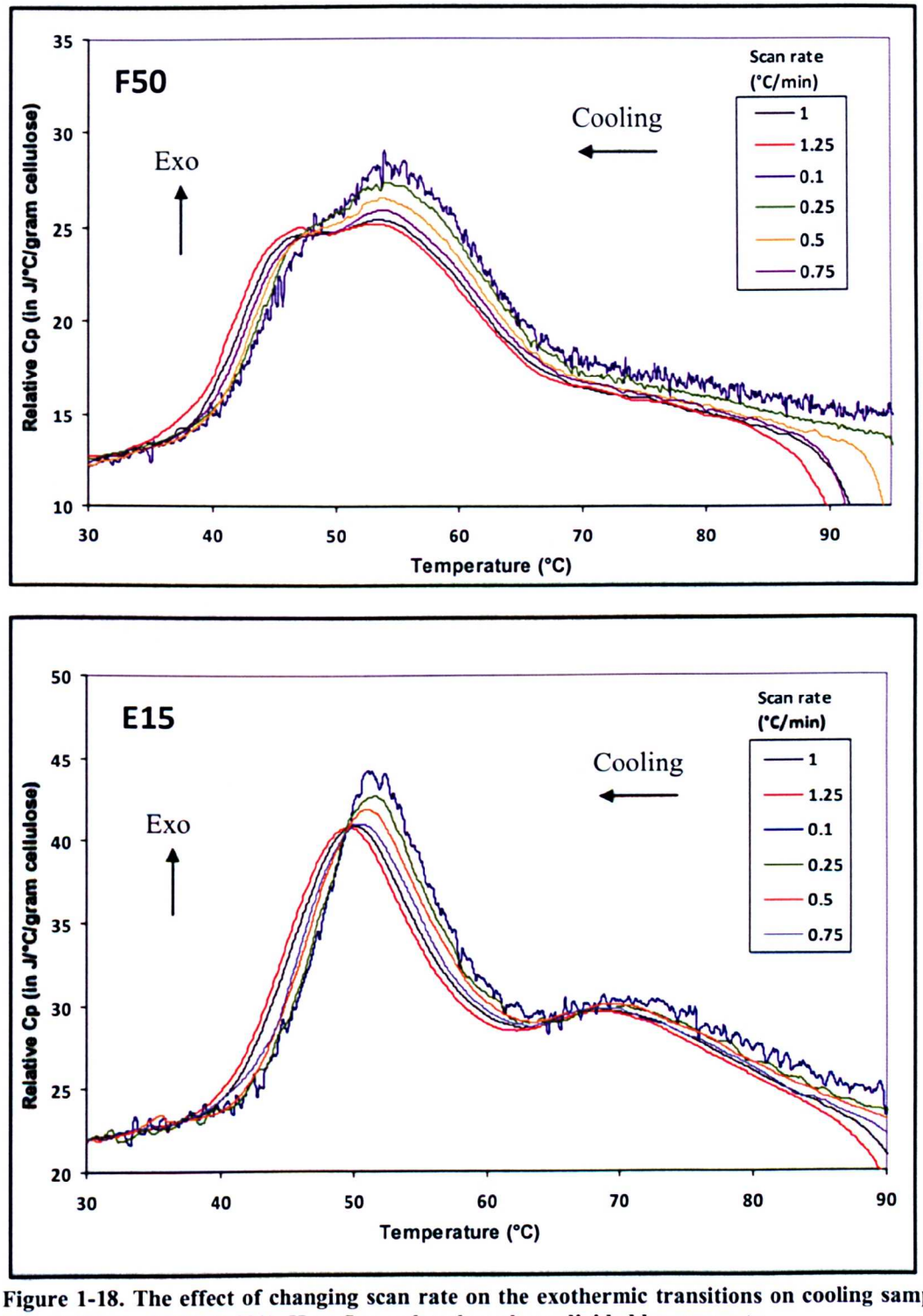


Figure 1-18. The effect of changing scan rate on the exothermic transitions on cooling samples F50 (5.6%) and E15 (7.0%). Heat flow values have been divided by scan rate.

As shown in Figure 1-19, the exothermic transitions observed at approximately 50°C correlate well with the changes in elastic and viscous modulus observed on cooling the higher molecular weight samples E4M, F4M and F220M – viscous modulus starts to increase at ~ 50 °C on cooling these samples, and there is a characteristic "blip" in elastic modulus at this same temperature. For the lower molecular weight

samples however, although the characteristic "blip" in viscous modulus observed in samples E50, E15 and F50 on cooling occurs at approximately this temperature, there is no change in the direction of elastic modulus at this temperature. Furthermore for sample E5, the peak exotherm temperature does not correspond with any changes in the direction of either the moduli, further showing the limitations of using rheological criteria to ascertain gelling and melting temperatures in this investigation.

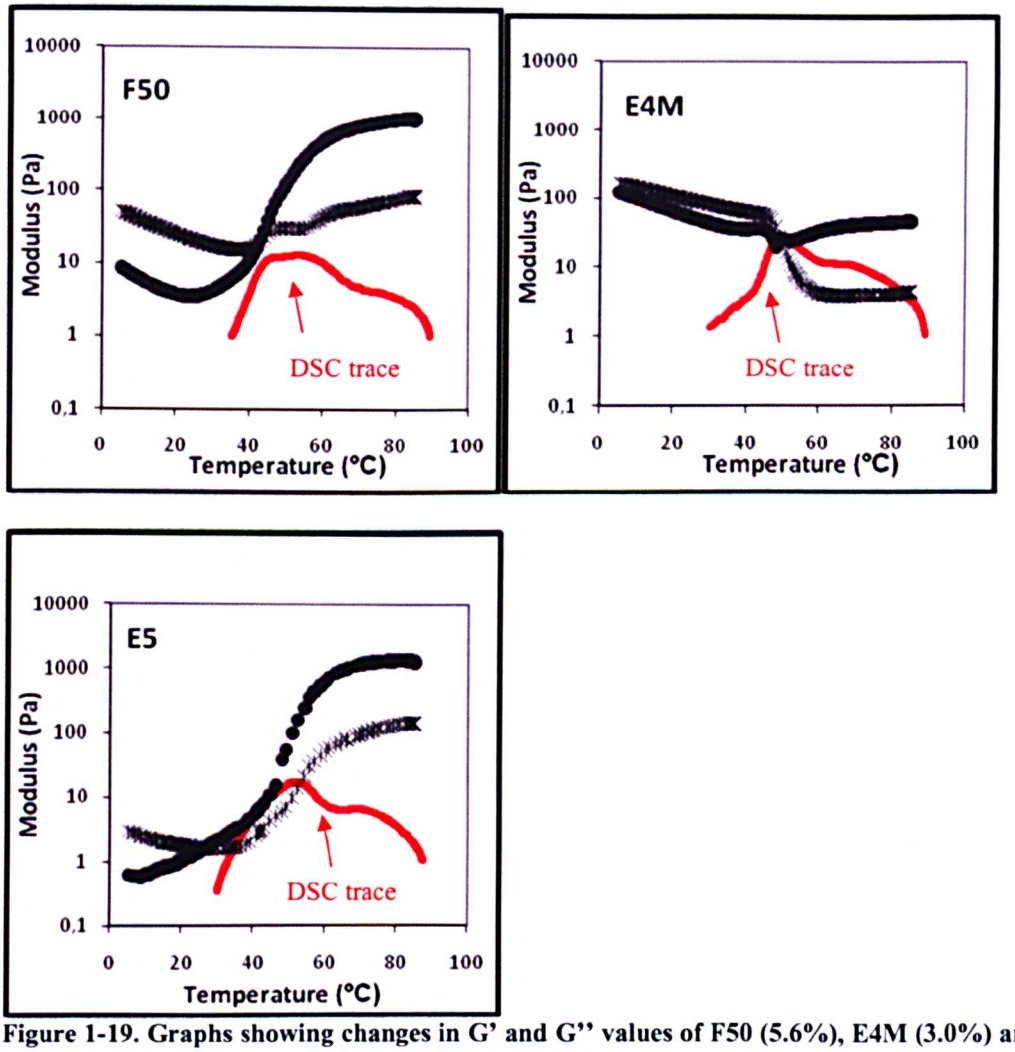


Figure 1-19. Graphs showing changes in G' and G'' values of F50 (5.6%), E4M (3.0%) and E50 (5.1%) with temperature. Only one of the repeated measurements are shown for each sample. Temperature was increased at 2°C per minute, with a 1% strain and a frequency of 10 rad s^{-1}. DSC traces are shown in red.

Some authors use transition mid-point temperatures to determine melting temperatures (Haque and Morris 1993; Haque, Richardson et al. 1993) instead of temperatures at maximum heat flow would, since this includes the contribution from any additional peaks. Using transition mid-point temperature for our samples here

would, if anything, increase the values of melting temperature for the samples being studied here, since the minor peak is at a higher temperature. Since the main objective of this study was to ascertain whether gels melted at temperatures above 36.3°C, inspection of the exothermic graphs show that for all samples, events involving energy changes had stopped occurring by 36.3°C (see Figure 1-20), suggesting that melting had indeed occurred for all samples at this temperature. Therefore, based on the results from the calorimetric studies carried out here, all eight samples are possible candidates for the "melt-in-the-mouth" gel.

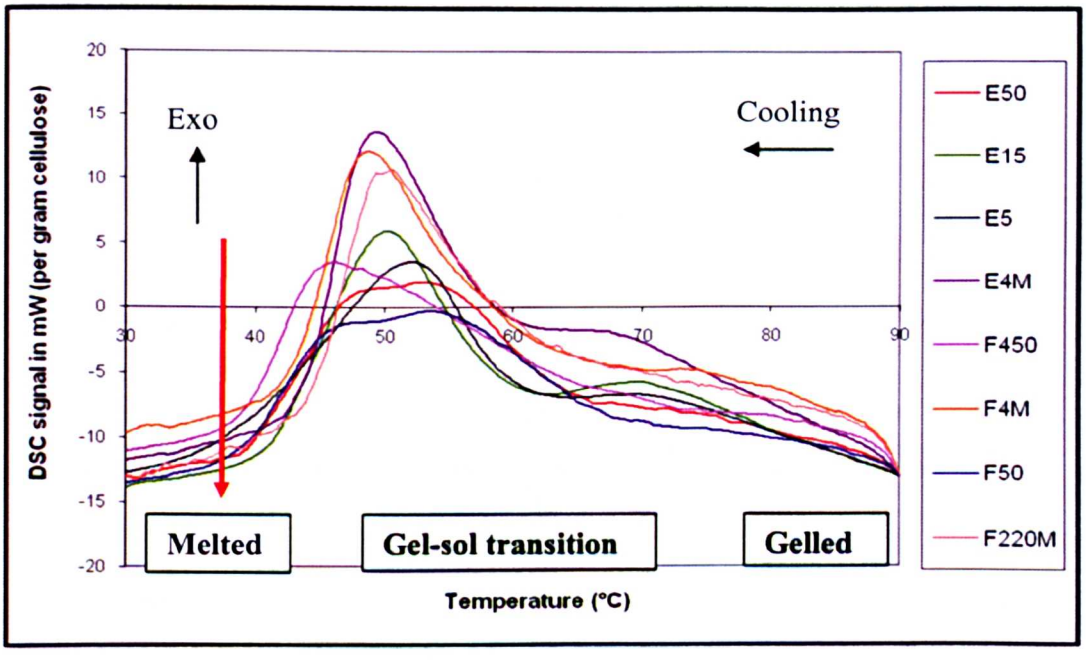
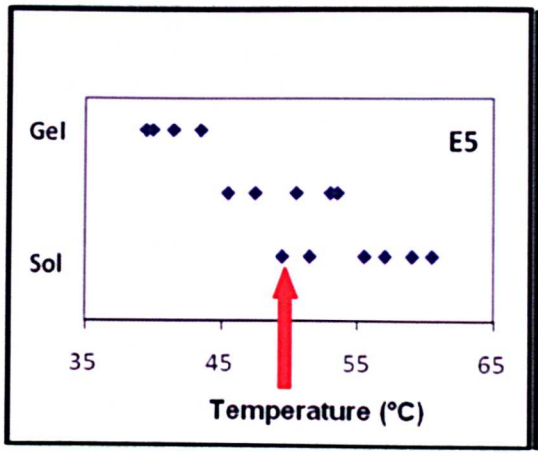


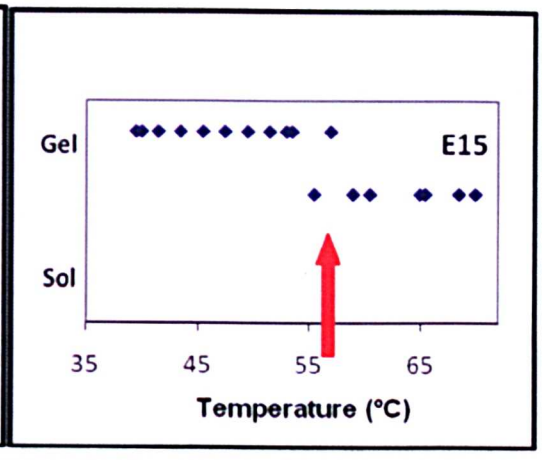
Figure 1-20. Graph comparing exothermic transitions of the eight HPMC samples to average mouth temperature. The thick red line indicates mouth temperature. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%), F220M (1.5%).

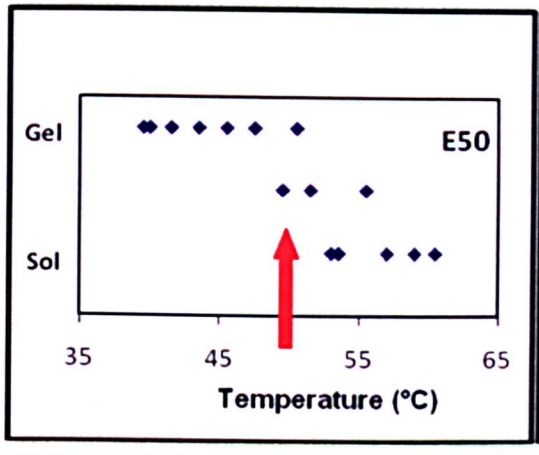
As has been explained in the introduction, although providing valuable information into temperatures at which gel-sol transitions occur, the method of DSC is not very chef friendly, and could not be used in the future by the restaurant to characterise gel melting temperatures. Therefore, results for melting temperature as determined using the more kitchen friendly method the "tip-test" will be compared to those obtained from calorimetric studies, to see if this method could act as a suitable alternative.

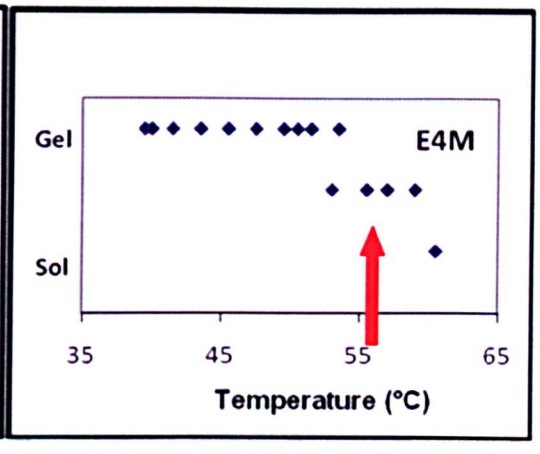
1.5.2.3. The use of the tip-test to measure gelling and melting temperatures

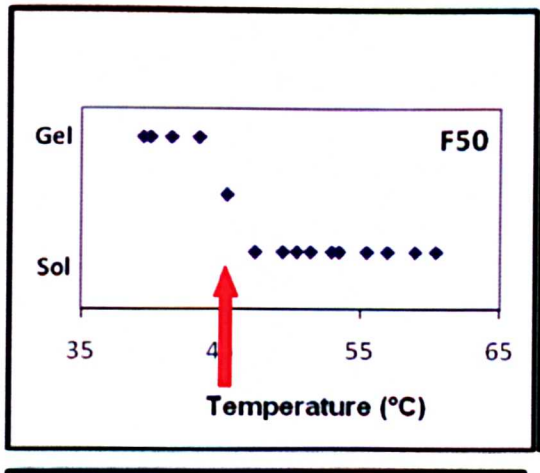
Graphs showing the temperatures at which transitions from the gelled to melted state were occurring as monitored by the tip-test are shown in Figure 1-21.


Gel
Sol
E5
35
45
55
65
Temperature (°C)


Gel
Sol
E15
35
45
55
65
Temperature (°C)


Gel
Sol
E50
35
45
55
65
Temperature (°C)


Gel
Sol
E4M
35
45
55
65
Temperature (°C)


Gel
Sol
F50
35
45
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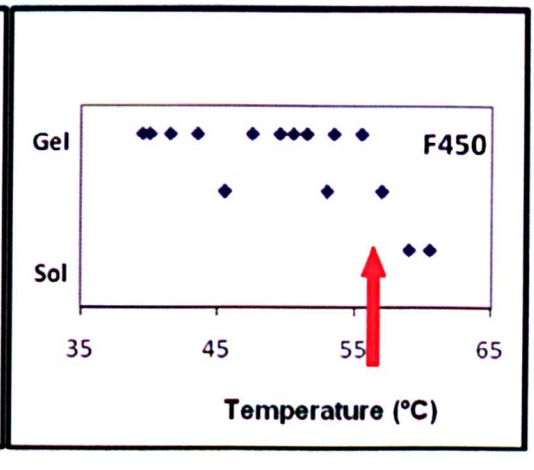
65
Temperature (°C)


Gel
Sol
F450
35
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Temperature (°C)

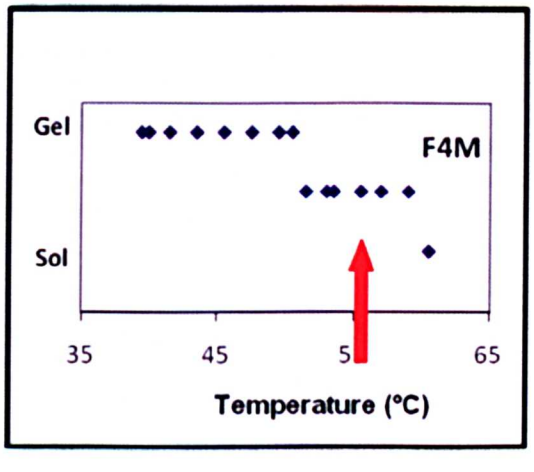

Gel
Sol
F4M
35
45
55
65
Temperature (°C)

Figure 1-21: Graphs to illustrate the value chosen for melting temperature according to the tip-test. Note – sample F220M could not be characterised by this test because it was too thick. Samples contained HPMCs at the following concentrations: E5 (8.5%), E15 (7.0%), E50 (5.1%), E4M (3.0%), F50 (5.6%), F450 (2.9%), F4M (3.0%).

Temperatures at which it is thought the gel-sol transition occurred according to these visual assessments are marked with a red arrow, and as Figure 1-21 shows all samples melt between 45–60°C. These values for melting temperature were compared to those obtained from rheological studies, calorimetric studies, and the cloud test, as is shown in the bar chart below (see Figure 1-22). Raw data for the cloud test and the temperatures at which melting was thought to occur based on these results is shown in the appendix (see Table 6-1).

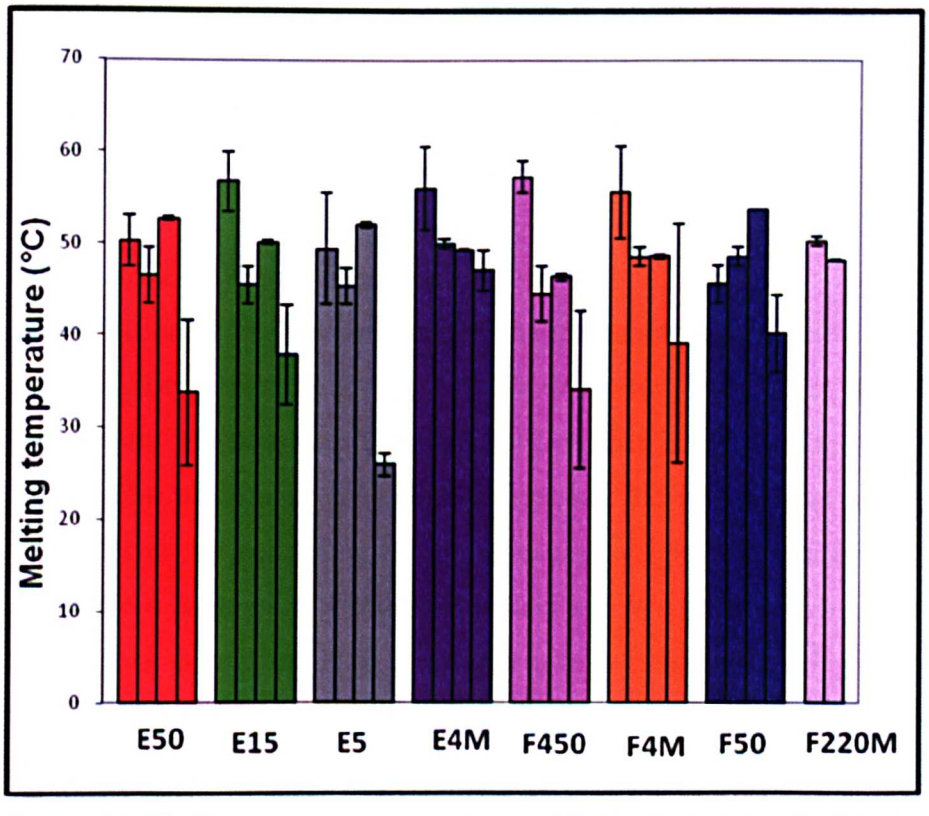


Figure 1-22. Melting temperatures as determined by the tip-test, the cloud-test, calorimetric and rheological measurements respectively, for each of the HPMC samples. Error bars are given as +/- s.d for calorimetric and rheological values, and as areas of ambiguous "state" for the cloud test and tip test. Note: sample F220M was too viscous too be characterised by the tip test, so is only measured by calorimetric and rheological measurements.

Melting temperatures assigned using the tip test were consistently higher for samples than those determined by the cloud point (with the exception of sample F50). Practically, this means that as a gel melts, it is able to move freely as a solution

before it loses its turbidity. This phenomenon has previously been seen by Hussain, Keary et al. (2002), who observed that the clouding seen on heating a solution of 2% E4M occurred at a temperature lower than the temperature at which the thermal transition (i.e. the decrease in G' and G'') was observed.

As shown in Figure 1-22, all values for melting temperatures as determined by these empirical methods are fairly consistent with those results from calorimetry, suggesting that both the cloud-test and the tip-test are suitable alternative kitchen-friendly tests that give accurate results for melting temperature (within experimental error). While the large error bars in the results obtained with these empirical tests could be interpreted as the tests not being very accurate, as both rheological and calorimetric studies have shown the transitions that occur during the gelation and melting of modified cellulose solutions occur over a temperature range, rather than at one particular temperature, which would give rise to the fairly large temperature ranges of uncertainty observed with the empiricial tests.

1.5.2.4. General conclusions

In conclusion, it has been reported by the manufacturers that the F-type HPMCs melt at ~15°C lower than the E-type HPMCs. However, when all of the data for melting temperature was considered, we did not find such a significant difference in melting temperature according to substitution type, nor did we find that melting temperature was affected by concentration or molecular weight, even if these factors greatly affected the patterns of rheological transitions. We have shown here that gels made from all the E-type and F-type HPMCs at their minimum gelling concentration melt at similar temperatures, and moreover that they melt over a temperature range, rather than at one specific temperature, suggesting a stepwise process of melting. Finally, we can conclude that all the gels investigated here melt at temperatures above 36.3°C, and therefore will all melt at mouth temperature.

1.5.3. Factors affecting melting events

1.4.3.1. Effect of maximum heating temperature on subsequent melting events, as measured using rheological and calorimetric methods

Increasing maximum heating temperature of sample F450 from 75 to 95°C on the calorimeter increased peak height on subsequent cooling (and therefore it is thought enthalpy), and although it appears to increase maximum endothermic peak temperature and therefore melting temperature for both 3% and 4% F450 (see Figure 1-23, which shows 4% F450 as an example), this increase is only significant for 4% F450 ($p=0.02$) and not 3% F450 ($p=0.22$). Desbrieres and Rinaudo (1998) found that exceeding a certain temperature on heating caused a double exotherm to be observed on cooling, but we did not see this.

The results obtained here suggest that heating to a higher temperature allows for more water cages to be broken, since this would result in a larger energy release on cooling due to the reformation of more cages. After heating the sample to different temperatures and then cooling, the subsequent gelling transitions are completely identical, showing that this gelation process is indeed truly reversible, and appears to have no memory.

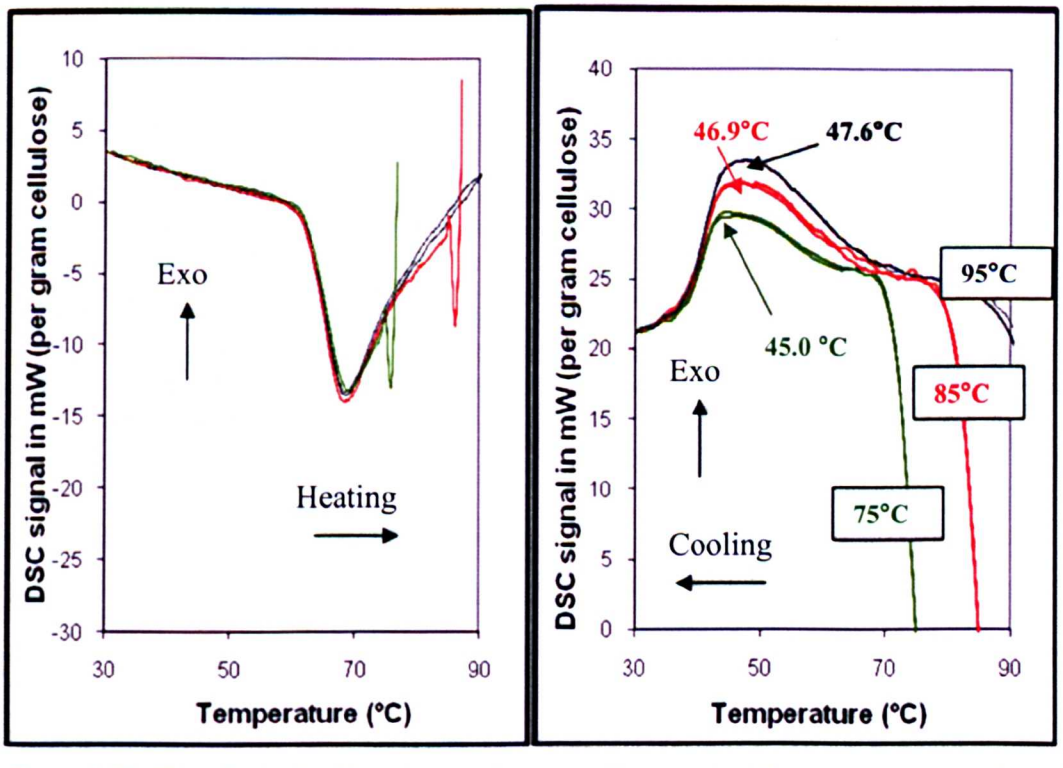


Figure 1-23. The effect of gelling temperature on subsequent melting events, as monitored by calorimetry. 4% F450 is shown as an example. Average temperatures of peak heights are shown.

The overall patterns of changes in G' and G'' on cooling, as monitored by rheology, did not appear affected by increasing gelling temperature from 75 to 85°C (see 4% F450 in Figure 1-24 as an example), although actual values for the moduli in the gelled state were higher for both 3% and 4% F450 – it is thought that heating to a higher temperature allows more hydrophobic interactions to form, or a more favourable rearrangement of bonding, producing a stronger network, which would increase values for elastic modulus. Values for elastic modulus in the solution state after cooling were also higher after heating to a higher temperature, which could be because some structure is remaining, however the high variation in general in repeated rheological measurements makes it difficult to form firm conclusions.

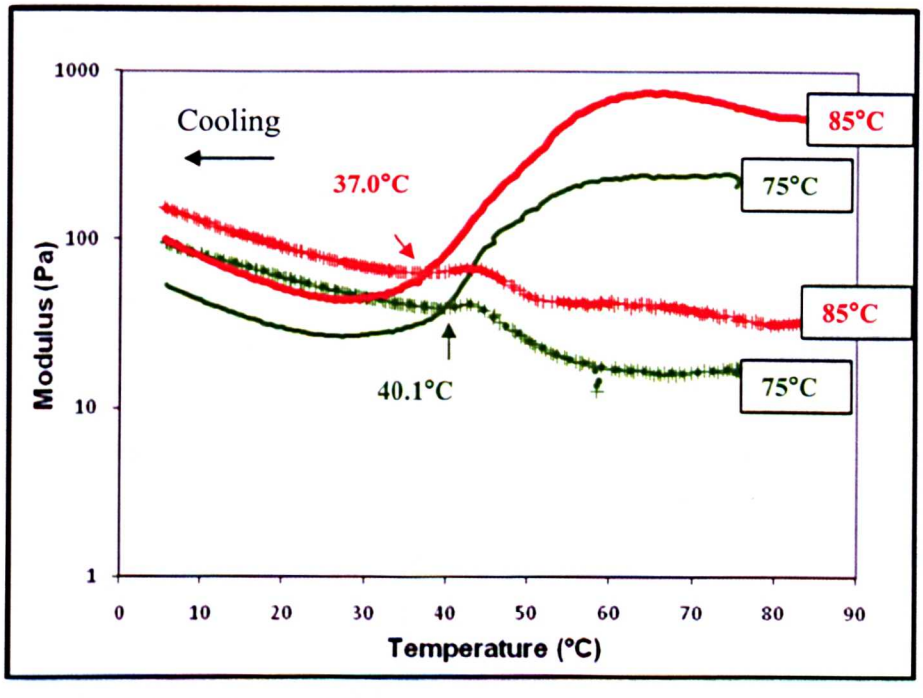


Figure 1-24. The effect of gelling temperature on the changes in elastic and viscous modulus on cooling for 4% F450. Green indicates heating to 75°C, red indicates heating to 85°C. Crosses represent viscous modulus. Continuous lines are elastic modulus. Samples were cooled at 2°C per minute, with an angular frequency of 10 rad s^{-1} and a shear strain of 1%. For simplicity, only one repeat for each sample is shown. Temperatures indicate where values for viscous and elastic modulus cross.

In general, heating to higher temperatures resulted in a lower melting temperature for both 3% and 4% F450 when rheological techniques were used. This could be explained by the stronger gel that is formed (which would need to be cooled more before it could melt). However the difference is not statistically significant (but this could be due to the highly variable nature of repeated measurements). Furthermore, the trend observed was opposite to that observed with calorimetry (where higher

gelling temperatures resulted in higher melting temperatures). Due to the lack of a consistent trend relating maximum gelling temperature to melting temperature, it can be concluded that while enthalpy and moduli values are affected, temperatures at which events occur on melting are unchanged by maximum heating temperature.

1.5.3.2. Effect of holding time on subsequent melting events, as measured by rheological and calorimetric methods

Holding isothermally also did not significantly affect the temperature at which exothermic transitions occured on cooling, regardless of whether the sample was held for the 40 minutes or not, either at 75 or 85°C, and for either concentration (see Figure 1-25 for an example, where 3% F450 is either held or not at 75°C). However, the enthalpy change on cooling after holding appeared greater, even when the sample was only held for the short periods allowed at 95°C. As when maximum heating temperature was increased, it is thought that holding the sample at elevated temperatures allows more water cages to break, resulting in a greater formation on cooling, and therefore a greater energy release.

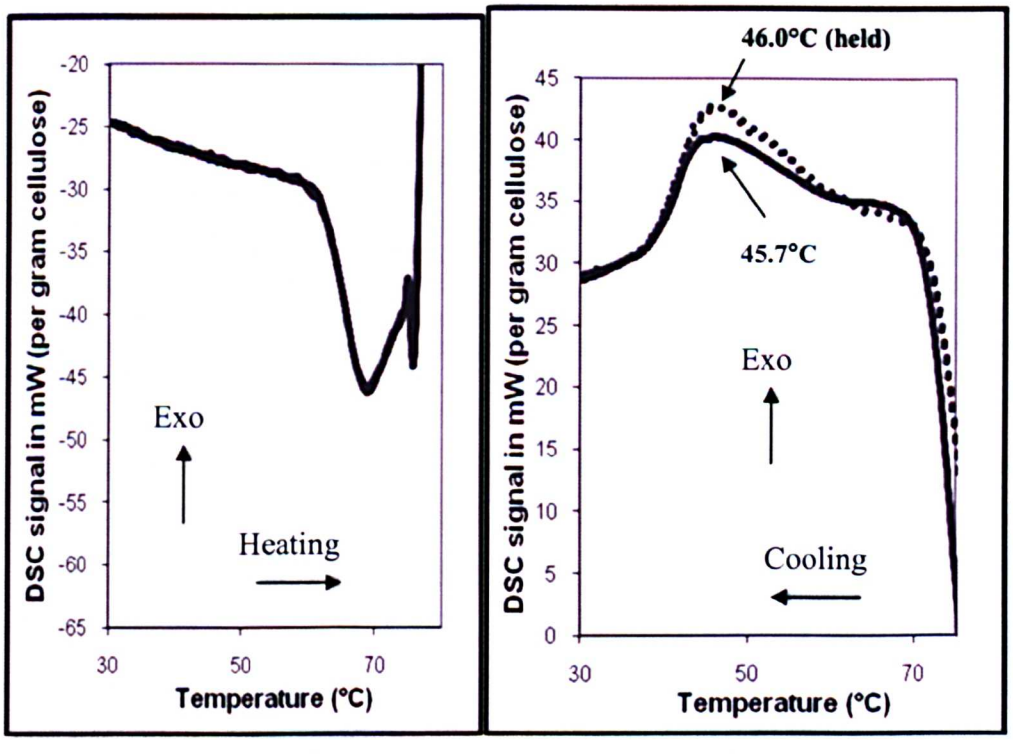


Figure 1-25. The effect of holding isothermally 3% F450 at 75°C for 40 minutes. Dotted lines indicate samples that have been held. Average temperatures of peak heights are shown.

After holding at a high temperature and then cooling, (like for heating to high temperatures and then cooling), the subsequent endotherms on heating are

completely identical, showing that this gelation process is indeed truly reversible, and has no memory.

The effects of holding isothermally had pronounced effects on rheological changes on cooling, similar to the effect of maximum heating temperature. As an example, the rheological transitions for 3% F450, either held or not at 75°C, are shown (see Figure 1-26).

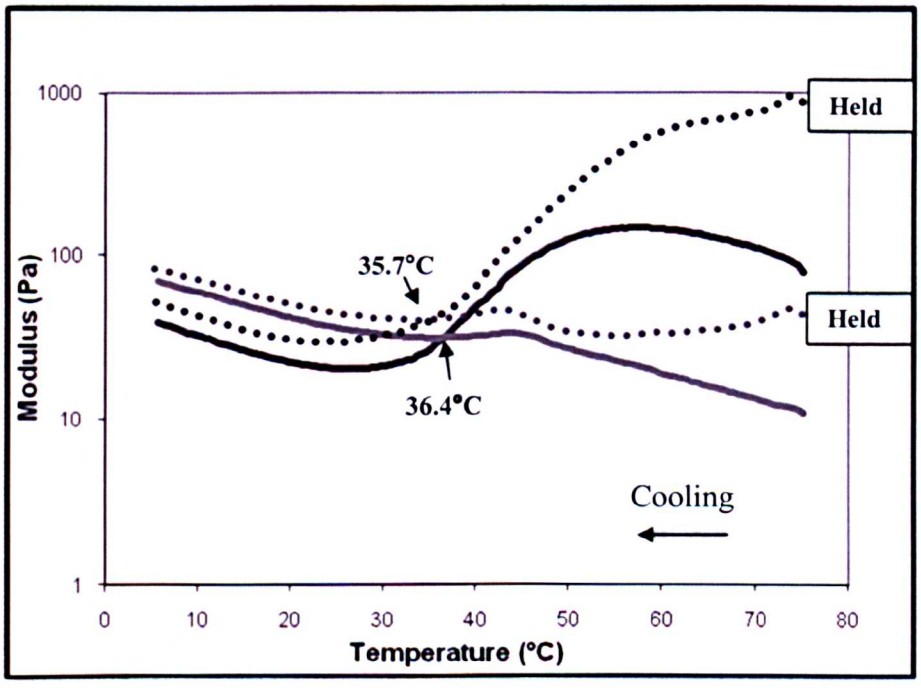


Figure 1-26. The effect of holding isothermally at 75°C on subsequent cooling events for 3% F450, by rheological methods. Dotted lines represent data from samples that have been held for 40 minutes. Grey represents the viscous modulus, and black the elastic modulus. Samples were cooled at 2°C per minute, with an angular frequency of 10 rad s^{-1} and a shear strain of 1%. For simplicity, only one repeat for each sample is shown. Temperatures indicate where values for viscous and elastic modulus cross.

As Figure 1-26 shows, values in elastic and viscous moduli after holding are also much higher than those when the sample was not held, which makes subsequent changes in moduli seem more prominent for elastic modulus, and less prominenent for the viscous modulus. However, with the exception of 4% F450 at 75°C, where the temperature at which G' and G'' cross was significantly lower after holding ($p=0.01$), consistent with a stronger gel having formed, all other values for melting temperature (as determined by G'G'' crossover or exothermic peak temperature), were not significantly affected by holding, and furthermore for some of these samples holding increased melting temperature, and for others it decreased it. Therefore, as for maximum heating temperature, it can be concluded that the main

effects are on the structure of the gel (which affects enthalpy and moduli values), rather than the temperatures over which changes occur.

It has previously been reported that holding methylcelluloses isothermally for long periods of time cause an increase in both G' and G'', with little variation in tan δ (Haque and Morris 1993). It is postulated that during the holding process, the hydrophobic groups have more time to form bonds, strengthen existing bonds, and rearrange the bonding network, all of which would result in an increase in gel strength, or G'. We found an increase in both elastic modulus and viscous modulus during isothermal holding at all temperatures and concentrations, with the changes in elastic modulus being more pronounced than the changes in viscous modulus. The development of these moduli on holding is shown in Figure 1-27. Heat flow also increased during holding (unpublished data), suggesting that changes also occur in the structure of water cages around the HPMC molecules during holding.

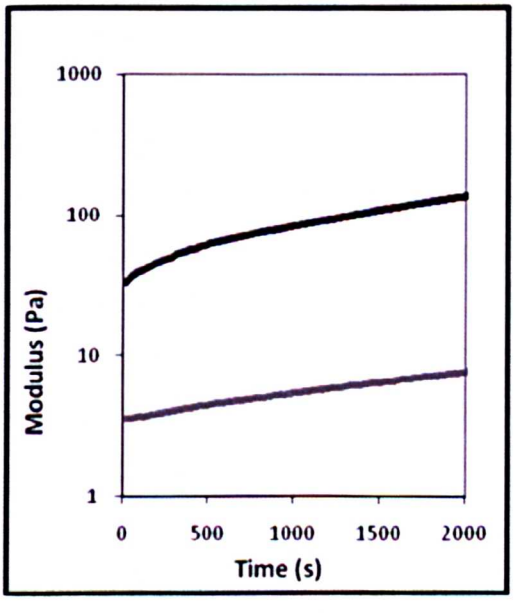


Figure 1-27. Development of moduli and DSC signal while holding 3% F450 at 75°C. Black lines indicate elastic modulus values. Grey lines indicate viscous modulus values.

Haque and Morris found that the increase in G' and G'' with time, although not negligible, was small in comparison with the orders of magnitude of change that they observed as the sample was heated, demonstrating that the gelation process of the modified celluloses is predominantly temperature dependent rather than time dependent (Haque and Morris 1993). We found that for some samples, the development of moduli with time was as great as the development over the whole

temperature range, showing the important impact of holding on gel formation (see Table 1-5).

Table 1-5. The development of elastic moduli as a function of time and temperature for sample F450. Each different number indicates a different repeat.

Sample	Increase in elastic modulus with time	Increase in elastic modulus with temperature
3% 75	14 fold, 5 fold	4 fold, 3 fold, 3 fold
3% 85	8 fold, 10 fold	4 fold, 6 fold, 15 fold
4% 75	5 fold, 3 fold	4 fold, 3 fold, 4 fold
4% 85	5 fold, 3 fold	11 fold, 13 fold, 9 fold

As these results show, holding the gel above its gel point allows a development of elastic modulus, suggesting that a more stable gel network is formed, either due to more hydrophobic interactions forming, or due to a rearrangement of existing bonds into a network that is more favourable. Since enthalpy changes measure the energy required to form and break the water cages, the gradual increase in enthalpy value on holding suggests that during the holding step, more water cages melt (probably cages that are more stable, that were hindered from falling apart without the added holding) which requires more heat to be supplied, and thus a greater enthalpy change on cooling as this heat is released on reforming the cages. A more extensive hydrophobic network could then form during holding as a direct consequence of more water cages breaking, which would cause the increase in elastic modulus values observed on holding.

1.5.3.3. Effect of concentration on subsequent melting events, as measured by rheological and calorimetric methods

The effect of increasing concentration on both the endothermic and the exothermic transitions during the thermogelation and melting of F450 is shown in Figure 1-28. We found that increasing HPMC concentration appeared to increase the overall enthalpy of both the gelling and melting transitions, but by a relatively small amount compared to when maximum heating temperature, or holding time, was increased. It is therefore thought that the larger number of HPMC molecules in more concentrated systems is increasing either the energy needed initially to separate the bundles, or to break the water cages (and therefore increases the energy released on reforming the

water cages or the bundles on cooling) but to a lesser extent to when maximum heating temperatures or holding times were increased.

The peak temperature of the exotherm on cooling for 3% and 4% F450 was only significantly different when samples were heated to 95°C (where more concentrated samples had higher melting temperatures, p=0.02), but not for any other temperatures, suggesting that in general peak temperatures are unaffected by concentration. This is consistent with the results found by Li, Shan et al (2002), where the height and breadth of both the exothermic and the endothermic peaks increased with increasing polymer concentration, but the peak temperature remained the same. Nisharini, Hofmann et al (1997) found that the endothermic peak increased in sharpness with increasing methylcellulose concentration, but it is difficult to conclude if a difference in sharpness exists here. Furthermore, Li, Shan et al (2002) observed that the appearance of a second peak became more prominent with increasing concentration on gelling, but we did not observe a difference in the pattern of the transition on increasing concentration, only in the height (Figure 1-28).

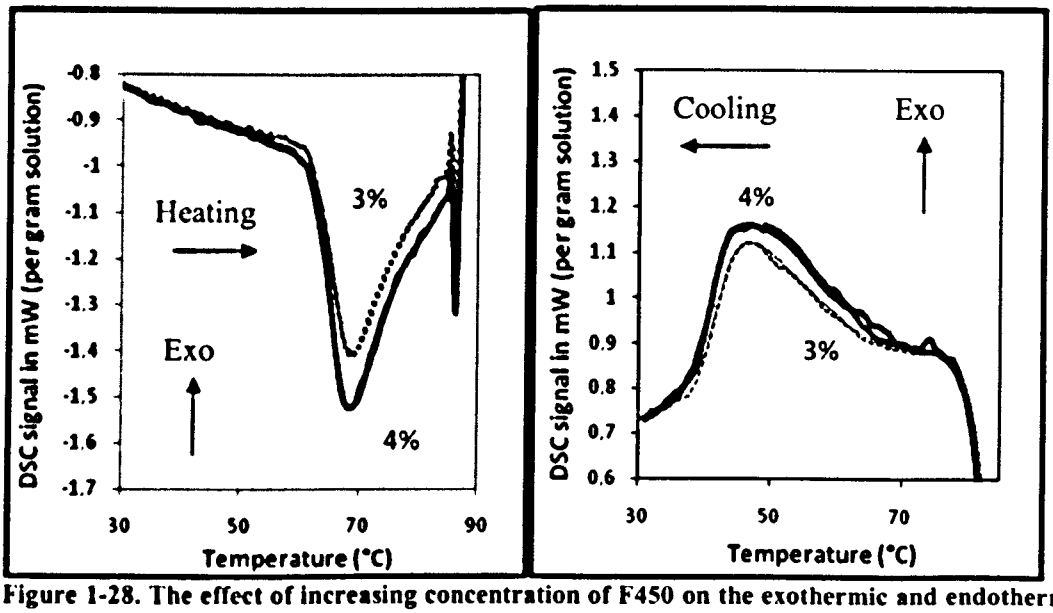


Figure 1-28. The effect of increasing concentration of F450 on the exothermic and endothermic peaks on heating to 85°C and subsequently cooling without holding

Inspection of the rheological graphs also shows that increasing concentration affects actual values for G' and G'' (see Figure 1-29), but does not seem to affect the patterns in which they change, which was surprising since we have previously shown that patterns in transitions change dramatically on increasing the concentration of modified celluloses, due to the contribution of entanglement coupling. Nishinari,

Hofmann et al (1997) observed that the temperature at which G' deviates from the baseline was shifted to lower temperatures with an increase in methylcellulose concentration, but we did not observe this here. Although the value of G'G'' crossover on cooling is signficiantly higher for 4% compared to 3% after heating to 75°C (p=0.04), there is no signficant difference on cooling when samples were heated to 85°C (p=0.82), however there was a large variation in repeated measurements at this temperature. Furthermore, increasing concentration increased gelling temperatures (as monitored by G'G'' crossover) signficantly when heated to 85°C (p=0.01), yet did not affect gelling temperature when heated to 75°C (p=0.99). Increasing concentration has previously been observed to *lower* gelling temperature as determined by rheological changes (Nishinari, Hofmann et al. 1997), and these authors proposed that in solutions of increased concentration, the number of substituted chain segments per unit volume is larger, and therefore the network structure can be formed at even lower temperatures than in dilute solutions. However, the high level of heterogeneity in the repeated measurements made it hard to see if this was occurring with our samples.

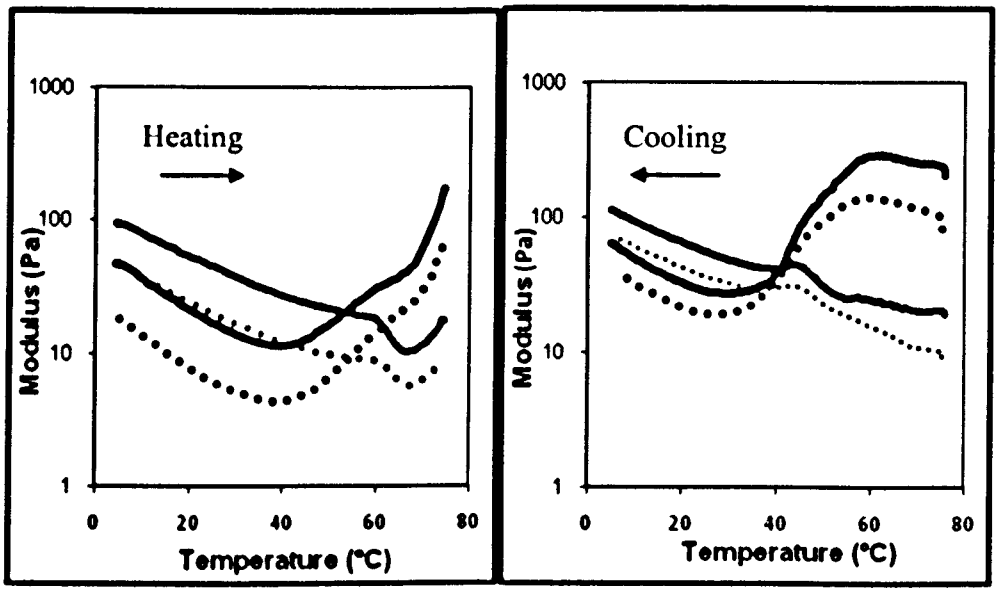


Figure 1-29. The effect of increasing concentration on rheological changes at all temperatures. 4% solutions are continuous lines. 3% solutions are dotted. Elastic moduli are shown in black, and viscous moduli in grey. The sample were heated to 75°C at 2°C per minute. Samples were cooled at 2°C per minute, with an angular frequency of 10 rad s^{-1} and a shear strain of 1%. For simplicity, only one repeat for each sample is shown.

The higher values for elastic and viscous modulus at 4% is probably because increasing concentration causes an increase in number of groups available for bonding and therefore gel strength (hence elastic modulus increases) and increasing

concentration is known to make a sample more viscous, which may explain the increase in viscous modulus. It is therefore somewhat surprising that the enthalpy values do not differ more when concentration is changed, since this suggests that a higher HPMC concentration is not really affecting the level of water cage breakage and formation as much as it affects the rigidity of the gelled sample.

It therefore appears that in general, increasing gelling temperature, gelling time and concentration affects values of moduli (as measured by rheology) and enthalpy (as measured by calorimetry) more so than it affects the patterns of changes in moduli on heating and cooling, or the temperatures at which the changes occurred, but this was when each factor was considered independently. Therefore, the effects of all these factors combined on melting temperature (as measured by exothermic peak temperature in calorimetry) was analysed using a 3-factor univariate ANOVA test and results showed that when considered altogether, some of these factors do have a significant effect on subsequent melting temperature – increasing concentration significantly increased subsequent melting temperature ($p=0.003$), as did increasing maximum heating temperature ($p=0.002$). Holding time, however, did not have an effect ($p=0.40$). The strong gel formed as a result of increasing concentration and heating to higher temperatures would be expected to take longer to disassociate on cooling, and therefore have lower melt-back temperatures, but this is contrary to what we found. The R^2 value was 0.83, suggesting the data fitted well with the model. However, the range of peak temperatures was small, ranging from 44.5 to 47.6°C, so it is thought that any effect on melting temperature due to changing these factors is small.

As shown in Figure 1-30, it is interesting to note that gelling the 4% sample at 75°C resulted in an extremely low melting temperature, as did holding the 4% sample at 85°C.

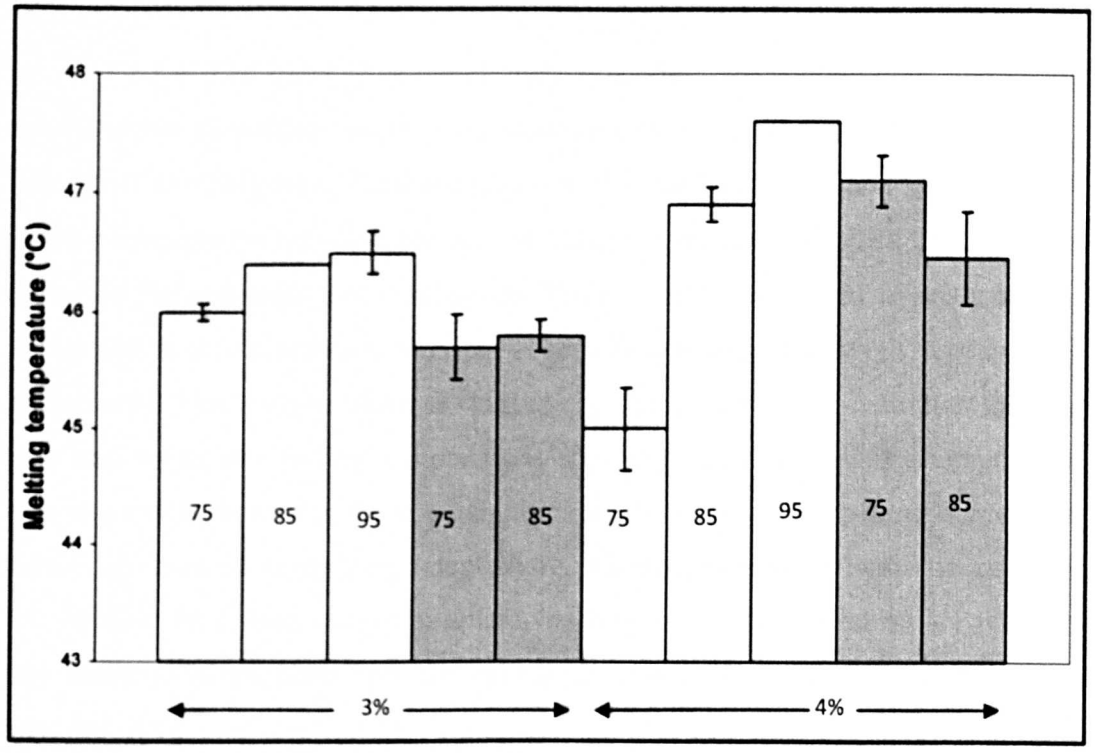


Figure 1-30. Bar chart showing the effect of gelling temperature, concentration and gelling time on melting temperature. Grey bars indicate those that have been held isothermally, white bars represent samples that have not been held. Numbers on each bar correspond to the maximum heating temperature. Error bars correspond to ± half s.d.

The effect of maximum heating temperature, holding time and concentration on the endotherm peak temperature on gelling was also compared using a 3-factor univariate ANOVA. The results showed that as expected, only concentration had a significant effect on gelling temperature ($p=0.03$) - holding time or maximum heating temperature did not have a significant effect ($p= 0.06$ and 0.91 respectively). The R^2 value was fairly high at 0.7, suggesting a good fit. Holding time and maximum heating temperature will only make a difference *after* the endothermic peak on heating, so it is of little surprise that they have no effect. Each time the sample is gelled, it gels at the same temperature, regardless of what has happened before i.e. there is no memory effect. This is consistent with the observation that this process of thermogelation is completely reversible.

Although changes in the temperature at which G'G'' crossed as a result of changing maximum heating temperature, holding time, and concentration, were also monitored by rheological methods, there was not a sufficient quantity of good data to perform a statistical test.

1.5.3.4. The effect of changing gelling temperature, gelling time, and gelling concentration on melting temperature, as measured by the tip test

The effect of changing gelling temperature, time and concentration on subsequent melting temperature was also carried out using the tip test. Raw data for this test is shown in the appendix (see Table 6-2). Design Expert was used to analyse these results (since the experiment was not repeated, a statistical analysis could not be carried out). The analysis showed that all the three factors (concentration, holding time and maximum gelling temperature) significantly affect melting temperature, with concentration having the most significant effect. Increasing gelling temperature caused an increase in melting temperature, whereas increasing both concentration and holding time decreased subsequent melting temperature. The model proposes that melting temperature can be calculated using the following equation (see Equation 1-2):

$$\text{Melting temperature} = 75.3 - 9.2 \times \text{concentration} - 0.047 \times \text{time} + 0.12 \times \text{temperature}$$

Equation 1-2. Value of melting temperature as predicted from results of the tip test.

The R^2 value for the model, including concentration, gelling temperature and time, is very high at 0.99, suggesting a very good fit. This equation also indicates that changing concentration has the largest effect on the melting temperature.

Although an increase in gelling temperature increasing melting temperature was consistent with the results from calorimetry, the other trends were not consistent. Although the tip test is a fairly crude method, it is thought this discrepancy in findings is probably because the effect these factors have on melting temperature are fairly insignificant, and so any changes are minimal. Therefore, in terms of the preparation of this dish in the restaurant, changing these parameters will not have too much of an effect on subsequent melting temperature, so slightly changing the preparation conditions each time is unlikely to affect the overall melting temperature of the gels. However, the results shown here indicate that changing preparation conditions may well affect gel texture, which may affect the consumption experience by changing the perceived texture in mouth.

As well as concentration, gelling time and gelling temperature, most additives (including sucrose, glycerine, and most electrolytes) lower the gel point of a modified cellulose solution, because they have a greater affinity for water and therefore dehydrate the modified cellulose. A few compounds, such as ethanol and propylene glycol, actually elevate the gel point (Sarkar 1979). The presence of such additives may therefore affect subsequent melting temperatures when contained in the final recipe. This will therefore need to be tested, to ensure that the gel will still melt in the mouth when different ingredients are added.

1.5.4. The temperature of the gels on removal from the oven

Temperatures of all eight gels were measured each minute after removal from the oven, and their temperatures were compared at 5 minutes after removal. A one factor ANOVA test on the data showed that the different gels did not differ significantly from each other in their temperature after 5 minutes, regardless of molecular weight, substitution type, or concentration. Although oven temperature was subsequently determined to actually be at 81°C and not 85°C when obtaining these results, when the experiment was repeated for half of the samples at the higher temperature of 85°C, a significant difference in temperature after five minutes was still not observed. The average drop in temperature after removing gels from the oven is shown in Figure 1-31 below.

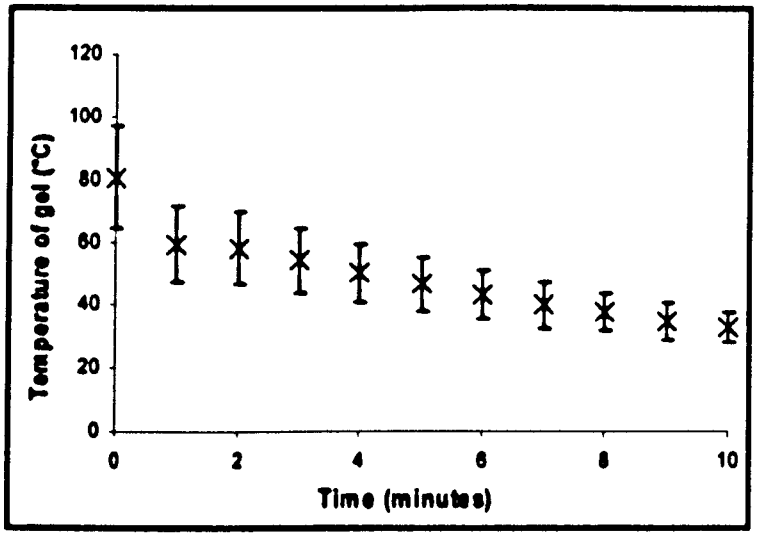


Figure 1-31. Changes in temperature of a gel with time. Values given are averages for the four samples tested - E5 (8.5%), E50 (5.1%), F50 (5.6%) and F220M (1.5%).. Error bars indicate ± half s.d.

1.5.5. Acceptable temperature to drink a hot sample/hot drink

The temperature of the hot drink at which people were prepared to take their first sip ranged from 67–75°C, with an average of 72°C. The temperature at which people were happy to actually drink the beverage ranged from 61-72°C, with an average of 66°C. Although some subjects were happy to consume the liquid at 65°C without discomfort, others still found this temperature too hot, so a lower temperature of 60°C was accepted as the best representation of the maximum temperature at which a hot product can be consumed. It is assumed that this temperature will be the same for a hot solid. Once removed from the oven, gels took 3 minutes to reach 60°C - the temperature at which they could be comfortably consumed. At 3 minutes, all samples were still gelled (unpublished data), suggesting that this would be the best time at which to serve the gels after removal from the oven.

1.6. Overall conclusions

The work presented here uses a number of different techniques to measure melting temperatures of gels prepared from all of the E-type and F-type HPMCs available from Dow Chemicals Ltd. It has also been shown that the conventional criteria of using the crossover of elastic and viscous modulus as an indication of gelling and melting temperature was unsuitable here, because changes in the transitions of the moduli was greatly affected by the relative contribution of entanglement coupling, which made values for gelling and melting temperatures highly dependant on molecular weight. Temperatures as obtained by calorimetric measurements seemed more indicative of true melting temperatures, when compared to results from the more empirical methods, and showed that in general melting temperatures did not follow any sort of trend dependant on concentration, substitution, nor molecular weight. Since all samples melted at temperatures greater than 40°C, it is predicted that all eight HPMC samples can be used to prepare hot gels which can be consumed without burning the mouth, and, once placed in the mouth, will melt as the mouth acts to cool it, in an analagous way to gelatine.

The effect of concentration, gelling temperature and holding time on subsequent melting temperatures was also investigated, and while these factors do affect the values of rheological moduli and enthalpy, they do not appear to have an effect on the temperatures at which the gel-sol transitions occur, suggesting that while gel texture may change, the melting temperatures of the gels will remain fairly constant, despite preparation methods chaning.

The potential use of the modified celluloses as texture modifiers that produce an interesting in-mouth experience will be further investigated in the following chapter, by assessing their suitablility in terms of mouth-feel and flavour release.

1.7. Further work

Further work could include a more extensive investigation into how different factors affect melting temperature – for example, it has been mentioned in section 1.5.3.4 that certain additives affect gelling, and therefore it is thought melting, temperatures of modified cellulose gels. Since these additives include salt, sugar and alcohol, all common ingredients in restaurant dishes, the effect of such additives on melting temperature could be tested using the techniques described here, since this will provide useful information with regards to recipe development.

Secondly, it may be interesting to see how the mixing of different HPMCs, or even HPMCs with various MCs, affects gel texture and melting events, since this may allow for a greater range of gel textures and melting temperatures to be achieved in the final gels, which may increase their potential uses.

2. Melting behaviour of the gels

2.1. Aim

To characterise the mouth-feel, flavour release and melting patterns of the HPMC samples, to see how they compare to gelatine.

2.2. Introduction

2.2.1. Good flavour release and mouth-feel

Many of the food additives currently available, including the modified celluloses, will thicken a solution when present, and it is generally agreed that an increase in viscosity through the addition of thickeners causes a reduction in flavour perception (Baines 1987). Various authors have shown that this suppression of flavour only occurs above a certain hydrocolloid concentration (known as the critical concentration, c*), and this phenomenon has been studied by a variety of authors for different hydrocolloids (Baines 1987; Hollowood 2002; Cook 2003), since it has important implications for the food industry. Flavour perception is due to the detection of both tastants and aromas, but it has recently been shown that the aroma release at hydrocolloid concentrations at which flavour suppression occurs remains unaffected (Hollowood 2002; Cook 2003), suggesting that either tastant release is affected, or somehow that the change in texture is acting at a cognitive level to modify flavour perception.

Gelatine solutions are known to display good flavour release. Recently, Koliandris, Lee et al (2008) showed that gelatine solutions display good tastant (salt) release, and furthermore they have been shown to mix efficiently with water (mimicking salivary mixing). It is presently thought that the ability to mix well with saliva allows efficient and rapid tastant release, and this could explain the good flavour release properties. Other authors (Ferry 2006) characterised various starch suspensions and showed that those displaying good mixing behaviour, as well as displaying good

tastant release and flavour perception, were also reported to have a pleasant mouth-feel. It is therefore thought that good mixing efficiency is also responsible for desirable mouth-feel and this would also explain the pleasant mouth-feel displayed by gelatine solutions. In order for the modified cellulose solutions under investigation here to show as desirable flavour release and mouth-feel properties as gelatine solutions, it is thought that they should also mix well with saliva.

Solutions thickened with the HPMC K4M, above its c* concentration, have previously been shown to display poor mixing efficiency, reduced salt and flavour perception, and have a fairly unpleasant mouth-feel (Ferry 2006). However, K4M is a fairly large molecule, whereas here, solutions containing a number of different molecular weights and concentrations are under investigation, both of which could be affecting mixing efficiency. The mixing efficiency of the eight solutions will therefore be examined.

An understanding of *why* some solutions mix better than others could be useful in helping to predict the mixing efficiency of uncharacterised solutions. Gelatine solutions are relatively non-viscous (Wulansari, Mitchell et al.1998), so it is thought that the low viscosity provided by gelatine solutions could allow for their good mixing. Different viscosity grades of the HPMCs impart different viscosities, so the viscosity of the modified cellulose solutions was therefore investigated, since it is thought that viscosity could be directly related to mixing efficiency and therefore flavour release. However recently, Ferry, Hort et al (2006) have pursued the hypothesis that the decrease in flavour perception with reduced mixing is not fundamentally related to viscosity, but is connected to solution structure - the difference in mixing efficiency of iso-viscous solutions of different types of starch is thought to be because of different levels of chain entanglement. Solutions of wheat starch and modified waxy maize starch, which have been shown to maintain their granular structure in water, display good mixing even above their c* concentration, whereas solutions of unmodified waxy maize, which behave more like a random coil polymer solution, mix less efficiently as a result of entanglement between chains.

In general, c* is defined as the concentration at which individual polymer chains interpenetrate and start to overlap significantly (Morris, Cutler et al. 1981). It is

dependent on the number and space occupancy of polymer molecules. Below the c* concentration, the individual polymer chains are free to move independently, making mixing more efficient (Baines 1989), whereas at concentrations above their critical concentration, aqueous solutions of polysaccharides almost invariably show pronounced shear rate dependant viscosity behaviour, which is typically shear-thinning (i.e. the viscosity decreases with shear). Disruption of entanglements with shear rate is generally cited as the reason for this behaviour (Morris, Cutler et al. 1981; Wulansari, Mitchell et al.1998). Gelatine is a noteable exception to this rule, since solutions show pronounced Newtonian behaviour even above its c* (Wulansari, Mitchell et al.1998), implying a lack of entanglement, which is thought to also be responsible for its good mixing efficiency (Koliandris 2008).

Since a concentration relative to c* is not a direct indication of whether entanglement is occurring, the rheological behaviour of these solutions at 37°C was investigated over a range of shear rates to elucidate whether shear thinning, and therefore entanglement, is occurring. Any solutions showing bad mixing efficiency were not be characterised further, since they are predicted to display undesirable mouth-feel and flavour release properties.

2.2.2. Inherent flavour

As well as affecting the release and perception of added flavours, the addition of HPMC to a solution is likely to impart its *own* flavour and texture. Although untreated gelatine can add a porcine flavour to a final dish, this is easily disguised by the addition of flavours – the same should be true for the HPMC containing solutions. Sensory descriptive tests are often used to obtain a detailed description of the sensory properties of foods and beverages, such as aroma, flavour, and/or oral texture, as well as when a comparison among several products is desired (Lawless and Heymann 1998). Quantitative Descriptive Analysis (QDA) was developed during the 1970s to correct some of the perceived problems associated with the Flavour Profile Analysis - the data is not generated through consensus discussions, panel leaders are not active participants, and unstructured line scales are used to describe the intensity of rated attributes. During QDA training sessions, subjects generate a set of terms that describe differences between products. Then, through

consensus, subjects develop a standardised vocabulary to describe the differences between the samples. The subjects also decide on the reference standards and/or verbal definitions that should be used to anchor the descriptive terms. In addition, during the training period, the panel decides the sequence for evaluating each attribute. The perceived sensory parameters which define the product are thus generated, and then the degree to which each of the characteristics is present is then measured (Meilgaard, Civille at al. 1999).

2.2.3. Pleasant melting patterns

Amongst its other desirable properties, gelatine gels have been reported to display a fast breakdown on consumption, and melt evenly when compared to other gel systems (Szczesniak 1975). It is therefore important that the modified cellulose gels also melt quickly enough to be of relevance to the eating process, and that this melting is even, or the melt-in-the-mouth sensation may not be achieved. Therefore, the melting time of these hot HPMC gels at 37°C will be measured and compared to the melting time of gelatine gels at 37°C, using a variation of the tip-test described in chapter 1. The effect of gel size on melting time will also be considered here, as well as the effect of factors such as gelling temperature, holding time at the gelling temperature, and concentration, since these may have important implications for the dimensions and the preparation process to be used for the final gel product.

Furthermore, the rheological behaviour of the samples after bringing them rapidly down from their gelled state (at 85°C), to 37°C, to mimic what would happen on consumption of the hot gel will be investigated, since this will help predict how they would melt in the mouth.

If results from this research indicate that as well as melting at mouth temperature, solutions containing the modified celluloses match the flavour release, mouth-feel and melting patterns of gelatine solutions, we can feel confident in offering them as suitable gelatine replacements.

2.3. Objectives

- To characterise the mixing efficiency of the samples at 37°C, in order to predict flavour release and mouth-feel properties, and exclude any inefficient mixers.
- To use sensory analysis to characterise the flavour and textural properties of the sample.
- To obtain data on melting time and rheological behaviour at 37°C after rapidly cooling from 85°C, in order to predict the melting behaviour of these gels in mouth.

2.4. Materials and methods

2.4.1. Preparation of solutions

HPMC solutions were prepared at their minimum gelling concentration as previously described (see Section 1.4.2), with the exception that for sensory analysis, sodium azide was not added, and solutions were prepared no more than 2 days before each session.

2.4.2. Mixing efficiency of the solutions

Five millilitre of each of the eight HPMC solutions, coloured with red food colouring (Supercook, Leeds, UK) at 0.2% v/w, to easily visualise mixing (was carefully added to the bottom of a 50ml glass beaker containing 20ml of distilled water, pre-heated to 37°C, by the means of a plastic syringe. The solutions were rapidly stirred by hand with a plastic spoon for 2-3s in a circular motion and photographed before, immediately after, and five minutes after, stirring. Based on the results of this test, samples E4M, F4M and F220M were excluded from further analysis.

2.4.3. Sensory characterization

Six subjects (all female, age range 25-40), each with an adequate experience of sensory testing, were recruited from the Division of Food Science (University of Nottingham). The profiling consisted of four training sessions and a fifth session to collect the final data set. The first and fifth sessions were carried out in sensory booths, designed according to the British Standards "Guide to design of test rooms for sensory analysis of food" (BS 7183:1989). The middles sessions were carried out in the training room. Samples E5, E15, E50, F50 and F450 were used for this sensory characterisation.

2.4.3.1. Training

During the first session, subjects were each provided with approximately 10ml of each sample, in a unique random balanced order. The subjects were seated in sensory booths with appropriate and controlled ventilation and lighting. Each sample was assigned a random 3 digit code, and subjects were asked to cleanse their palate with

water (Brecon Carreg, Carmarthenshire, UK) and cracker (Carrs, United Biscuits, UK). Subjects were asked to make notes on the appearance and the odour of each sample before sampling, and then the flavour and texture in-mouth after sampling.

The next three sessions were discussion sessions, where subjects were provided with an ample supply of all of the five samples, as well as palate cleansers. The sessions involved definition of the terms produced in the first session, in order to separate antonyms from synonyms. A number of references were provided to aid term definition. These are listed in the appendix (see Table 6-3). Many attributes were generated, but only some were seen as discriminatory – these were referred to as "rateable" attributes, and are shown in Table 2-1 below. All other attributes are listed in the appendix (see Table 6-4).

Table 2-1. Table showing all the flavour attributes generated during the first profiling session of the QDA. Rateable attributes refers to those that it was thought discriminated samples

Appearance	
Rateable Attributes:	*Scale anchors:*
Colour (i.e. intensity of straw colour).	Not to Very
Thick (shown by dropping a spoon worth back in the pot).	Low to High.
Odour	
Rateable Attributes:	*Scale anchors:*
Musty (i.e. damp, mouldy, earthy, wet-paper, papier mache).	Not to Very
Chemical (i.e. furniture polish, dilute nail varnish, medicinal, tar).	Not to Very
Taste/Flavour	
Rateable Attributes:	*Scale anchors:*
Damp paper (similar to chewing paper).	Not to Very.
Chemical (i.e. tar, lapsang souchong, smokey).	Not to very
Floral (old flowers, flavour lab fridge).	Not to Very.
Bitter	Not to Very.
Mouth-feel	
Rateable Attributes:	*Scale anchors:*
Thick (i.e. resistance when pushed against roof of mouth, gloopy).	Low to High.
Mouth-coating (i.e. how it coats all round the mouth).	Low to High
Sticky (i.e. does it stick to your lips).	Not to Very.
Cloying (i.e. how easy is it to clear the sample, swallow).	Low to High

Once the definition of a particular attribute had been agreed, subjects were asked to rate each sample individually for this attribute. After sharing their scores, the group agreed on a score for each sample for each attribute, that at least 5 of the 6 subjects agreed on, to facilitate consistent scale usage. This was repeated for each of the chosen attributes, and for each of the samples.

2.4.3.2. Data collection

Each sample was assigned a random 3 digit code, and palate cleansers were provided. Subjects were asked to rate each sample on all of the agreed attributes, using a continuous line scale. This was repeated three times, with 15 minutes breaks in between sessions to prevent fatigue. Data was captured using the computer software FIZZ (Biosystèmes, France), and data was subjected to a two factor (subject, sample) Analysis of Variance (ANOVA) to determine if significant differences existed between samples. Where a significant effect was found, a Tukey test was used to identify which samples were significantly different to which others.

2.4.4. Melting time

2.4.4.1. Preparation of gels

HPMC gels

20g (±0.1g) of HPMC solution E5, E15, E50, F50 and F450 were poured out into 100ml beakers, and beakers were covered in foil and placed in a preheated oven for an hour to gel them. Oven temperature was recorded at 84.4 ± 0.6°C.

Gelatine gels

A hot gelatine solution (bovine skin, type B, ~225g bloom, Sigma Aldrich) was prepared at 4.8% (w/v) as has been described previously (Blissett 2007) since at this concentration gels that were neither too mushy nor too firm were formed (Dr. Annie Blissett, personal communication). 20g (±0.1g) of this hot solution was poured out into 100ml beakers, and left to set at 6°C for 24 hours before testing.

2.4.4.2. Measuring melting time of gels

HPMC gels

After removal from the oven, beakers containing the HPMC gels were immediately placed in a water bath set at 37°C. Every two minutes, samples were removed

according to a balanced order and assessed for gelation via visual measurements as previously described (see Section 1.4.3.1). Placing the hot HPMC gels in the water bath increased the temperature initially to a maximum of 38.7°C.

Gelatine gels

Beakers were covered in aluminium foil and placed in the water bath at 37°C. Every two minutes a sample was removed and assessed for gelation via visual measurements. Placing gelatine gels in the water bath slightly reduced the temperature to a minimum of 33.8°C.

2.4.5. Factors affecting melting time

To investigate this, a similar experiment was carried out as described in Section 1.4.4.1, with the exception that melting time was measured (as described in Section 2.4.4.2. above) instead of melting temperature. Data was analysed using Design Expert to find any correlations between temperature, time and concentration on subsequent melting time.

2.4.6. Rheological methods

In general, rheological methods were conducted as previously described (see Section 1.4.3.2).

2.4.6.1. Viscosity measurements

Temperature of the rheometer was set to 37°C. Viscosity was measured for step-wise increasing shear rates for all of the eight HPMC solutions.

2.4.6.2. Oscillatory measurements to mimic in mouth behaviour

A temperature ramp was performed at a rate of 2°C per minute between 5 and 85°C for samples E5, E15, E50, F50 and F450, during which moduli and phase angles were monitored. After reaching 85°C, the temperature of the rheometer was dropped suddenly to 37°C at the fastest cooling rate allowed, calculated at 0.56 (± 0.05)°C/s. (However the sample was probably at a higher temperature than that recorded by the software at the end of this cooling step, due to a time lag.) Rheological properties of the solution at this time point was then monitored by successive oscillatory and viscometry measurements at 37°C. For some samples, values of moduli did not

change on heating as expected from the work carried out in the previous chapter, so data from these experimental runs was not used in the analysis (unpublished data).

2.5. Results and Discussion

2.5.1. Melting efficiency of melted solutions

The photos displayed below in Figure 2-1 show beakers containing the eight red modified cellulose samples and water at 37°C. Photos were taken 5 minutes after stirring.

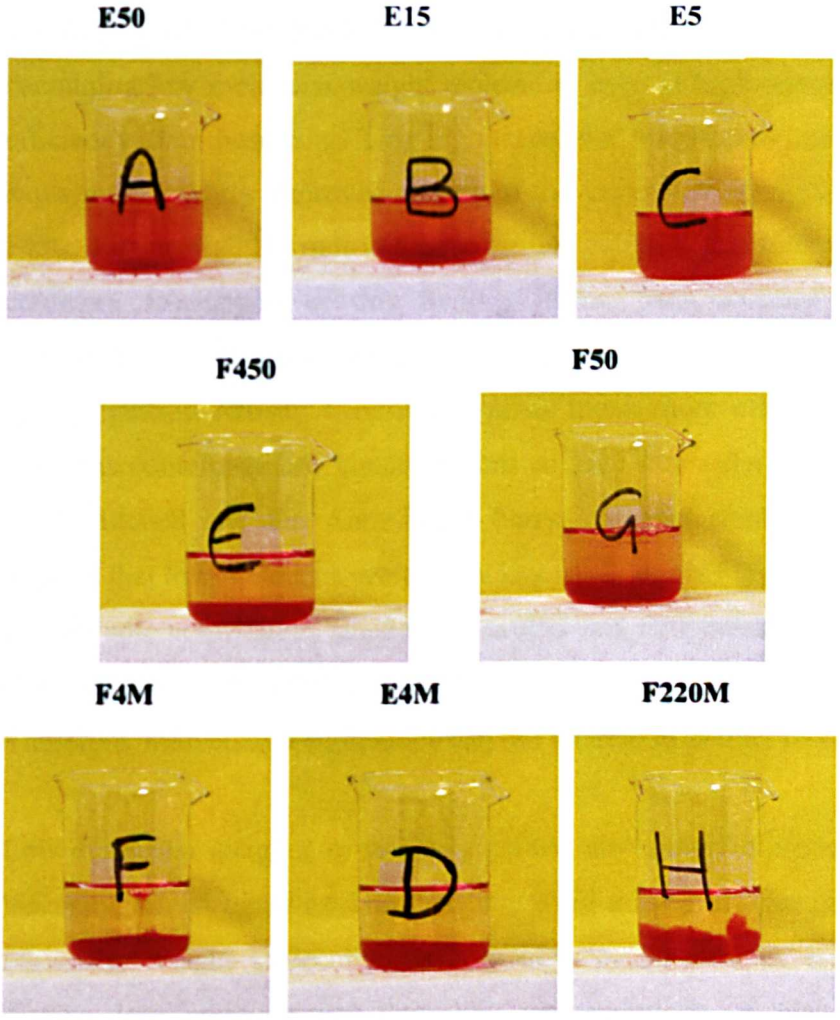


Figure 2-1. Photographs of HPMC solutions five minutes post-stirring at 37°C.

From these photos the mixing efficiency of the solutions was deduced - samples E50 (A), E15 (B) and E5 (C) are mixing efficiently, whereas for the other five solutions, mixing is poor – the coloured HPMC remained a separate phase after mixing.

Since the samples that are mixing the most efficiently are all of the E-type class, it was thought that perhaps the difference in substitutions of the two different types was affecting their hydrophilicity, and therefore their ability to mix well with water.

According to the manufacturers guidelines, the E-type HPMCs do contain a higher percentage range of hydroxypropyl substituents, which may favour better mixing (due to an increase in ability to form hydrogen bonds). However the exact percentage substitutions of the two types is unknown, so firm conclusions on this cannot be drawn.

The three samples that are mixing well also all contain HPMC molecules of low viscosity grade (and therefore low molecular weight), so it seems that solutions containing low molecular weight molecules, even at high concentrations, mix more efficiency than those containing high molecular weight molecules. (This would also explain the slightly improved mixing of the lower viscosity grade F50 compared to F450, but the slightly crude and uncontrolled nature of this test makes this difficult to confirm). In support of this finding, it has been recently shown that highly concentrated solutions of a low molecular weight alginate molecule, used to make the indigestion remedy *Gaviscone*, mixes much more efficiently than iso-viscous solutions containing low concentrations of high molecular weight molecules (Prof. John Mitchell and Dr. Anne-Laure Ferry, personal communication). It therefore appears that low molecular weight is a key factor driving good mixing efficiency (but not the sole contributor, since samples E50 and F50, which contain similarly sized molecules but at different concentrations, differ in their mixing efficiency). Therefore, molecular weight alone can not be used to predict mixing efficiency.

Low molecular weights, or viscosity grades, are known to produce solutions of low viscosity, which *could* explain their improved mixing efficiency. Solutions of small molecular weight molecules, even at high concentrations, are also predicted to display less entanglement than low concentrations of high molecular weight molecules, as exemplified schematically in Figure 2-2, and this lack of entanglement could be responsible for the good mixing efficiency. Work carried out by Ferry, Hort et al (2006) on iso-viscous starch solutions, as described in the introduction, suggests that the latter is more important in affecting mixing efficiency (although the two are invariably linked).

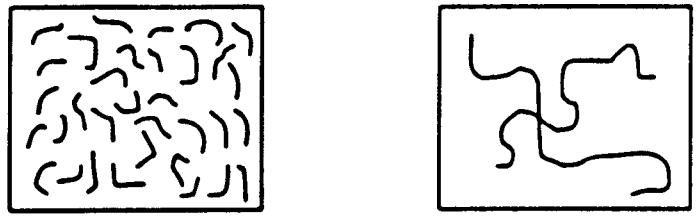


Figure 2-2. Schematic diagram suggesting how the level of entanglement could be linked to molecular weight.

In order to further understand what is responsible for the different mixing efficiencies, the rheological properties of the eight solutions were considered. Solutions in which polymers are entangling tend to show shear thinning behaviour with high shear rates. It is proposed that at low rates of shear, entanglements which are disrupted by the imposed deformation are replaced by new interactions between different partners, resulting in no net change in the extent of entanglement, and hence no reduction in viscosity - this results in a Newtonian plateau that is often observed for polymer solutions. The onset of shear thinning occurs when the rate of externally imposed movement becomes greater than the rate of formation of new entanglements, and thus the "crosslink density" of the network is depleted, and viscosity is reduced (Morris, Cutler et al. 1981). Although typical shear thinning behaviour was observed for solution F220M (see Figure 2-3), for some of the other HPMC solutions the patterns in the behaviour of the viscosity at very low shear rates (i.e. at shear rates of less than $1s^{-1}$) was peculiar, such as shear thinning behaviour at very low shear rates followed by a constant viscosity at higher shear rates, or a two step decrease in viscosity at low shear rates where a Newtonian plateau would be expected. Further studies using a MCR301 (Anton Paar, Austria), where careful control of the temperature was achieved using a Peltier temperature controlled bottom plate and an actively heated hood to cover the sample, showed that this behaviour could be due to the onset of structure formation which is detectable at these low shear rates. At high shear rates it is assumed that this structure was destroyed. Since these solutions gel on heating, it is possible that at 37°C some structure formation is starting to occur that although *not* detectable in calorimetric measurements (deviations from the base line only occur at approximately 50-60°C) is detectable by rheology.

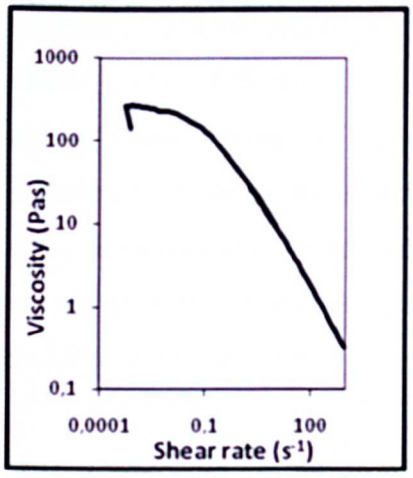


Figure 2-3. Changes in viscosity of sample F220M with increasing shear rate. Data is obtained for the sample coming from cold.

Therefore, to predict the level of entanglement (in order to see how well this relates to mixing efficiency), changes in viscosity of the eight solutions will be explored only for shear rates greater than $1s^{-1}$, as shown in Figure 2-4.

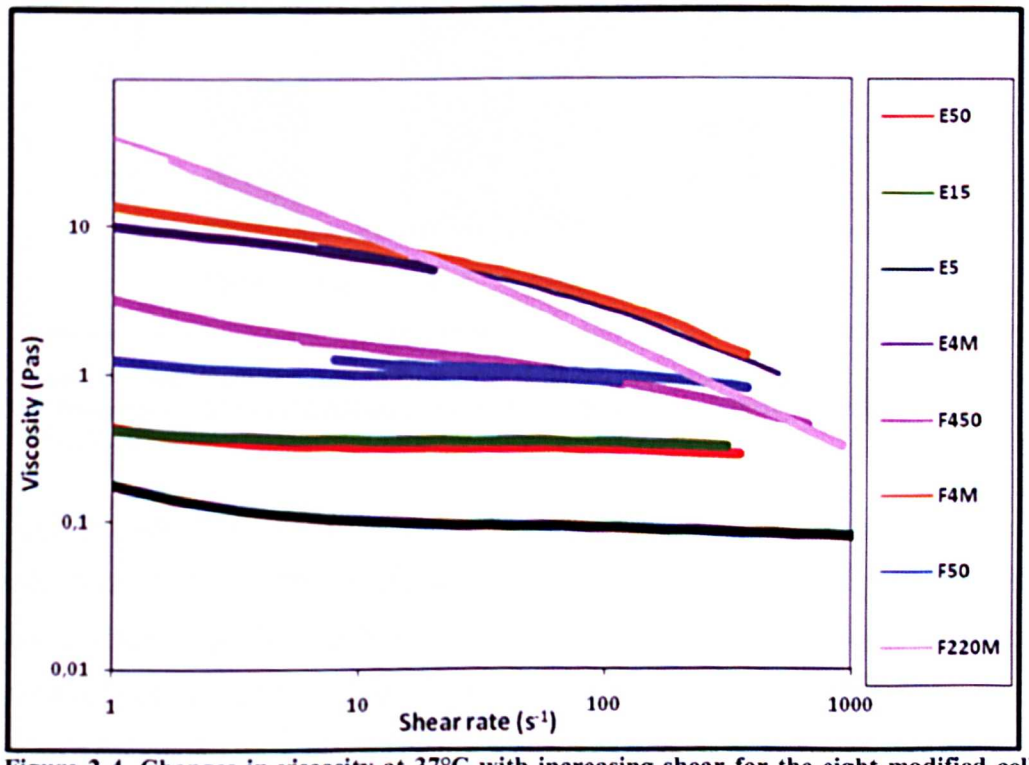


Figure 2-4. Changes in viscosity at 37°C with increasing shear for the eight modified cellulose samples

The gradient of a plot of log γ versus log η as shown above in Figure 2-4 is n, the power law index. For Newtonian fluids, viscosity is constant with shear rate – the value for n is therefore 1. For shear thinning materials, viscosity decreases with increasing shear rates, so the value for n will be less than 1. (For shear thickening

solutions, viscosity increases with increasing shear, so the n value will exceed 1). n values are therefore useful ways of quantifying Newtonian behaviour and therefore entanglement. Graphs of log η versus log γ were therefore plotted for the eight solutions over the range of shear rates of 10-100 s^{-1} (thought to represent a range of shear rates similar to those found in mouth) to calculate n (see Figure 2-5 below). A univariate ANOVA was also carried out on the data, and results from the analysis are included in the bar chart.

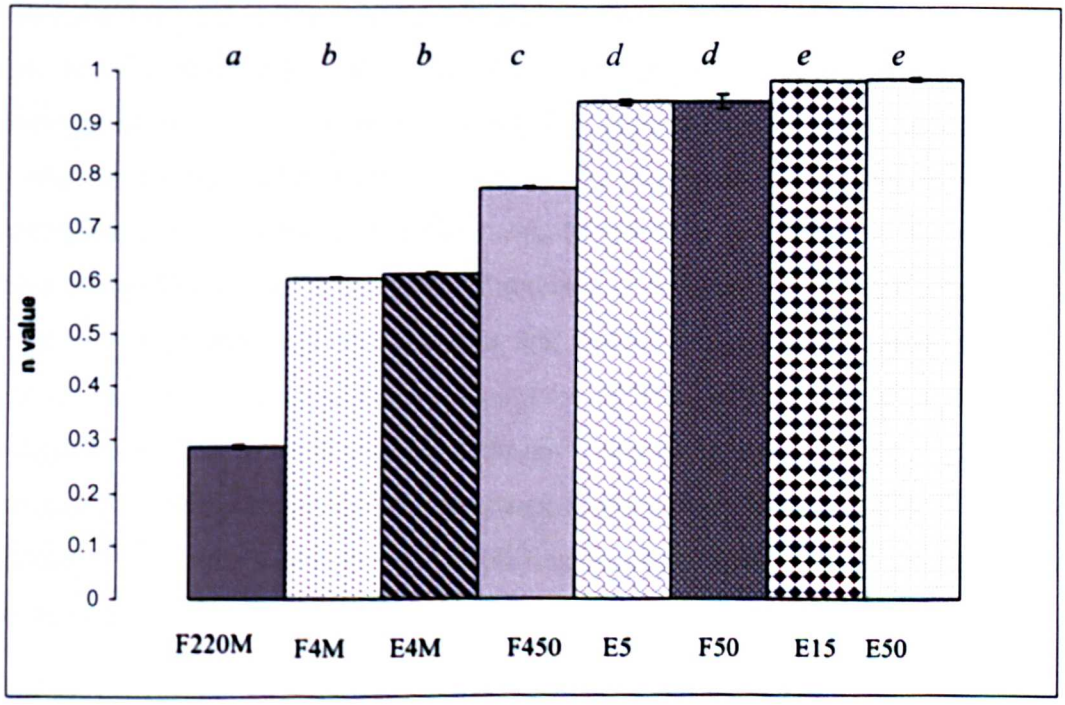


Figure 2-5. Bar chart showing the n values of the eight solutions. Error bars represent +/- half s.d. Samples that differ significantly from one another are indicated by different letters (Tukey test, p=0.05).

As the values for n show, solutions of large molecular weight molecules do show pronounced non-Newtonian behaviour over the range of shear rates investigated here, since their n values are much lower than 1, indicating that significant entanglement is occuring. This is supported by the rheological results from chapter 1, where the masking of certain rheological transitions for these large molecular weight solutions was suggested to be due to the contribution of entanglement coupling. The n value for sample containing F220M (the largest molecular weight HPMC molecule) is significantly lower than for the other samples, and samples E50, E15, E5 and F50 all display close to Newtonian behaviour, with n values much nearer 1, suggesting a direct relationship between molecular weight and deviation from Newtonian behaviour, and that entanglement only predominates above a particular

molecular weight, which corresponds to that of F450. Szczesniak and Farkas (1962) recorded similar findings – for both modified cellulose and alginate solutions they found that the shorter the chain length of the molecule, the closer to Newtonian the viscosity behaviour, whereas the longer the chain, the greater the dependence of viscosity on the rate of shear.

It is somewhat of interest that solutions E5, E15, E50 and F50 all display Newtonian-like behaviour, since it is thought that all the eight HPMCs under investigation here are at concentrations exceeding their c* (Linda Bellekom-Allen, private communication), and therefore would be expected to display entanglement. However, gelatine solutions have been shown to display Newtonian behaviour at concentrations above their c* (Wulansari, Mitchell et al.1998), and these authors have proposed that this is due to the formation of tightly coiled helical structures in solution, which prevents entanglement and therefore allows Newtonian behaviour (Wulansari, Mitchell et al.1998). Therefore HPMC molecules with a molecular weight below that of F450 (which, although it has never actually been calculated, is expected to have a molecular weight range slightly exceeding 120,000-150,000 Da (Sarkar 1979)) are somehow not displaying entanglement, even at concentrations exceeding their c*.

It is also of interest that samples E50 and E15 have n values significantly higher than E5, despite containing molecules of a larger molecular weight, however structure formation at low shear rates seems particularly pronounced for sample E5, and this may be responsible for the generation of a lower value than expected.

When the n value of the eight solutions is compared to their mixing efficiency, it does seem that solutions displaying closer to Newtonian behaviour tend to also show good mixing efficiency, suggesting that entanglement and mixing efficiency are linked. F50, however, is a noteable exception to this - according to the rheological data, this solution is displaying near Newtonian behaviour over the range of shear rates considered (n value = 0.94), suggesting that entanglement is not occuring, however this solution does not display good mixing, implying that the link between entanglement, as predicted from deviation from Newtonian behaviour, and mixing efficiency is perhaps not so straightforward.

Therefore, their mixing efficiency was directly compared to their viscosity, to see if this relationship was more direct. According to Shama and Sharman (1972), the approximate range of shear rates generated when stirring the contents of a container have been reported to be $10^{1.5}$ to 10^{2} s^{-1}. Inspection of the data displayed in Figure 2-4 suggests that values for viscosity at these shear rates seem to be more correlated with mixing efficiency (since sample F50 has a more similar viscosity to F450 than to E5, E15 or E50, which matches more directly with the trend in mixing efficiency), however the slighlty improved efficiency of mixing of sample F50 compared to F450 suggests that viscosity values at lower shear rates may be more relevant in predicting mixing efficiency.

These results suggest that a low viscosity value (i.e. <0.5 Pas) at low shear rates is a good indication of good mixing efficiency, and seems more directly linked to mixing efficiency than entanglement as measured by deviation from Newtonian behaviour. Furthermore, molecular weight, although key in determining how Newtonian-like the sample behaves, can not be used alone to predict mixing efficiency (compare the mixing efficiency of F50 and E50).

Although the solutions investigated here had not been pre-gelled, it is assumed that their mixing behaviour at 37°C prior to gelling would be similar to the behaviour at 37°C post gelation (which represents the solution in the mouth after melting), since the thermal gelation displayed by these solutions is reversible. Due to their poor mixing efficiency, samples E4M, F4M, and F220M were excluded from future characterisation, since poor mixing implies poor mouth-feel and flavour release. Samples F450 and F50 however, despite displaying poor mixing behaviour, appeared to mix better than samples E4M, F4M, and F220M, so were included in the subsequent analysis for comparative purposes.

2.5.2. Sensory characterisation

An inspection of all the attributes generated during the initial discussion session, both rateable and not, suggests that these HPMC solutions had a fairly strong flavour, and

one that was not particularly pleasant. An ANOVA analysis showed that the five HPMC samples were found to differ significantly for 9 out of the 12 rateable attributes. Although for all of these attributes a significant subject:product interaction was found ($p<0.05$), some samples were rated as very similar for some of the attributes, which sometimes resulted in subjects ranking the samples slightly differently and this led to crossovers. Results also showed a significant difference between subjects ($p<0.05$) for 11 of the 12 attributes. This suggests that despite the training, individuals still used a varying range of the scale to score the flavour attributes during data collection.

2.5.2.1. Flavour and appearance

The flavour and appearance attributes straw colour, chemical odour and chemical flavour were all seen to differ significantly between products, and the five samples were placed in a similar order for each of these attributes (see Table 2-1).

Table 2-1. Table showing values of mean score out of 100 (± s.d), calculated from the position of the marking along a line-scale, for all samples for the attributes straw colour, chemical odour and chemical flavour as obtained by QDA. Samples in any one column with different superscript letters are significantly different (p=0.05)

Sample	Attribute		
	Straw colour	Chemical odour	Chemical flavour
E5 (8.5%)	93.6 (±4.6)^{a}	79.2 (±20.8)^{a}	79.2 (±22.3)^{a}
E15 (7.0%)	41.3 (±22.8)^{b}	53.4 (±30.8)^{b}	55.3 (±29.2)^{b}
F450 (3.0%)	19.2 (±14.3)^{c}	42.4 (±33.1)^{bc}	40.4 (±31.7)^{c}
E50 (5.1%)	6.3 (±5.2)^{d}	33.4 (±26.7)^{cd}	41.7 (±30.0)^{c}
F50 (5.6%)	4.5 (±5.1)^{d}	28.0 (±20.9)^{d}	35.2 (±28.0)^{c}

Although subjects are relatively consistent in their mean values for straw colour across the five samples, with all subjects giving sample E5 a significantly larger mean score, inspection of the line graphs for the attributes chemical odour and chemical flavor indicate that different subjects are giving the different samples different values for these attributes, and while 5 out of the 6 subjects gave sample E5 the highest score for these attributes, one subject did not, and the level of crossover was large (see Figure 2-6). An investigation of panel performance shows that almost half of the subjects could not discriminate the samples for these chemical attributes,

so this data should be interpreted with caution.

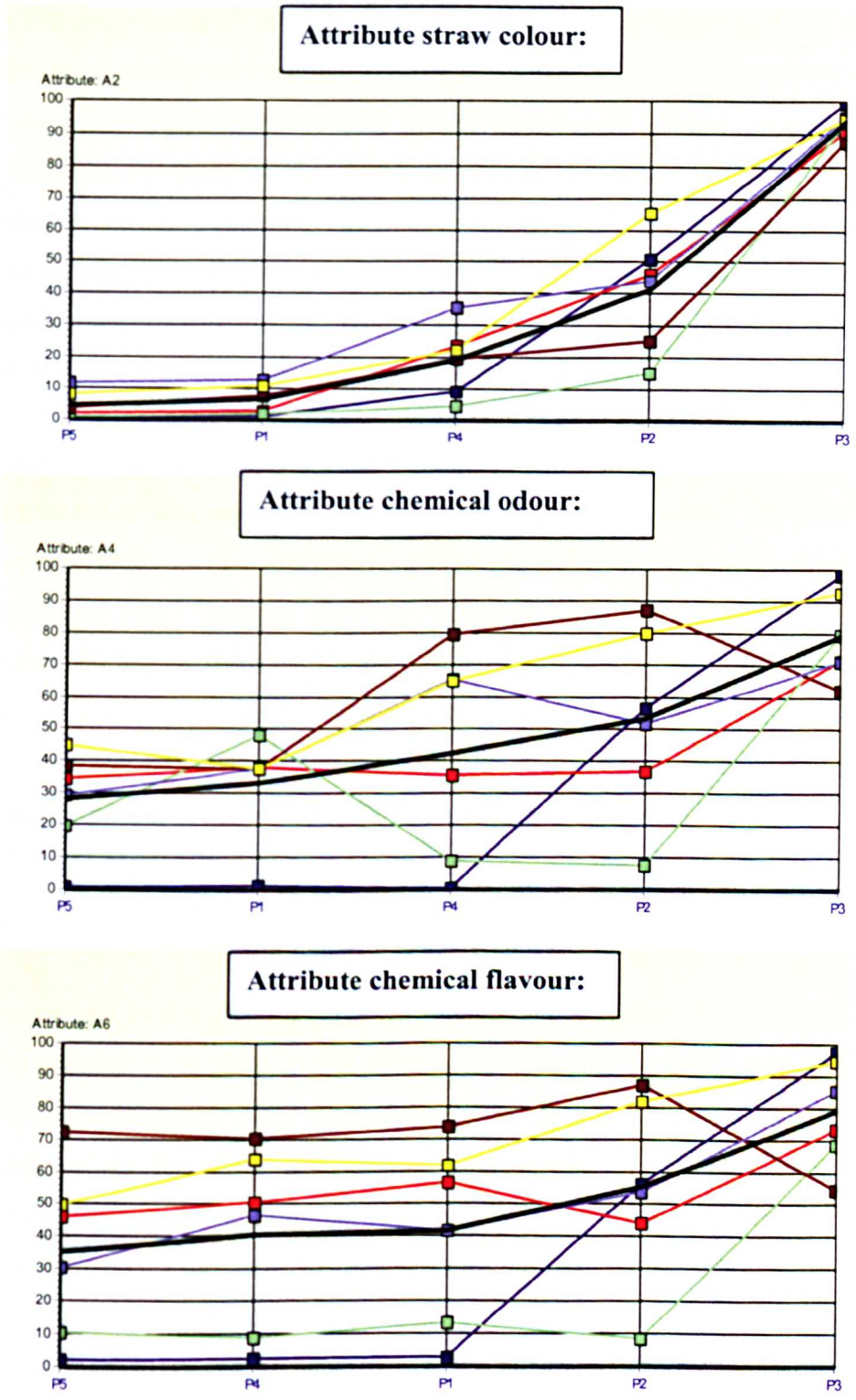


Figure 2-6. Line graphs showing the mean score out of 100 (as measured on position on a line scale) of the three repeated measurements for each of the six subjects for the five samples. Different coloured lines indicate mean values from different subjects, and the thick black line represents the panel mean. P1 is E50 (5.1%), P2 is E15 (7.0%), P3 is E5 (8.5%), P4 is F450 (3.0%), and P5 is F50 (5.6%).

So with the exception of one, all subjects gave sample E5 the highest mean value for these three attributes, suggesting that the generation of high values for these attributes (especially for straw colour) seems linked to concentration - sample E5 is the most concentrated sample. This trend, however, is not directly related, since the less concentrated F450 (3.0%) scores significantly higher values for all three attributes compared to E50 (5.1%) and F50 (5.6%). When these sensory scores were plotted against concentrations in nM (which takes into account concentration in percentage as well as molecular weight) a much better trend is observed - straw colour, chemical odour and flavour all show a very good positive correlation with molar concentration, with R^2 values of 0.90, 0.83 and 0.93 respectively.

Since a high HPMC concentration seems to give high values for these flavour attributes, it was expected that the most concentrated sample, E5, would also have higher values for the other flavour characteristics (musty smell and flavour, bitter taste and floral flavour). However, although sample E5 scored the highest mean value among all the samples for the attributes musty smell and flavour, the five samples were not seen to differ significantly for the attributes musty smell, musty flavour and floral flavour ($p=0.09$, $p=0.12$ and $p=0.50$ respectively). Inspection of panel performance shows that almost all subjects cannot discriminate these attributes ($p=0.05$) from each other, and the mean score for the whole panel is fairly constant across the samples.

In contrast to this general trend for flavour characteristics, for the attribute "bitter", the samples were rated in the reverse order (see Table 2-2), suggesting that large molecular weights, rather than high concentrations, are responsible for generating high values for bitter taste, however inspection of the line graph shows that crossovers are very prominent, and that 4 out of the 6 subjects cannot discriminate the samples for this attribute. Furthermore, during training subjects did indicate that they did not feel as comfortable discriminating samples for this attribute compared with the other attributes.

Table 2-2. Table showing values of mean score out of 100 (± s.d), calculated from the position of the marking along a line-scale, for all samples for the attribute bitter taste as obtained by QDA. Samples with different superscript letters are significantly different (p=0.05)

Sample	Attribute bitter
F50 (5.6%)	48.3 (±28.8)[a]
F450 (3.0%)	44.8 (±28.3)[ab]
E50 (5.1%)	38.6 (±23.2)[ab]
E15 (7.0%)	32.2 (±30.7)[bc]
E5 (8.5%)	17.9 (±18.7)[c]

The results shown here suggest that for most of these flavour attributes, the panel find it hard to discriminate the samples – this could either be due to the samples not in fact differing, or because the subjects did not fully understand the attribute and therefore couldn't discriminate the samples for it. Subjects only participated in four training sessions – it is possible that more training is required. However, the results do suggest that a high HPMC concentration causes higher values for most of these attributes, especially for straw colour.

2.5.2.2. Texture attributes

For thick appearance and the four mouth-feel attributes however, the samples are rated in the opposite order to that for flavour. As shown in Table 2-3, highly concentrated solutions show low values for these attributes. All subjects were able to discriminate the samples for these attributes, suggesting that these textural attributes are more discriminatory overall than the flavour attributes, and that subjects are more comfortable using these attributes.

Table 2-3. Table showing values of mean score out of 100 (± s.d), calculated from the position of the marking along a line-scale, for all samples for the attributes thick appearance, and the textural attributes thick, mouth-coating, sticky and cloying as obtained by QDA. Samples in any one column with different superscript letters are significantly different (p=0.05)

Sample	Attribute				
	Thick appearance	In-mouth thick	In-mouth mouth-coating	In-mouth sticky	In-mouth cloying
F50 (5.6%)	80.0 (±15.8)^{b}	87.7 (±10.8)^{a}	87.8 (±7.8)^{a}	81.4 (±14.6)^{a}	92.4 (±5.8)^{a}
F450 (3.0%)	91.2 (±7.6)^{a}	75.7 (±19.2)^{b}	80.5 (±15.6)^{b}	73.9 (±12.9)^{a}	76.8 (±15.8)^{b}
E50 (5.1%)	54.7 (±21.8)^{c}	63.6 (±21.8)^{c}	72.2 (±15.2)^{c}	60.6 (±24.2)^{b}	71.6 (±18.0)^{bc}
E15 (7.0%)	49.9 (±18.3)^{c}	49.2 (±20.3)^{d}	67.1 (±13.1)^{c}	58.2 (±21.8)^{b}	66.3 (±17.0)^{c}
E5 (8.5%)	15.2 (±15.3)^{d}	13.3 (±14.8)^{e}	21.9 (±15.1)^{d}	15.3 (±13.0)^{c}	15.5 (±16.1)^{d}

Highly concentrated samples contain low viscosity grades, so the results observed here indicate that high viscosity grades (or large molecular weight molecules) produce high values for these textural attributes, even at the low concentration at which they are present. Panel mean values for all these textural attributes, like for the flavour attributes, show the best correlation with concentration in nM than either molecular weight or percentage concentration alone (but in the opposite direction to that for flavour attributes) showing that it is the combination of molecular weight and concentration in % that drives the generation of these flavour and texture attributes. R^2 values showed a strong negative relationship when panel mean scores for these textural attributes were plotted against concentration in nM, with values at 0.85, 0.86, 0.92, 0.90, and 0.88 for thick appearance, in mouth thickness, in-mouth mouth-coating, in-mouth stickiness and in-mouth cloying respectively. The opposite direction of the trends observed here compared to those for the flavour attributes suggests that samples that gave high flavour scores also give low scores for texture. Also, the statistical subgrouping differs depending on the attribute in question, which suggests that different properties of the solutions are important in generating different attributes. Therefore, the different attributes will be considered separately.

Visual thickness:

Sample E5, which represents the lowest viscosity grade, has the lowest score for visual thickness, and F450, the highest viscosity grade of the five samples, has the highest score. This suggests that visual thickness is directly related to viscosity grade, or molecular weight. Samples E15 and E50, which both contain differently sized molecular weight molecules at different concentrations, were seen as iso-viscous in appearance, suggesting that the concentration of a solution also affects the perception of visual thickness. The order of visual thickness correlates well with the order of viscosity as postulated from zero shear viscosities, indicating that this rheological parameter may be linked to visual thickness, however plots of panel mean values for visual thickness against mean viscosity values of the five samples at higher shear rates have higher R^2 values than when plotted against mean viscosity values at low shear rates. This suggests that knowledge of viscosity values over a range of shear rates could be potentially used to predict visual thickness.

The mouth-feel attributes are also rated in an order that in general follows viscosity grade/molecular weight, however sample F450 consistently receives a lower panel average for all the mouth-feel attributes compared with the smaller molecule F50, suggesting that the relationship between molecular weight and mouth-feel attributes is more complicated, and other factors may be playing a role.

Perceived in-mouth thickness:

Each of the five samples differed significantly from each other in their perceived in-mouth thickness. Samples E15 and E50, which were perceived as iso-viscous visually, were perceived as significantly different for in-mouth thickness, and samples F50 and F450 were rated in the opposite way for these two attributes, implying that different stimuli are responsible for generating visual and perceived in-mouth thickness. Tactile perception is almost certainly affected by swallowing and by saliva, both of which alter conditions in mouth (Kokini 1977), and this could be responsible for this difference.

Much work has been carried out in the past to try and predict what rheological parameter corresponds to viscosity as it is perceived in mouth. Original work by Wood correlated the perceived texture of hydrocolloids with their flow properties

and concluded that the stimulus associated with the oral evaluation of viscosity was a shear stress developed in mouth at a constant shear rate of ~ $50s^{-1}$ (Cook 2003) . When the viscosity of the five solutions at $50s^{-1}$ was compared with their perceived in-mouth thickness, we did not find a direct correlation. Figure 2-7 shows how the viscosity of the five samples differed at this shear rate.

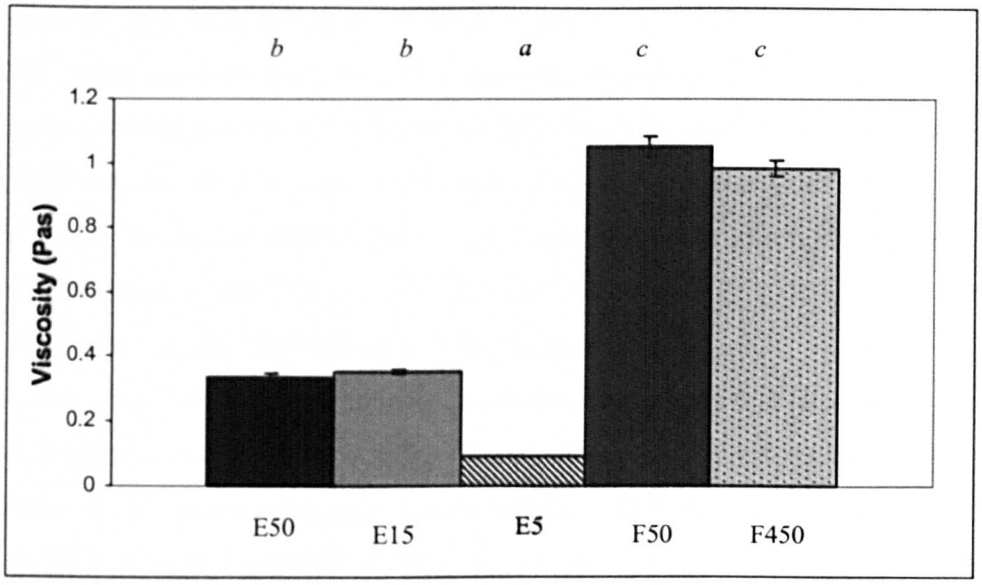


Figure 2-7. Bar chart showing the viscosities of the five samples at a shear rate of $50s^{-1}$. Samples with different superscript letters are significantly different (p=0.05)

The order of viscosity at $50s^{-1}$ does not correlate exactly with the order in which samples were rated for in-mouth thickness, since viscosity values for the pairs of samples E50 and E15, and F450 and F50, were not seen as significantly different, whereas all subjects but one gave sample E50, and F50, a higher score for perceived in-mouth thickness than their iso-viscous equivalent, however the R^2 value of a plot of panel mean score for in-mouth thickness and viscosity at $50s^{-1}$ is fairly high at 0.76. (Interestingly, the order in which the samples were placed based on viscosity at $50s^{-1}$ correlates well with the order in which the samples were placed for the attribute stickiness, although the R^2 value for this plot is slightly lower, at 0.72). This suggests that viscosity at $50s^{-1}$ may *not* be the best indication of perceived in-mouth thickness. Indeed many authors have recently criticised the use of $50s^{-1}$ as a representation of perceived oral viscosity, and have spent much time researching the exact shear rate that corresponds with perceived in-mouth viscosity. Kokini, Kadane et al (1977) and Shama and Sherman (1972) were key players in this research, and they propose that

the value associated with a particular food depended on the flow characteristics of the food itself.

Shama and Sherman (1972) studied the oral perception of viscosity for a wide range of foods, and found it to range from viscosity values at a shear rate of $10s^{-1}$ to $1000s^{-1}$, the exact shear rate depending on the properties of the food itself. For liquid foods, viscosity at a shear stress of approximately 10Pa corresponds to the perceived viscosity in-mouth, whereas for viscous foods, the viscosity value at shear rates of $10s^{-1}$ were more relevant. Between these two extremes, a curved space defining the limits of oral viscosity exist. The rheological data for the five solutions was therefore superimposed on this curve published by Shama and Sherman (1972) (see Figure 6-1) and the results showed that the predicted perceived in-mouth viscosity was that at shear rates of $58s^{-1}$ for sample E50, $58s^{-1}$ for sample E15, $161.5s^{-1}$ for sample E5, $25.3s^{-1}$ for sample F450 and $28.5s^{-1}$ for sample F50.

A few years later, Kokini and his co-workers suggested that the sensory perception of thickness could be predicted by viscosity at the Kokini oral shear stress. They assumed that liquid perception occurs between the tongue and the roof of the mouth (mimicked as two parallel plates). These two are slowly squeezed together by a normal force, W, which is constant during each assessment. Simultaneously, the plates move steadily relative to each other at velocity V. It is assumed that all these forces are constant for each sample, and that the resulting predicted shear stress that corresponds to perceived in-mouth viscosity is dependant on the rheological properties m and n (the consistency index and the power law index respectively) of each sample (Kokini, Kadane et al. 1977). Based on his model, perceived in-mouth viscosity of the five samples should correspond with the viscosity value at the following shear stresses: 12.5Pa for sample E50, 13Pa for sample E15, 6.2Pa for sample E5, 27.0Pa for sample F450, and 23Pa for sample F50. Viscosity values at shear rates and stresses as determined by these models, as well as viscosity values at $50s^{-1}$, for all five samples, are shown in Figure 2-8.

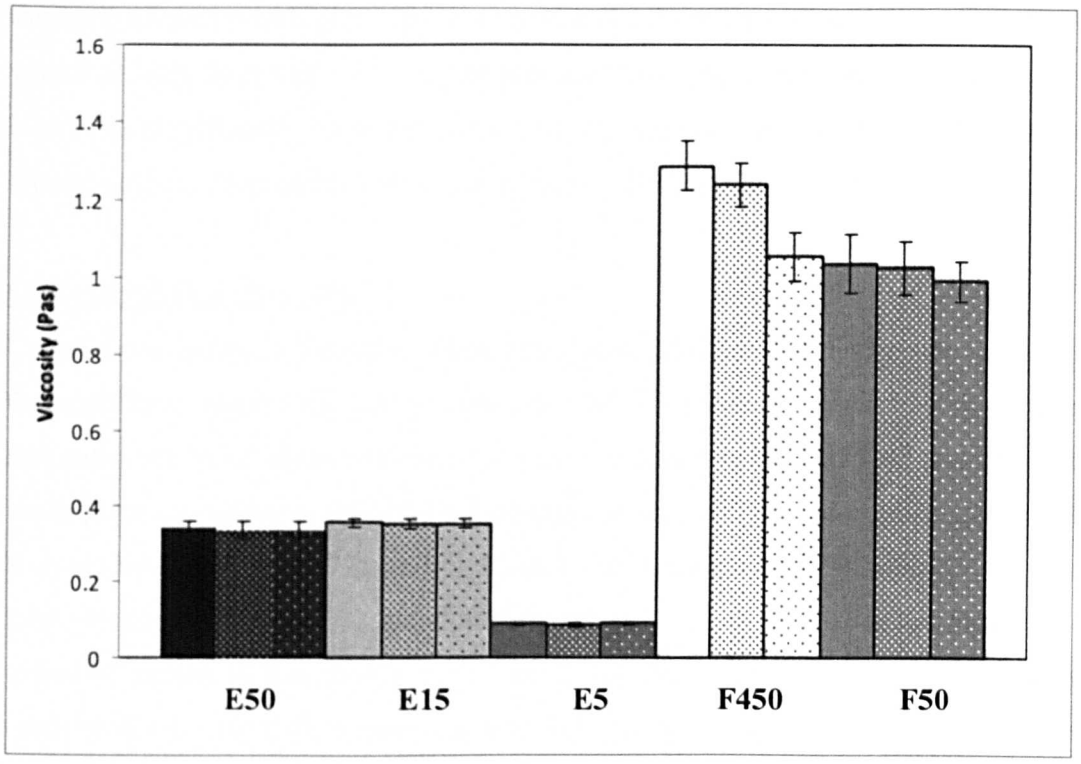


Figure 2-8. Bar chart comparing the predicted in-mouth viscosity from three different models. The first bar for each sample indicates in-mouth thickness as predicted by the Kokini, Kadane et al model (1997), the second bar indicates that obtained by the Shama and Sherman (1972) model, and the third bar indicates viscosity at 50 s^{-1} (the model by Wood (1968)).

However when these values were compared to the sensory data for in-mouth thickness, using the model of Shama and Sherman (1972) and Kokini, Kadane et al (1977) actually predicted sample F50 to have a significantly *lower* viscosity than sample F450, so the data fitted even less well than when viscosity at a shear rate of $50s^{-1}$ was used. Plots of panel mean scores for in-mouth perceived thickness against mean viscosity values at $50s^{-1}$, Kokini shear stresses, and Sherman shear rates, have R^2 values of 0.76, 0.67 and 0.67 respectively, further supporting that viscosity values at $50s^{-1}$ correlate best with the data for perceived in-mouth thickness compared to viscosity at those shear rates and stresses deduced by Kokini, Kadane et al (1977) or Shama and Sherman (1972).

Due to the shear thinning behaviour of F450, viscosity values for sample F50 will actually be higher than for F450 at high shear rates compared to at low shear rates, indicating that perhaps viscosity values at higher shear rates are more relevant to perceived in-mouth thickness. This theory has also recently been suggested by other authors, who report that the shear rate important in generating the perception of in-

mouth thickness is as high as 10^5 s^{-1} (Nicosia and Robbins 2001). However, viscosity values at high shear rates will still be identical for samples E50 and E15. Since E50 is seen as significantly more viscous in mouth, it appears that viscosity values alone are not entirely responsible for perceived in-mouth thickness.

Other mouth-feel attributes:

Correlations between the other three mouth-feel attributes and viscosity values at these different shear rates and stresses showed that panel mean values for all these attributes are most directly linked to mean viscosity values at $50s^{-1}$ compared to mean viscosity values at any other shear rates or stresses (such as those suggested by Kokini, Kadane et al (1977), and by Shama and Sherman (1972)), with R^2 values at 0.65 for mouth coating, 0.72 for stickiness, and 0.62 for cloying. Correlation between viscosity values at low shears and these mouth-feel attributes is poor (R^2 values consistently below 0.4), suggesting that the generation of these three mouth-feel attributes, like in-mouth thickness, are most closely linked to viscosity at $50s^{-1}$ compared to other viscosity values, and may also suggest that panellists are actually interpreting all the different mouth-feel attributes as one.

Since these solutions are not entirely viscous, it was thought that viscoelastic properties at 37°C may be important in generating these mouth-feel attributes. Therefore, differences in phase angle, elastic modulus, and viscous modulus between the five solutions at 37°C were compared (see Table 2-4).

Table 2-4. Subsets for the effect of sample on the values for phase angle, viscous modulus, and elastic modulus at 37°C. Samples in different subsets are significantly different (Tukey test, p=0.05)

Sample	Phase angle (°)	Viscous modulus (Pa)	Elastic modulus (Pa)
E5 (8.5%)	49.4^a	1.11^a	1.11^a
E15 (7.0%)	82.4^b	3.4^b	0.4^a
E50 (5.1%)	84.4^b	3.7^b	0.4^a
F50 (5.6%)	84.6^b	9.5^c	0.9^a
F450 (3.0%)	73.5^b	11.8^d	3.5^b

As Table 2-4 shows, it is possible that generation of the attribute "cloying" could be linked to phase angle, since only sample E5 has a significantly lower value for both the attribute "cloying" and phase angle, while all the other samples were grouped as not significantly different. (Although sample F50 was actually rated as significantly higher for cloying but not for phase angle, it had the highest value for phase angle, even if not significantly higher). Furthermore, plots of the panel means for the attribute cloying against mean values for phase angle for each of the five samples showed a good correlation, with an R^2 value of 0.83.

Phase angle is one of the few rheological parameters (with the exception of viscosity at very high shear rates) where sample F50 has higher values than all the other samples (although the difference in phase angle between sample F50 and the second highest sample is only by 0.2°C, and not significantly higher), which is consistent with the general trend in mouth-feel data. Since phase angle represents the relative contribution of viscous and elastic modulus, or "solid-like" and "liquid-like" proportions in a sample, it is easy to imagine how this could be linked directly to the generation of certain mouth-feel properties – although not grouped in the same significance sub-groups, the attributes in-mouth thickness, mouth coating and stickiness also correlate well with values of phase angle, with R^2 values at 0.66, 0.80, and 0.74 respectively.

Generation of the attribute "mouth-coating" could be linked to the value for viscous modulus at 37°C because with the exception of sample F450, samples are grouped similarly for both viscous modulus and this attribute, however a plot of panel mean values for mouth-coating against values for viscous modulus for all five samples shows a very poor correlation, with an R^2 value of 0.07. This shows that although samples are rated in the same order for this attribute and this property, the relationship between the two is poor. In fact, all plots of the mouth-feel attributes against values for elastic and viscous modulus are below 0.2, showing that while phase angle values are related to these mouth-feel attributes, their appears little relationship between values for viscous or elastic modulus alone and these attributes.

Kokini, Kadane et al (1977) have proposed a sequential evaluation of textural attributes in the mouth – they suggested that the generation of the attributes "thickness", "slipperiness" and "smoothness" depends on the amount of liquid

separating the tongue and the roof of the mouth. Initially, this separation is complete because the tongue is only in contact with the liquid. Only viscous forces are assessed, and the consumer perceives "thickness". Later, the tongue begins to touch the mouth, and both frictional and viscous forces are assessed – the consumer perceives "slipperiness". Still later, the tongue extensively touches the mouth, so feels almost exclusively the frictional force, and the consumer perceives "smoothness" (Kokini 1977). This therefore suggests that the generation of different attributes is actually due to different forces acting on the sample, and therefore cannot be predicted by its rheoloigical behaviour alone. Furthermore, mastication is a process involving the grinding of lumps of food to a fine state, mixing them with saliva, and converting them into a liquid slurry at approximately body temperature. This process of mixing, wetting with saliva, and the breaking up of food are not well mimicked in rheological measurements, and furthermore, the perception of moistness, size, shape and roughness of food particles are important factors contributing to the perception of texture, but these properties are not measured by rheology (Bourne 1977). Therefore, it seems that generation of the mouth-feel attributes of these solutions cannot be related to just rheological measurements.

Relationship with mixing efficiency:

Ferry, Hort et al (2006) found a relationship between mixing efficiency of a solution with water and its mouth-feel. Samples displaying poor mixing efficiency were described by their panel as sticky and unpleasant and harder to swallow than samples which mixed well. Our data agreed with this in general, because the poorer mixers, namely solutions F450 and F50, had much higher values for the mouth-feel attributes sticky, mouth-coating, and cloying (cloying was defined by our panel as hard to swallow) compared to the good mixers E50, E15 and E5, and informal discussion with my panel indicated that samples with high values for stickiness, mouth-coating and cloying were undesirable. However, sample F50 appears to mix slightly better than F450, yet has consistently higher values for these mouth-feel attributes – in order to further understand this relationship, a method to quantify mixing efficiency is needed. It is of interest, though, that for the attribute cloying, samples E50 and F450 are seen as equally cloying, despite mixing efficiency differing substantially, suggesting that mixing efficiency alone is not related to the generation of this attribute.

Relationship with level of Newtonian behaviour:

Other authors have found a direct relationship between mouth-feel and level of Newtonian behaviour. Szczesniak and Farkas (1962) found that those samples that displayed a large decrease in viscosity with increasing shear rate tended to be defined as much less slimy (slimy was defined as "thick, coats the mouth, and is difficult to swallow") than those with behaviour closer to Newtonian. They suggest that the faster the solution decreases in viscosity under the revolving motion of the tongue, the faster and easier it can be swallowed - the slower the change in viscosity in the mouth however, the more difficult it is to swallow (Szczesniak and Farkas 1962). Although this may explain the consistently higher values for the mouth-feel attributes for sample F50 compared to sample F450, in general our findings show the opposite trend to this – those samples with near Newtonian behaviour according to their n value (i.e. samples E50 and E15), had consistently lower values for these unpleasant mouth-feel attributes than sample F450, which deviates from Newtonian behaviour, indicating that Newtonian behaviour causes more desirable, rather than undesirable, mouth-feel properties. Plots of panel mean values of the mouth-feel attributes against average n values display negative correlation as we might expect, but R^2 values are extremely low, with all values at less than 0.1, suggesting little correlation.

In conclusion, from the sensory results presented here, it seems that samples containing high concentrations of the low molecular weight HPMCs have prominent inherent flavour characteristics, but mouth-feel is more pleasant. Samples containing low concentrations of the higher molecular weight molecules, however, have low values for the flavour attributes but high values for the mouth-feel attributes. Although concentration and molecular weight appear responsible in part for the generation of these flavour and texture attributes respectively, the relationship is not clear cut. Furthermore, while good mixing efficiency does seem to correlate generally with pleasant mouth-feel properties, as reported by the panel, the rheological data, while suggesting that there are certain parameters underpinning the perception of certain mouth-feel attributes (such as viscosity at $50s^{-1}$ corresponding to "stickiness" and the attribute "cloying" corresponding to phase angle value), clear links between rheological properties and specific mouth-feel attributes have not been found.

2.5.3. Melting time

The melting time of the five successful candidates was compared to that of gelatine. As Table 2-5 shows, with the dimensions used in this experiment, HPMC gels melt in approximately 4 minutes, and gelatine gels in approximately 6 minutes. Although these may not be the exact dimensions that will be used in the final dish in the restaurant, and despite the large error bars indicating the uncertainty of the state of the sample, the melting time of the gels is clearly similar for the two types, suggesting that the HPMC gels are melting within a reasonable time scale. Furthermore, all of the five candidates are melting within the same time range, suggesting any one of them could be used in the final application.

Table 2-5. Melting time of HPMC gels compared to gelatine gels. G indicates that the sample was gelled, M indicates that the sample was melted, and ? indicates a state of uncertainty.

	State of gel					
Time (minutes)	E50	E15	E5	F450	F50	Gelatine
2	G	G	G	G	G	G
4	?	?	?	?	?	G
6	M	M	M	M	M	?
8	M	M	M	M	M	M
10	M	M	M	M	M	M

When gelatine gels are eaten, a smaller quantity than 20g is consumed each time, and furthermore the process of mastication will break the gel down into smaller parts. It has been shown here that decreasing gel dimensions decreased melting time for both gelatine and the modified celluloses gels (unpublished data), so on consumption of smaller quantities of hot HPMC gels, it can be assumed that as for gelatine, the shorter melting time could be sufficiently quick to allow melting, relative to the eating and swallowing process, in the mouth

Melting time was also affected by the gelling conditions, as shown in Figure 2-9.

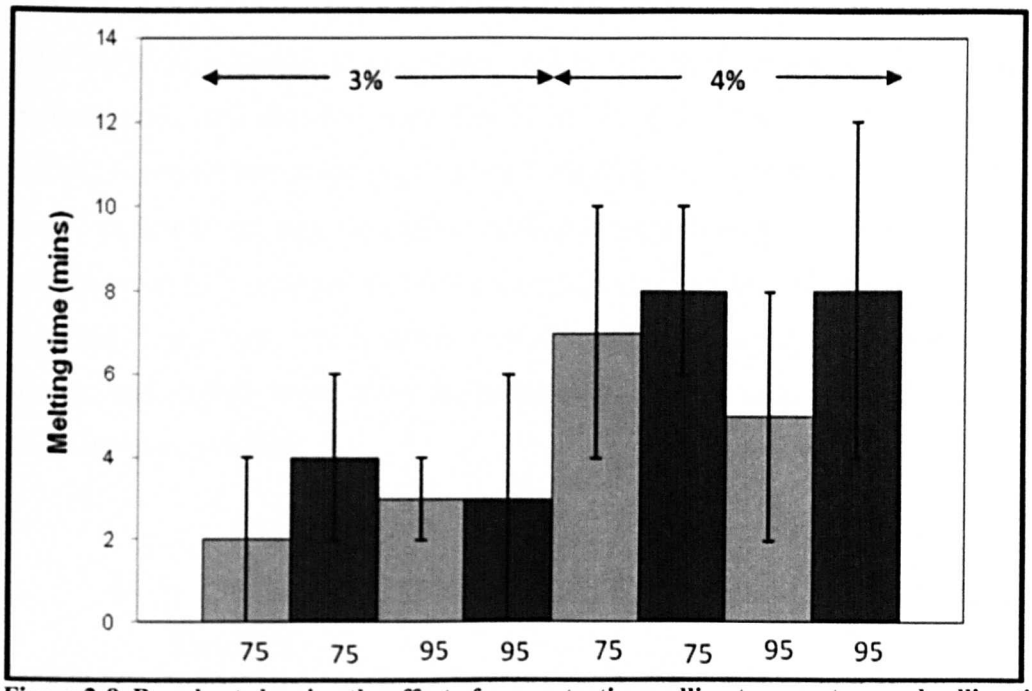


Figure 2-9. Bar chart showing the effect of concentration, gelling temperature and gelling time on subsequent melting time for sample F450. Dark grey bars correspond to samples that have been "held" (i.e. gelled for 80 minutes). Light grey bars have only been gelled for 40 minutes. Numbers below the bar indicate gelling temperatures in °C. The error bars here indicate the temperature range over which the state of the sample was unclear.

From this graph, it appears that increasing concentration causes a general increase in melting time, as does isothermal holding, but gelling temperature does not have an effect. As polymer concentration is increased, it is thought that more bonding groups are available for network formation, so a stronger gel is formed. Similarly, a long gelling time could allow more time for optimal network bonding due to bond rearrangement, which could also result in a stronger gel. The stronger the gel network formed, the longer the time taken for the network structure to break down again, which could explain how increasing concentration and gelling time increased melting time. The lack of effect of gelling temperature on melting time suggests that as long as the critical gelling temperature is exceeded, the same gelling time and polymer concentration will produce a similar gel. When this data was analysed using Design Expert, the significant effect ($p<0.01$) that both concentration and gelling time had on melting time was modelled by the following equation (see Equation 2-1). This model fits the data well with an R-squared value of 0.91.

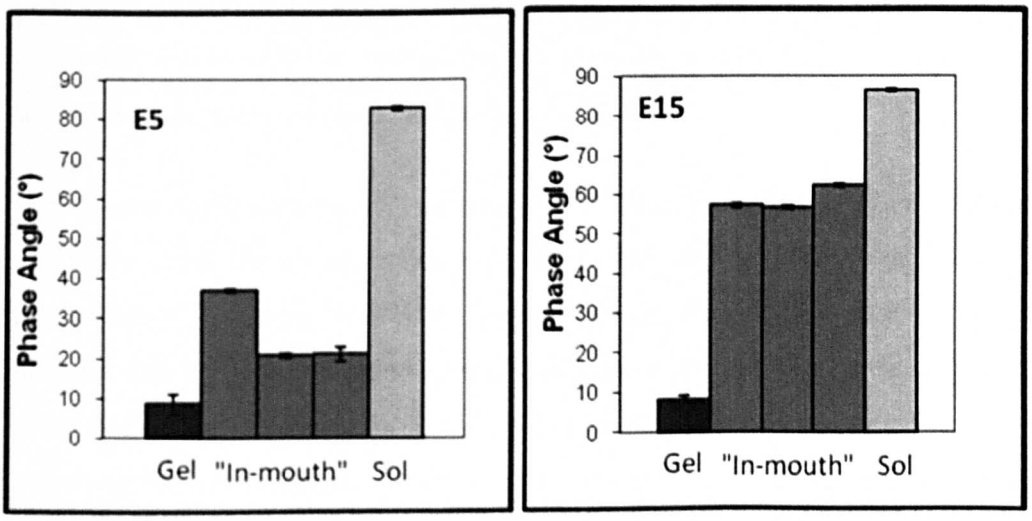
$$\text{Melting time} = -11.25 + 4 \times \text{concentration} + 0.038 \times \text{time in oven.}$$

Equation 2-1. Equation showing the relationship between concentration, gelling time and melting time

This equation indicates that concentration is having the most significant effect on melting time, and therefore care should be taken that the correct concentration of HPMC is used when preparing this gel for a dish. A controlled gelling temperature is not as important as a controlled gelling time, although a minimum temperature clearly needs to be reached to allow proper formation of the gel. However, the tip test is a fairly crude test, which makes it hard to draw many firm conclusions from the results, and slightly more advanced methods need to be used in order to understand the relationship further.

2.5.4. Predicting sensorial melt-in-the-mouth behaviour using rheology

Rheological techniques were used to characterise the viscoelastic properties of the samples after cooling to 37°C, to predict their in-mouth melting. The figure below (Figure 2-10) shows values for the phase angle of the samples after bringing gels immediately down to 37°C from their gelled state at 85°C, and these values are compared to phase angle values both in the gelled state (at 85°C) and the solution state (at 5°C), to see whether their viscoelastic properties at 37°C (called their "in-mouth" state) were more similar to those for their gelled state or their melted state.



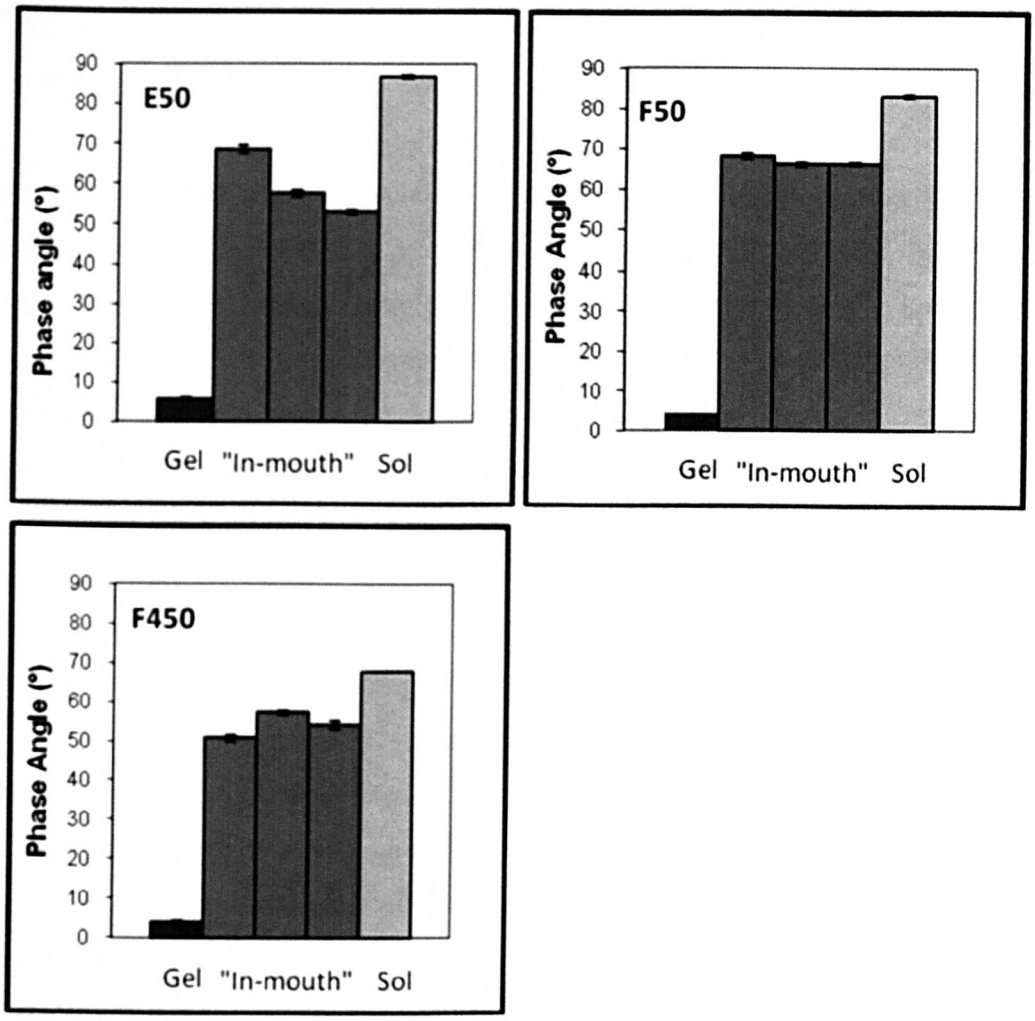


Figure 2-10. Graphs showing phase angle of samples after cooling quickly from 85 °C on the rheometer. Black bars correspond to the phase angle in the gelled state, and the lightest grey correspond to samples in the solution state. Intermediate shaded samples correspond to the phase angle value at 37°C, after gelation. Each of the three bars corresponds to the average value of phase angle over the time-course of the experiment.

When phase angle exceeds 45°, the sample will have more liquid-like than solid-like properties. With the exception of sample E5, all samples had phase angle values greater than 45° in their "in-mouth" state. The low phase angle value for sample E5 indicates that it has predominately elastic (solid) properties, suggesting that it is still somewhat gelled. Samples E50 and F50 had phase angle values closest to 90°, indicating that these samples had properties most similar to a liquid, however their average phase angle was still less than 70°, indicating that some elastic components were still present in the sample, which may be undesirable. It is assumed that gelatine melts back to a liquid with fully viscous properties (i.e.a phase angle of 90°), and this suggests that the hot gels under investigation here may not be fully melted at this point. However, even in their fully liquid state, phase angle values are not 90°,

indicating that even when fully melted, some elastic components remain in the sample.

In order to understand the relative efficiency of melting, phase angle values "in-mouth" were expressed as a proportion of the phase angle value in the fully liquid state (unpublished data). Samples F50 and F450 had the highest value, suggesting that these gels had melted almost completely at this "in-mouth" point. Sample E5 however, had the lowest proportion, indicating that phase angle was still quite different at this point compared to in its fully melted state, indicating that melting was far from complete for this sample at this stage.

These findings suggest that F50 would be display the most efficient in mouth melting, since it has a phase angle "in mouth" most similar to its phase angle value in solution, indicating melting is almost complete, and in addition it has the highest value for phase angle compared to the other samples, suggesting that this sample had the highest proportion of "liquid-like" properties. With the exception of E5, the other samples also show adequate melting, however this technique is only a crude model, and information from sensory analysis on actually consuming the hot gels is needed to confirm this.

After carrying out these oscillatory measurements at 37°C for approximately 4 minutes, the viscosity of the samples was then measured over a range of shear rates. The viscosity behaviour at this point is compared to the viscosity behaviour at 37°C, without preceding gelation and melting (see Figure 2-11).

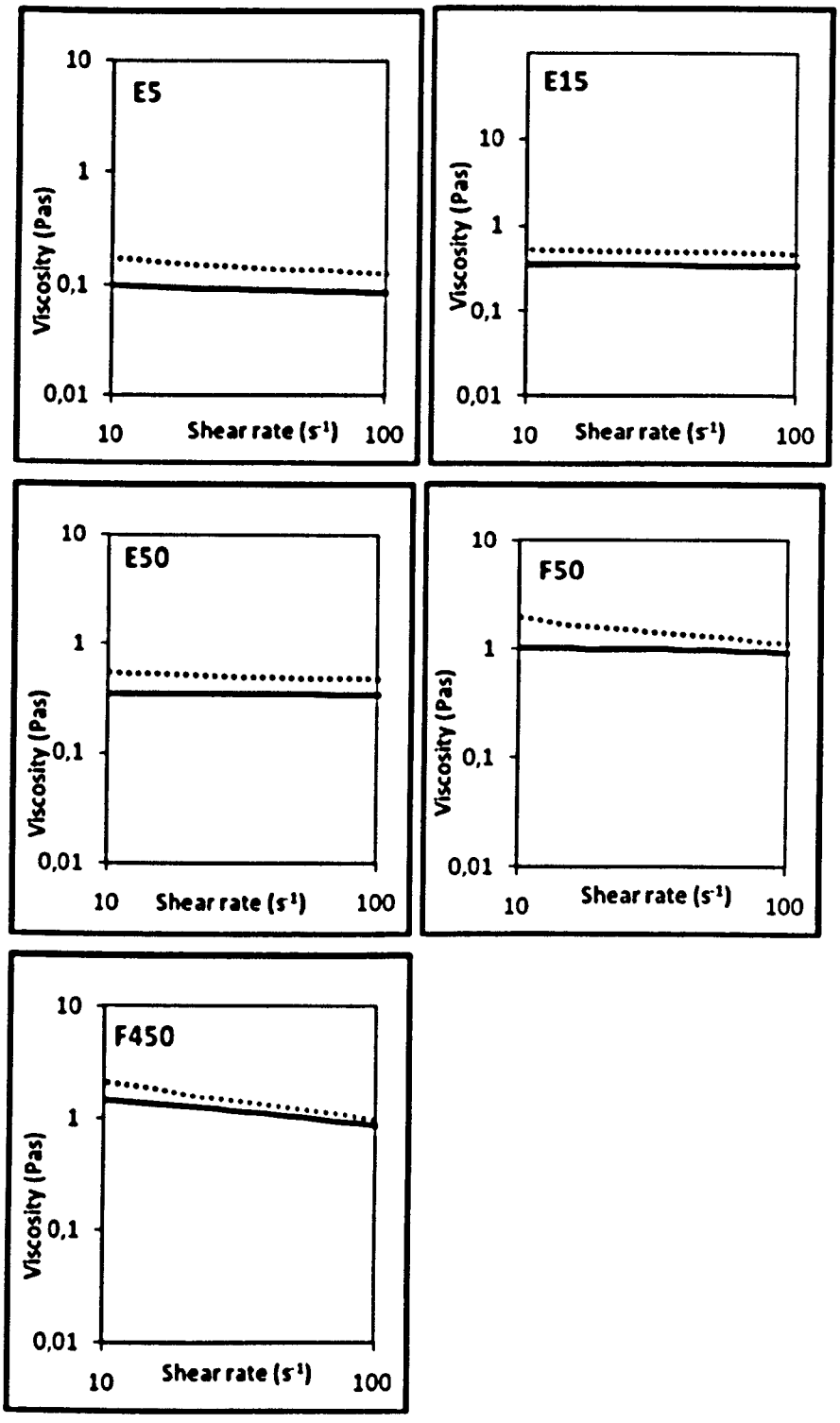


Figure 2-11. The viscosity behaviour of the solutions 4 minutes after melting to 37°C over a range of shears from 10 to 100s^{-1}, compared to their rheological behaviour without prior gelation. Dotted lines indicate post gelation. Black lines indicate samples without prior gelation.

With the exception of sample F50, the rheological behaviour post-gelation is generally similar to that pre-gelation, however viscosity values post gelation are slightly higher. This is thought to be due to some structure formation remaining in

the samples after gelation, which will take some time to destroy, increasing their viscosity. Sample F50, however, actually shows shear thinning behaviour on melting, whereas pre-gelation its rheological behaviour was close to Newtonian. This may be because sample F50 still had some structure in it after melting, yet inspection of its phase angle actually suggests that it melts the most efficiently of all the samples. Szczesniak (1975), who investigated the rheological behaviour of gelatine and carrageenan gels after heating them stepwise to 37°C, found that sensory subjects reported that gelatine gels, which displayed Newtonian behaviour once melted, had the fastest and smoothest breakdown. The disintegrated carageenan gels, however, showed shear dependant behaviour, and their breakdown was described as slower and less smooth, and even slightly slimy (Szczesniak 1975). These findings suggest that E50, E15 and E5, whose melted solutions all display Newtonian behaviour on melting, are breaking down more evenly and pleasantly in the mouth than those displaying non-Newtonian behaviour, however this contradicts the results obtained for phase angle (especially for sample E5) at this temperature.

2.6. *Technology transfer*

2.6.1. Sampling the gels

Samples of what was predicted to be the most successful hot gel candidates were prepared for consumption by Blumenthal and his developmental team, to assess their eating experience. Gels were prepared from sample E50 (at its minimum gelling concentration of 5%) and sample E5 (at both its minimum gelling concentration of 8.5 and at 10%, since the latter formed a more manageable gel than the former, beneficial to the final preparation of the ravioli). While sample E5 had been shown to have the lowest values for the more undesirable textural attributes, sample E50 had lower values for many of the flavour attributes, so was expected to have less of a natural flavour, despite a more pronounced in-mouth texture. These two samples were therefore chosen to compare the relative contributions of inherent flavour and texture to the final concept. 20g of each sample was prepared in glass beakers and gelled at 85°C. The samples were consumed while hot with a spoon, and noticeably melted in the mouth, as predicted, mimicking the sensation of consuming gelatine. However, there are various limitations with using this hot HPMC gel for a potential ravioli dish – the opaqueness of the gels would prevent the consumer from seeing the ravioli contents, the gels were delicate and quite difficult to handle - preliminary work showed that it was difficult to produce and mould tangible gelled sheets from these solutions, and the flavour of the samples was strong and unpleasant.

2.6.2. Flavouring the gels

As the HPMC solutions were described by the panel as sticky, a toffee flavouring was tried since it was thought that it might be congruous with the inherent texture, enhancing overall flavour perception. The full perception of toffee flavour requires sugar, so the sweetener aspartame was also added, instead of sugar, since the presence of sugar at the concentrations needed to produce an equal sweetness to aspartame might change the gel structure and affect its melting properties.

Toffee flavouring (Firmineeh, Swirtzerland) at 6 x 10^{-4}% (w/w) and aspartame (Sigma-Aldrich) at 4 x 10^{-6}% w/w was added to the two samples E5 and E50,

however there were still strong hints of unpleasant damp paper flavour as well as a lingering sweetness and toffee flavour in the final sample. A number of stronger flavours were therefore added instead (at approximately 50% w/w), including wasabi, honey, Dijon mustard, marmite, peanut butter, stock cubes, soy sauce, coffee, hot chocolate powder, Thai green curry sauce, mulberry jam, balsamic vinegar, milk, and tomato ketchup. Although some succeeded in masking the natural flavour, for the dish in mind the chef wanted to be able to choose the flavouring of the gelled ravioli sheet, and not be limited to only a few strong flavours. Furthermore, the addition of such substantial quantities of ingredients to the HPMC solution is likely to have an effect on the structure of the final gel, and therefore possibly its melt-in-the-mouth properties.

HPMC levels could be reduced by combining it with another gelling agent, as Chef Wiley DuFrense, head chef of WD50 in New York, has done in his "oatmeal pannacotta" and a celery root "ricotta", which combine methylcelluloses with carrageenan (Chef Wylie DuFrense, personal communication). However, the inclusion of carrageenan into HPMC gels, although allowing gelation with a reduced HPMC concentration, will keep the gel solid at lower temperatures, preventing the gel from melting on cooling. Preliminary work showed that a solution combining equal quantities of E4M and a gelling agent displaying thermal melting (gelatine) did not gel properly when heated (unpublished data).

It was therefore concluded that hot HPMC gels were not suitable for this ravioli concept, mainly due to the inherent texture and flavour properties. However, the unique concept of thermal induced gelation displayed by these modified cellulose solutions could have other potential uses in the restaurant. Examples include: methylcellulose gels with liquid gelatine centres, however preliminary work has showed that this may be difficult to achieve; modified celluloses solutions that could be squeezed into hot water to form instant and hot gelled spaghettis, balls, or even moussed balls; drinks of different flavours, where the layers are kept separate by a gelled modified cellulose solution; or visual products, for example using hot coloured methylcellulose gels to mimic an egg "un-frying" as the gel cooled and melted. However, the unpleasant natural flavour of the HPMCs at their gelling concentration may limit the success of such concepts.

A few months after sampling the hot melt-in-the-mouth gels with the sponsor, Blumenthal was asked to prepare a dish which would recreate a scene from Victor Velasco's film *Barefoot in the Park* (a more detailed explanation can be found in the Big Fat Duck Cook Book). In order to recreate this, Blumenthal wanted to use a hot modified cellulose gel, that could be placed on a plate along with other ingredients, and after five minutes would melt, "leaking" into and contaminating the other ingredients on the plate. Although naturally bitter, quinine was added to the gel to increase its bitterness in the final dish. For this application, the E-types and F-type HPMCs again seemed the most likely candidates - the SG and A types take a long time to melt unless actively cooled, so would not naturally melt after 5 minutes. The K types would have too mushy a texture to form a manageable gel to be used in the final dish.

Approximately 20g of various E-type and F-type solutions were therefore coloured and gelled at 85°C. Gels were removed from the oven after an hour and placed on plates. Photos were taken every minute to monitor the melting of the gel. Photos of samples E50 and E5 at 1min 15s and 4min 15s are shown in Figure 2-12 below:

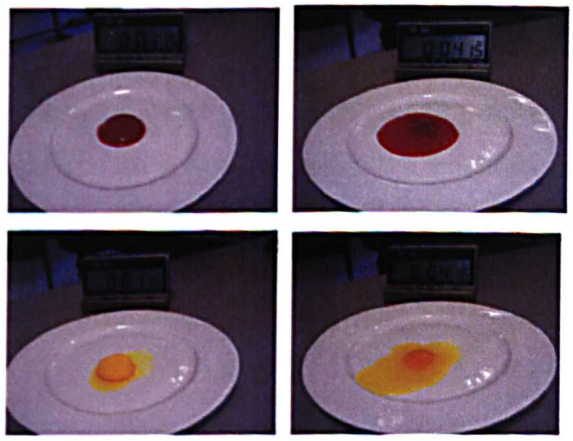


Figure 2-12. Gel at 1min 15s (left), and at 4min 15s (right) after removal from the oven and placing on a plate. The gel made from sample E50 is coloured red, and that from sample E5 is coloured yellow.

As these photos show, gels take approximately 5 minutes to melt. However, the lower viscosity of sample E5 causes it to spread out more around the plate as it melts, so it may mix with and contaminate the other foods in less than five minutes. Higher viscosity grades were therefore thought to be more suitable, since they produce more viscous solutions, but too high a grade may produce such a viscous solution that it

may not "leak", even after fully melting. The melting gel needs to pose a "threat". Therefore, it was thought that sample F450, which contained a medium high viscosity grade, would be the most suitable, so its melting pattern was videoed and sent to the sponsors. The video showed that the gel took five minutes to melt.

Ideally, the gel would remain gelled for a full five minutes before is started to melt, and it would then melt as fast as possible, in order to rapidly taint the food that hasn't been eaten sufficiently quickly. Increasing concentration of F450 to 4% formed a gel that started to melt at approximately 2 minutes, but increasing concentration further to 5% formed a gel that had not even started to melt after 10 minutes, which was far too long for the application, and furthermore, increasing concentration increased viscosity of the melted solution, preventing it from spreading out and tainting the rest of the dish once melted. Preheating the plate to 90°C prevented the gel made from the 4% solution from melting until 4 and a half minutes (at which point the temperature of the plate was 45°C), and therefore met the objective in hand. Therefore, it appeared that a 4% F450 gel, placed on a pre-heated plate was the most suitable candidate for the application, and one that was subsequently used by the sponsors (see The Big Fat Duck Cook Book for more details).

This is an example of how one starts out with one particular objective in mind (i.e. the hot melt-in-the-mouth gel) which, although unsuitable for its original application, turns out to have a number an alternative successful use.

2.7. Overall conclusions

The work described in this chapter involved a characterisation of the mixing efficiency of the eight HPMC solutions under investigation here and how this linked with their rheological properties. Large molecular weight HPMCs, even at a low concentration, showed poor mixing efficiency, while solutions containing the smaller molecular weight HPMC molecules showed good mixing efficiency. When mixing efficiency was compared with rheological properties, it was found that in general, those mixing well displayed Newtonian behaviour, suggesting a lack of entanglement, whereas those mixing poorly displayed non-Newtonian shear-thinning behaviour, however sample F50 was a notable exception to the rule. It appears that low viscosity values at low shear rates, rather than Newtonian behaviour (which was molecular weight dependant), was a better indication of good mixing, and therefore a better prediction of good flavour release. Gels prepared from the five samples that mixed the most efficiently (E5, E15, E50, F50 and F450) all melted within an acceptable melting time, supporting their potential to display melt-in-mouth properties. Although results from oscillatory experiments indicate that samples F450 and F50 will display the most efficient melting and sample E5 the least, based on changes in phase angle, the results from viscometry could be interpreted as suggesting the opposite. Therefore, further experimentation is needed to confirm this – this could include carrying out a sensory analysis as subjects actually consumed the hot gels and described its rate of melting. Samples F450 and F50 had the highest values for the fairly unpleasant mouth-feel attributes of all the five samples, and this is thought to be linked to their reduced mixing efficiency with water. Samples E50, E15 and E5, although rated higher for many of the flavour attributes, had lower scores for the more undesirable mouth-feel attributes, and as is predicted from their mixing efficiency to have better flavour release) than samples F50 and F450. Since the natural flavour of these gels could be more easily masked than the natural unpleasant mouth-feel (see section 2.6.2), it was concluded that gels made from higher concentrations of lower molecular weight HPMC molecules would provide the most suitable candidate for the melt-in-the-mouth ravioli under development here. Furthermore, gels made from these small molecular weight HPMCs have been

shown to form firmer gels (see chapter 1), which would aid the ease of preparation and serving.

2.8. Further work

Further work could include carrying out a sensory descriptive test as subjects actually consumed the hot gels and described their melting patterns in mouth, in order to consolidate the predictions made here.

In addition, varying gelling time could be investigated as a factor when calculating the minimum gelling concentrations of the HPMC samples. Results from chapter 1 indicated that gelling time had an effect on gel texture, so gels could possible be prepared at lower or higher concentrations by varying the gelling time. This could allow the concentrations of the samples to be varied, in order to further understand the effect of molecular weight by removing concentration as a confounding factor.

Finally, a method to quantify the mixing efficiency of the solutions could be used, in order to make more detailed links between mixing efficiency and other properties of the solutions.

Part B - Creating innovative flavour experiences

General introduction

An in-depth understanding of flavour perception can be very beneficial in the food industry, where manufacturers want to ensure overall perceived flavour is unchanged when certain ingredients are substituted (eg for cheaper, or lower calorific ingredients). In the context of this thesis, the knowledge of flavour perception was used to innovate and create potential restaurant dishes which provide unique flavour experiences.

Flavour perception

Flavour is defined as "the complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting" (Sensory Analysis – Vocabulary. ISO 5492, 1992). Volatile aroma molecules that are released on mastication are predominantly hydrophobic, and are detected by olfactory receptors located in the nasal cavity - this is known as retronasal perception. In contrast, orthonasal perception occurs when aroma molecules released from the surface of the food are detected *before* the food is placed in the mouth. So far, over 17,000 different odour compounds have been identified (Meilgaard, Civille et al. 1999). Taste molecules, in contrast, are non-volatile, and during mastication they dissolve in the saliva and are subsequently detected by receptors located primarily on the tongue's surface. A large number of taste molecules exist, but they are normally classified into five major taste groups - salty, sweet, sour, bitter, or umami (the "savoury" or "meaty" taste elicited by monosodium glutamate). As well as taste and aroma, sensations arising from trigeminal stimuli such as hot, pungent and "biting" are detected by pain, tactile and temperature receptors. These receptors are located in the mouth, nose and eye, and when activated they send messages to the brain which also contribute to the perceived flavour. The texture of the food, its appearance, and the sound it makes as it is being consumed, also play a role (Fisher 1997). Changing just one of these components can have a large impact on the final perceived flavour.

Each food that we eat is associated with a unique flavour that is specific to that food. Although the flavour profile of a food may change slightly as it is consumed, for

example flavour intensity may decrease or increase (like the decline in mintiness as chewing gum is consumed), a dramatic change in the flavour during the consumption process is rare. Willi Wonka, in Roald Dahl's famous Charlie and the Chocolate Factory, creates a "three-course-meal" gum, which changes flavour drastically during consumption from tomato soup, through to roast chicken and all the trimmings, culminating in the blueberry pie (Dahl 1998), which amazes Violet Beauregarde (until things go wrong). Is there a reason why this extraordinary experience only exists imaginatively, in a fictional story? Would it be possible to create a similar concept of dynamic flavour release in a product which could be served in a restaurant setting?

Theoretically, to create a product similar to Willi Wonka's bubble gum a spherical delivery system could be developed, where different flavoured layers of gum were assembled successively around a central core – flavours contained within the outer layers of the gum would be experienced first as they were released, followed by flavours in the next inner layer, and finally culminating in the release and subsequent perception of flavours in the central layers. In order for this concept to work in reality, the consumer would need to chew the gum in a homogenous fashion, ensuring even dissolution of each layer and therefore flavour before the next, thus allowing the flavours to be perceived one after each other. If the consumer was to chew the gum in an uneven fashion, the flavours may not be released sequentially – the second flavour may start to be released while the first layer of flavour is still being perceived, which may cause unpleasant mixing of the flavours contained (such as the mixing of gravy and blueberry pie). People actually have very different patterns of eating and chewing when it comes to confectionery. Blissett, Hort et al (2006) used a number of different techniques to characterise the chewing and swallowing profiles during the consumption of two different confectionary chews (gum Arabic-based and carrageenan based). Subgroups of subjects displaying different eating characteristics were identified for both products.

In order to realistically develop a product that could change flavour sequentially or dynamically, some simplifications of the model described above were required. Initial prototypes were therefore focused around more subtle changes in a food flavour, so that any potential, yet probably unavoidable, mixing of different flavours would not be as unpleasant as the mixing of gravy and blueberry pie. Therefore,

appropriate flavours needed to be chosen. Furthermore, to eliminate the effect of the natural variation in peoples chewing/sucking patterns, initial prototypes were made in the form of matrices, where flavour could be changed in a linear rather than a spherical progression, as shown in the Figure A.

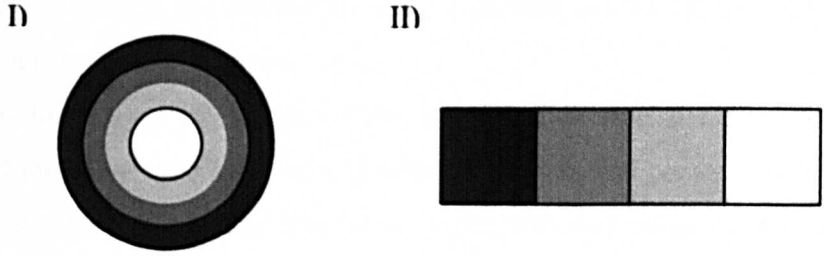


Figure A. Schematic diagram illustrating the difference between a spherical, and a linear, delivery system. I) Spherical delivery system. II) Linear delivery system. Each different shade of colour represents a layer of different flavour.

A simple way to deliver flavours linearly in such a way would be via a solid matrix – different sections could be prepared, each containing a different flavour, and the final sections could be assembled side by side in the final product. As the product was consumed from left to right, the flavour would change. Such an administration system would be easy to prepare, however it was thought that a more impressive and surprising way to deliver the concept would be in the form of a drink. Unlike with the solid matrix, consumers would be left wondering how this magical process had occurred. Although it was expected that there may be some variation in people's drinking patterns, it was thought that the variation would be less significant than with chewing or sucking patterns. Solutions of different flavours could be layered horizontally, and then as the drink was consumed, flavour would change dynamically as each layer was consumed in turn. However, using such a system of liquid delivery presented the challenge of finding ways to prevent the different layers from mixing, both during the preparation process, and during the subsequent drinking process. These issues therefore needed to be considered.

<u>The flavours to chose</u>

Tastants play a key role in driving flavour perception. Salt and monosodium glutamate (or MSG) are well known to enhance the flavour of many savoury foods – more specifically, according to McGee (2004) MSG "lends an added dimension of

flavour to foods that are rich in them" and salt "is a taste enhancer and modifier – it strengthens the impression of aromas that accompany it, and it suppresses the sensation of bitterness". The key factors driving the perception of mintiness and fruity flavours has been shown to be sugar rather than menthol or fruity aroma levels (Davidson 1999); (Hort 2004). It was therefore thought that a subtle, but detectable, way to change the flavour of a food product would be to sequentially change its tastant profile.

Few foods naturally contain molecules that elicit all of the five basic taste sensations, and therefore the choice of a flavour which was still pleasant and congruous even in the presence of all these five tastant molecules was restricted. However, tomatoes are a notable exception – all of the five classes of tastant have been detected in the tomato by biochemical analysis and/or sensory methods (Bucheli 1999). It was therefore decided to develop a tomato-flavoured drink where the perceived flavour would be sequentially modified by heightening each of the five taste sensations in turn, to give the diner a unique flavour experience.

For the second application, we decided to choose a food flavour which actually changed naturally over time, in a pleasant and desirable way. Flavours mimicking these changes could then be delivered sequentially in the product. The changes that occur to the flavour of most foods over time are mainly due to rancidity and the development of off-flavours (often simultaneous to the development of undesirable texture changes), which make the food less and less pleasant to consume with time. In contrast, some foods such as cheeses and fruits, improve in flavour with time – initially their flavour is mild and bland, yet a number of processes and reactions occur over time which actually improves the overall flavour. The ripening of fruit involves a highly coordinated, genetically programmed, irreversible phenomenon involving a series of physiological, biochemical, and organoleptic changes (Prasanna, Prabha et al. 2007). Skin colour changes, normally from green to a shade of yellow or red, due to the unmasking of previously present pigments by the degradation of chlorophyll. Taste develops due to an increase in sweetnesss (mainly caused by an increase in sugars, due to the hydrolysis of polysaccharides especially starch as well as a decrease in acidity). An increase in flavour and aroma occurs, attributed to the production of a complex mixture of volatile components such as ocimen and myrcene; as well as the degradation of bitter compounds, flavanoids,

tannins and other defensive compounds that have accumulated during growth to deter infection or predation. Enzyme mediated alterations in cell wall structure and composition, as well as the hydrolysis of starch and other storage compounds, result in a softening of the fruit texture. These changes all lead to the development of a softer, sweeter and more flavoursome product with desirable quality attributes (McGee 2004; Prasanna, Prabha et al. 2007). Most fruits therefore have a relatively short period of time where the flavour is optimal, and are therefore only consumed in this ripe state. Some fruits, like tomatoes, mangoes and papaya, are enjoyed in their unripe forms as well, but they are normally treated as vegetables in this state and used in salads or pickles (McGee 2004). Bananas are one of the few fruits that are consumed and enjoyed by people over the whole ripening process, although personal and cultural preferences for lesser or more ripe forms do seem to exist.

We therefore decided to create a banana-based drink with a flavour that ripened as it was consumed. This would provide a novel experience for the consumer because fruit normally ripens over a time scale of days not minutes. Speeding up a process that normally takes a number of days to one that would occur in minutes is similar to the concept developed by the artist Sam Taylor Wood in his film "Still Life", in which the slow rotting of a bowl of fruit, usually occurring over a number of weeks, is speeded up to merely a few minutes. His piece is featured in the Tate Modern (permanent exhibition), and was immensely popular.

Aims of part B

To use scientific knowledge to aid the development and creation of two products that changed flavour dynamically, in a desirable and pleasurable way, as they were consumed. The two specific applications that were chosen consisted of:

- a tomato flavoured drink that slowly changed flavour on consumption due to the sequential heightening of each of the five tastants,
- a banana flavoured drink that slowly changed flavour on consumption, mimicking the ripening process.

3. The changing flavour tomato drink

3.1. Aim

To use scientific knowledge to aid the development of a tomato-flavoured beverage that changed flavour as it was consumed due to a sequential modification of the tastant content.

3.2. Introduction

For the drink under consideration here, it is essential that each taste is detected before the next one is delivered. When a solution containing a tastant is applied to the tongue, the taste it elicits is generally detected very quickly (Bujas, Szabo et al. 1991, Kuznicki and Turner 1986, Yamamoto and Kawamura 1981). Reaction time to a taste is defined as the interval between initial stimulation of a receptor and the report of a reaction (Bujas, Szabo et al. 1991). It is generally agreed that the reaction time to salt is the fastest, and that to bitter is the slowest. The faster reaction time to salt is thought to be due to its rapid mechanism of transduction. The reaction time to umami has not been studied as extensively as for the other tastes, but it is hypothesised to be similar to those for the sweet and bitter taste, since they share common transduction mechanisms (Amerine, Pangborn et al. 1965). Mechanisms of taste perception have been extensively studied and can be found elsewhere (Rawson and Li 2004).

It has also been reported that adaptation to a previously administered tastant solution can affect reaction time to a subsequent taste. In 1991, Bujas, Szabo et al compared reaction times to tastants after adaptation to either a particular tastant solution or to water, and they found that preceding adaptation to some tastants significantly shortened or lengthened reaction time to the other tastes. It is therefore thought that the order in which the tastants would be administered in this final drink may have an effect on reaction time, and consequently may affect the detection of each taste. If

the order in which the tastes are administered *is* affecting the time taken to detect each taste, this may have important implications on the order in which the tastants should be served, as well as relative volumes, in the final drink.

During the normal consumption of a flavoured drink, swallowing is required in order to allow full flavour perception. Burdach and Doty (1987) showed that active alteration of the muscles of the mouth and throat, such as those caused by spitting and swallowing, were needed to produce an enhancement of retronasal intensity, and that diffusion alone was not very efficient. They suggest that alterations in air pressure that occur during the various stages of swallowing induce air currents within the oral cavity which causes the movement of aroma molecules to the nasal epithelium where they can be detected (Burdach 1987). As well as the presence of the different tastants, this drink would be tomato flavoured, and tomatoes contain a large array of aromas important to flavour perception. In order that the diner was able to perceive each different tomato flavour (modified by the tastant it contained) in turn, sufficient volumes of each tastant solution would need to be administered to ensure that the diner swallowed before the next flavour was delivered. It is desirable that volumes of each solution to be used in the final drink were as small as possible, since it was thought that if changes in flavour occurred over a relatively small volume this would give the most emphatic and impressive experience. Therefore, ways in which drinking rate can be slowed, in order to reduce the volumes needed while still ensuring swallowing in between the delivery of each flavour, will be investigated here.

3.2.1. The effect of tastant order on reaction time

To mimic flavour delivery that would occur in the drink, a system of continuous flow delivery, called Dynataste, was used, which mixes and delivers solutions directly into a subjects mouth at a pre-programmed rate (Hort and Hollowood 2004). Furthermore, this administration system would eliminate any variation in drinking patterns between subjects. Two tastants were supplied in each experimental run, and reaction times to the second tastant was measured each time, to see how it was affected by the identity of the first tastant.

Potential sensory subjects were screened and selected to ensure that they were capable of detecting all five tastes. Aguesia, the complete loss of taste sensation, is very rare, but some people are particularly insensitive to one or more tastes. People suffering from taste insensitivity were not suitable for the application here, so potential subjects were screened for taste sensitivity using a modification of the British Standards Test "Sensory Analysis of Food – Part 7: Investigating Sensitivity of Taste" (BS 5929-7: 1992).

The chosen panel also needed to perceive the concentrations of the five different taste solutions to be used in the final experiment as similarly intense. If one tastant is perceived as more intense than the others, it may overpower the reaction to the others tastants and skew reaction times. Preparing tastant solutions of equal perceived intensity is not straightforward, since compounds responsible for eliciting the different tastes have different relative intensities. Kemp and Beauchamp (1994) found that the following four tastes were easily detected and identified, and were perceived to be of moderate strength, at these concentrations:– sucrose (0.05M), citric acid (1.25×10^{-3}M), the bitter compound quinine sulphate (Q_2S0_4) (2.5×10^{-5}M), and salt (NaCl) (0.025M). Since a wide range in variation in taste sensitivity among a population (and therefore it is assumed among different sensory panels) is known to exist (Miller 1988), solutions of the tastants were initially prepared based on these concentrations, and the subjects selected for this investigation were asked to rate the tastant solutions for perceived intensity in order to optimise concentrations for the panel used in this study. Based on these results, concentrations of the five tastants to be used in the final experiment were modified accordingly.

Variation in taste sensitivity within a particular individual has also been reported (Amerine, Pangborn et al. 1965) - extraneous noise, time of day, physical condition, hunger level, tiredness, and smoking have all been shown to affect intensity values and the key findings are summarised elsewhere (for a review, see Amerine, Pangborn et al. 1965). Therefore, triplicate measurements, each carried out in a separate session, were taken for each subject.

3.2.2. The quantity of liquid consumed per swallow during drinking

The volume that people consume per swallow during normal drinking patterns will be crucial in developing the prototype, since it will have important consequences for flavour perception. The final drink will be served with a straw, since it was thought that the action of tipping the glass to take successive sips may cause undesirable additional mixing. During drinking with a straw, people need to stop drinking in order to allow breathing, and swallowing normally occurs at this point. It is therefore thought that slowing down drinking rate will encourage swallowing on the consumption of smaller volumes of liquid. It was thought that the diameter of the straw may have an effect on drinking rate, so this was tested. Furthermore, the difference in volume of liquid consumed per swallow while drinking through these different straws was measured, since this will have important implications for the straw dimensions and volumes needed in the final drink. It has previously been shown that sequential swallows differ physiologically from discrete swallows (Daniels 2001) and it is thought that this may affect swallow volumes. Since diners in the restaurant may display either of these two swallowing patterns while drinking, volumes consumed during both sequential and discrete swallowing was investigated.

3.2.3. Technology transfer

To consider how to prepare such a drink on a practical level, focussing on ways to reduce the mixing between the different layers, both during the preparation process, and subsequently.

3.3. Objectives

- To screen potential subjects for aguesia.
- To create solutions of each of the five tastants that were perceived to be equally intense by the chosen panel.
- To measure reaction times to the five different taste solutions using Dynataste to determine if a previously administered taste affects the reaction time to a tastant administered immediately afterwards. Results will indicate the optimum order in which to serve the five tastants in the drink, as well as relative volumes, to ensure that each tastant is detected before the next is delivered.
- To investigate the volumes swallowed on average during drinking with a straw, since this will be key to ensuring sufficient quantities of each flavoured solution are present in the drink to ensure full delivery of the tomato aromas to the nasal cavity (by swallowing), and therefore proper perception of each of the different tomato flavours.
- To suggest ways to layer up the differently flavoured solutions in the final drink.

3.4. Materials and methods

3.4.1. Materials

The following food-grade reagents were used to elicit the five tastes of salty, sweet, acidic umami and bitter respectively: sodium chloride (NaCl) (Asda-brand, Leeds, UK), sucrose (Tate and Lyle, Cheshire, UK), citric acid anhydrous (Fluka, Belgium), L-glutamic acid sodium salt hydrate, (monosodium glutamate (MSG)) (Sigma, Germany), and quinine monohydrochloride dehydrate (QHCl) (Sigma, Germany). Tastants were delivered in the medium of water rather than tomato flavour, to eliminate any effect of tastants naturally present in tomato on the perception of the added tastants.

3.4.2. Recruiting a suitable panel

3.4.2.1. Initial pre-screening of subjects

Initial preparation of solutions:

Tastant solutions were prepared using mineral water (Brecon Carreg, Wales) and salty, sweet and acidic solutions were initially prepared at the concentrations used by Kemp and Beauchamp (1994), although initial tasting indicated that some modifications were needed to make these three tastants easily detectable and identifiable, yet not too strong. Solutions of appropriate strength for the bitter and umami taste were prepared by serial dilutions of QHCl and MSG respectively.

Pre-screening of subjects:

Approximately 50 naïve subjects with no previous experience/training from within the Division of Food Science (University of Nottingham) were selected for this screening, based on their willingness to participate. Subjects were provided with samples of these five tastant solutions and asked if they could detect the five different tastes. 33 subjects could, so were taken onto sensory testing.

3.4.2.2. Sensory testing

Unless otherwise specified, sensory tests were carried out in sensory booths, designed according to the British Standards "Guide to design of test rooms for sensory analysis of food" (BS 7183:1989), and illuminated with Northern hemisphere lighting. Approximately 15ml of each solution was supplied in small plastic vessels, each labelled with a unique randomly generated three digit code. The order of presentation was randomised. Subjects were told to cleanse their palates between samples with plain cracker (Carrs, Long Eaton, UK) and mineral water (Brecon Carreg). The tastant solutions and the water were kept at room temperature (~ 20 °C) throughout the tests.

Screening for the sensitivity of taste

33 subjects (17 male) that had been successful in pre-screening were screened for taste sensitivity, based on the common British Standards Test "Sensory Analysis of Food – Part 7: Investigating Sensitivity of Taste" (BS 5929-7: 1992). Each subject was supplied with 11 vessels, each containing either one of the five tastant solutions or bottled water (the control). The tastant concentrations used were: salt (28mM), sucrose (50mM), acid (2.6mM), umami (5.6mM), and bitter (0.063mM). IOf the eleven samples, each subject received at least one of each tastant solution as well as one water control, and solutions were repeated randomly to make up the final five samples (but no more than one repeat of either bitter, umami or salt was ever given). The order in which samples were supplied was randomised. Subjects were asked to try the samples in the order dictated on their answer sheet and note down which taste they thought was elicited by the sample. Subjects were asked not to return to previously sampled solutions. Of all the subjects tested, 10 subjects correctly identified all the tastes, and 4 subjects misidentified one of the tastes, but correctly identified its repeat. These 14 subjects were therefore selected for the panel to use in the final experiment.

The errors made by the remaining subjects were divided into two types – either one particular taste was confused with water, or two particular tastes were mistaken.

Confusion of one particular taste with water: The inability of some subjects to differentiate a specific taste at the concentrations used in this experiment with water is probably due to the wide variation in recognition thresholds that exists, especially

for bitter compounds (Shallenberger 1993), which is consistent with our findings where the bitter taste was the one most commonly confused with water. Subjects that failed to recognise the salty, sweet or acidic taste were re-tested using solutions of slightly higher concentrations - two subjects correctly identified all tastes at these concentrations, so were selected for the final panel. Subjects that failed to recognise the umami and bitter tastes were not re-tested using higher concentrations and therefore not selected, since informal discussion with the successful subjects indicated that these solutions were already too strong. Based on these findings, tastant concentrations were modified slightly for the next test (i.e. salt, acid and sugar levels were increased, while bitter and umami levels were decreased).

Confusion of 2 or more particular tastes: Subjects who were unable to differentiate different tastes from each other were not used in the subsequent investigation. Confusion between tastants is not uncommon and has been reported previously in a review (Shallenberger 1993). Taste confusions observed here were between bitter and acid (4 subjects); bitter and salty (1 subject); and umami and salty (6 subjects). A frequent confusion between the bitter and acid taste, and the bitter and salty taste, was observed by Meiselman and Dzendolet (1967, according to Shallenberger (1993)). Although these authors didn't test the umami taste, umami is often referred to as the savoury taste, which may explain its frequent confusion with salt.

3.4.3. Creating tastant solutions of equal perceived intensity

Tastant concentrations were modified as a result of the previous test, and the following tastant concentrations were used for this investigation - salt (33mM), sucrose (56mM), acid (3.4mM), umami (4.8mM), bitter (0.045mM). 16 subjects (8 male) were provided with the five tastant solutions in a random order, and were asked to rate them according to the overall perceived intensity of taste on an unstructured line scale with verbal anchors ("no taste" to "very intense taste"). Subjects were allowed to go back and retest previously tasted samples to ensure intensity measurements were made relative to the different samples. Subjects repeated this test three times during different sessions. Ideally, subjects attended sessions on different days, but where this was not possible subjects attended sessions at different times in the day, with never more than two sessions per day. Values for perceived intensity of each solution were calculated as percentages, based on the

position of the subjects indication from the left anchor relative to the total line length. Only 12 of the 16 subjects that participated in this test were available for the final experiment, so only their data was examined.

As expected, subjects showed variation in their perceived intensity values for a particular solution (the greatest range between different subjects for the same solution was 49%, and this was for the sweet solution), as well as in their repeated measurements (the greatest range between repeated values given by a subject for the same solution was 69%). Mean intensity values for all 12 subjects for each taste are shown in Table 3-1.

Table 3-1. Average scores out of 100 for perceived intensity for each of the five tastants across the 12 subjects as measured as position along a line scale.

Tastant	Mean perceived intensity (calculated from the position of subject's indication from the left anchor on the line-scale relative to the total line length, expressed as a %) for all subjects	Std. Deviation
Salt	36	20
Sweet	43	19
Acid	44	17
Umami	54	19
Bitter	63	21

Based on these results, concentrations of umami and bitter were reduced further for the final test, to produce solutions that were perceived to be more equally intense. Although the salt solution was seen as slightly less intense than the other tastes, subjects indicated that increasing salt concentration would be unpleasant, so this concentration was not changed.

3.4.4. The effect of tastant order on reaction time

The 12 subjects (6 male) discussed above were used in this study. The following concentrations were therefore used in the final experiment: salt (33mM), sucrose (56mM), acid (3.4mM), umami (4.1mM) and bitter (0.035mM). Solutions were prepared fresh on each test day. For each experimental run, Dynataste was programmed to deliver one tastant solution from one pump for 20 seconds, followed

by a second tastant solution from the second pump for the next 20 seconds. The output from the pumps was combined at a manifold, and delivered continuously via a second tube (the feeder tube) to the subject's mouth. An illustration of this set up is shown below (see Figure 3-1):

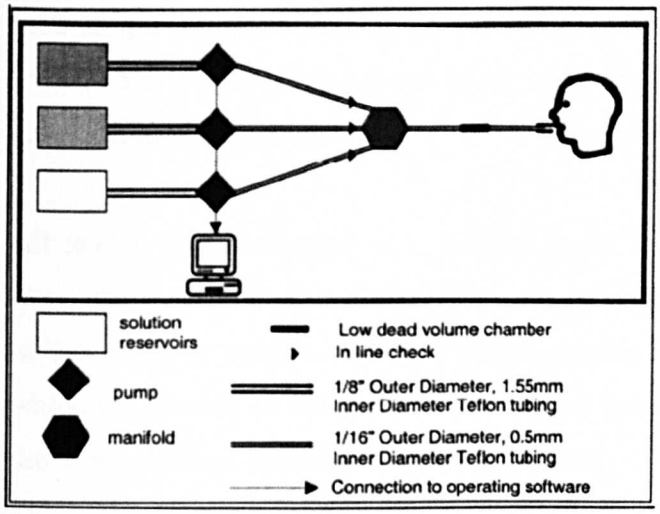


Figure 3-1. Schematic diagram of the Dynataste delivery system

In order that every taste was administered with every other taste, both in the first and second position, each subject needed to teste 20 possible binary taste combinations (see Table 3-2 for the full list). These 20 combinations were divided into 4 sessions, so that in every session, the five combinations given included each of the five tastes in both the first and the second position. Each session also contained a randomly chosen control (used to monitor panel performance), and in each session the identity of the tastant used as the control was changed. For the control runs, both pumps were connected to the same tastant solution, so that only one tastant was administered over the 40 second period. Therefore, each session contained six experimental runs. Subjects sat the four sessions in an order based on a Latin Square design, and the order of runs within each session was randomised. Subjects were told that a solution would be constantly administered into their mouth for a period of 40 seconds. They were told that the identity of the solution may, or may not, change during this time period. They were asked to press a button every time they thought the identity of the solution had changed, without worrying about actually identifying the change – therefore the time to detect the tastant, rather than recognise it, was measured. Subjects were also asked to ignore any changes in concentration. The button was connected to a light in the adjacent room, and times at which the light flashed were recorded using a stop clock. A flow rate of 14ml/min was used, since this rate has

previously been reported to provide sufficient sample for evaluation purposes (Hort and Hollowood 2004). Practice sessions were carried out using just water to acquaint the subjects with the method of solution administration. Subjects were told to hold the feeder tube in a way they found comfortable, allowing the solution to drip onto their tongue. Subjects were given a short break in between experimental runs, during which tubes were rinsed and pre-filled, and subjects were instructed to cleanse their palette during this break.

All testing was performed in a sensory booth where subjects had no sight of the Dynataste system other than the feeder tube. The feeder tube that linked the manifold to the subject's mouth was fairly long, creating a time delay between the time at which the identity of the solution leaving the pump changed, and the time at which the new solution reached the subjects mouth. This delay time was calculated as follows (see Equation 3-1).

Volume of feeder tube: $\Pi r^2 h = 0.4059 cm^3$

(Length of feeder tube = 170.86 cm, internal radius of feeder tube = 0.0275cm)

Flow rate of feeder tube: 14ml/min

Time taken for the fluid to pass along the feeder tube = 0.4059 x (60s/14ml) = 1.74s

The second solution therefore reached the subjects mouth at 21.74s.

Equation 3-1. Equation used to calculate the delay time. Adapted from Faurman (2005)

The time at which subjects indicated a change was subtracted from 21.74s to give a "real" value for the time it took to detect the new tastant from when it first entered the subjects mouth. A two factor univariate ANOVA test was carried out on the data to see if reaction times differed significantly from each other as a result of either the administration order, or the subject. Also, one factor ANOVA tests were carried out on the data, excluding either the identity of the first tastant or of the second tastant, to see if the identify of the first tastant, or the second tastant, was having a more significant effect. Where significant differences existed, an LSD (Least Significant Difference) analysis was carried out on the data after the initial analysis to see which samples differed significantly from each other (see Mahony (1986) for details).

Since there was only one change in tastant identity during each experimental run (excluding the controls), it was expected that subjects would indicate only one change, and this would be after 21.74s. However, subjects frequently indicated multiple changes, both before and after the actual change, as well as during the controls. For the purposes of data analysis, only runs where subjects indicated one and only one change after 21.74s were considered. Situations where subjects indicated a change where one had not occurred were examined and are discussed briefly below:

Multiple indications after the change: In 5% of experimental runs, subjects made more than one indication of a change after the actual change had occurred. This occurred most frequently when either salt or acid was being administered as the second taste, and never when bitter or umami was being administered, and appeared independent of the identity of the first taste. It is possible that aftertastes from the first tastant persist after the change, which may be causing these multiple indications. According to Amerine, Pangborn et al (1965), sweet compounds have been reported to have a bitter aftertaste and vice versa, a sweet taste is produced on washing the mouth with water after tasting a solution containing the salt potassium chloride, and after tasting dilute sulphuric acid distilled water tasted sweet. It is therefore possible that if, for example, the sweet taste was administered first, and then the salty taste, the subject may detect the presence of bitter in between the two, due to the bitter aftertaste of some sweet compounds, and the subject may indicate this as a change in tastant identity.

No indications after the change: In only 3% of the test runs, subjects failed to indicate a change even though one was occurring. This only ever occurred when the binary taste combination contained two of the following three tastes - umami, bitter and sweet - and never when it contained salt or acid (which tended to cause multiple indications rather than no indications at all, as mentioned above).

Runs where subjects indicated either no change or multiple changes, as described above, were seen as "missing data" for the purposes of data analysis.

Controls: Subjects indicated a change in tastant identity for 65% of the controls. These indications were more common for some subjects, suggesting that it could be related to human nature. False indications were made when all tastes were administered as the control, but occurred in a higher percentage for some tastes compared to others – 88% of times when acid was administered as the control, 82%

for salt, 70% for bitter, 56% for umami, and 30% for sugar. MSG has been shown to elicit sweet, sour, salty and bitter tastes (Kemp and Beauchamp 1994), which may explain the false indications when umami was administered as the control. The lower frequency of false indications during the sugar control could be because sucrose is the taste with which individuals are most familiar in its pure form. It is unclear why the false indications occurred most commonly with salt and acid. False indications may have been caused by expectation error (i.e. subjects became accustomed to a change occurring half way through the experimental run, so tended to expect a change even if one did not occur). Alternatively, these false indications could be due to subjects becoming adapted to the solution – it has been reported that the perception of taste with continuous stimulation diminishes with time, and eventually almost disappears. This is thought to be due to the exhaustion of receptors (Shallenberger 1993). Hahn (1934, according to Shallenberger (1993)) carried out tests on adaptation-recovery data for sodium chloride, and found that at 7% NaCl, adaptation occurs after 3 seconds. Although 7% is much more concentrated than the salt solution used here, it is possible that adaptation causes subjects to mistake the disappearance of a taste sensation as a change in tastant identity.

Indications before the change: In 28% of the test runs, subjects indicated a change before the actual change (i.e. before 21.74s). However, it was not specified in the instructions whether or not subjects needed to indicate when they detected the first taste (i.e. during the first few seconds) and this may explain the occurrence of indications before the change. In 3% of these cases, two indications were made before the actual change. Tastants which caused these double indications before the actual change were examined, but no particular trend was found; however it occurred most often with one particular subject, so could be explained by their responding more sensitively.

3.4.5. Investigations into swallowing volumes

For these experiments, subjects were recruited from the Division of Food Sciences, University of Nottingham, based on their willingness to participate. Subjects were given rubber tubes of various dimensions (to mimic straws), as well as small glass vials (33ml volume), each containing 30ml of bottled water (Evian).

3.4.5.1. Effect of straw dimensions on drinking rate

Fifteen subjects (3 male) were used in this study. Each subject was provided with 8 straws (silica tubing, platinum cured, Fisher Scientific, Leicestershire, UK) of different dimensions, varying both in diameter (1mm, 2mm, 3mm, and 4mm) and length (20cm and 30cm) and eight vials of water. Subjects were asked to consume the entire volume of water, with a different tube each time (according to a predetermined random balanced order), and the time it took them to drink the liquid with each straw was recorded. A one factor univariate ANOVA was carried out on the data, for both straw length and diameter, to ascertain whether drinking rate was affected by either straw length or diameter. Where significant differences were found, an LSD test was carried out to identify significantly different subgroups.

3.4.5.2. Effect of straw dimensions on swallowing volumes, both during isolated and continuous swallowing.

Ten subjects (3 male) were given straws of both 1.5mm and 2mm internal diameter (Smiths medical clear PVC tubing, Portex Ltd, Kent, UK). Subjects were asked to naturally drink the water in the vial, using each straw in turn, until they had swallowed either once, twice, or until they had finished the water. For this last test, subjects were asked to count the number of times they swallowed during drinking. The volume remaining in the glass vials after one or two swallows was measured, and this was subtracted from the initial volume to calculate the volume of water consumed in one, or two, swallows. Each subject repeated each experiment 3 times. A two factor univariate ANOVA test was carried out on the data to investigate whether straw diameter or subject were having an effect on either the volume consumed in one swallow, or the volume consumed in two swallows.

3.5. Results and discussion

3.5.1. Effect of order of tastant administration on reaction time

The average reaction time to the second tastant in each of the 20 binary taste combinations is shown in Table 3-2.

Table 3-2. Table to show the average reaction time across all subjects for all of the 20 solution combinations administered.

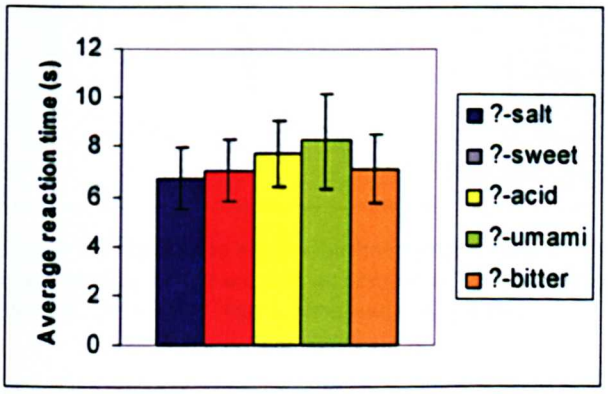
Taste combination		Mean reaction times (s)	S.D
First tastant administered	**Second tastant administered**		
Salt	Sweet	5.5	1.3
Salt	Acidic	6.2	1.6
Salt	Umami	6.4	3.0
Salt	Bitter	5.8	2.2
Sweet	Salt	5.5	1.6
Sweet	Acidic	8.3	2.7
Sweet	Umami	8.7	4.3
Sweet	Bitter	7.4	2.4
Acidic	Salt	8.1	2.6
Acidic	Sweet	8.7	3.9
Acidic	Umami	8.5	4.5
Acidic	Bitter	6.8	3.4
Umami	Salt	7.1	2.6
Umami	Sweet	7.4	1.8
Umami	Acidic	7.6	2.3
Umami	Bitter	8.4	2.9
Bitter	Salt	6.1	2.1
Bitter	Sweet	6.5	1.0
Bitter	Acidic	8.7	3.5
Bitter	Umami	9.4	3.1
Average		7.4	2.9

A two-factor ANOVA test on this data showed a significant difference in reaction time ($p<0.05$), both with respect to binary tastant combination, and subject. The taste combinations salt to sweet, and sweet to salt, produced the fastest reaction times to the second tastant, whereas reaction time was longest for the umami taste after bitter administration. Reaction times given here are much longer that those described previously (Kuznicki and Turner 1986), but reaction time is known to vary inversely with concentration (Amerine, Pangborn et al. 1965; Yamamoto and Kawamura 1981) and Kuznicki, Turner et al. (1986) used much higher concentrations than utilised here. Yamamoto and Kawamura (1981) measured reaction times to a range of tastant concentrations, covering the concentrations used here, and still found reaction time to be much faster (even for the lowest concentration of the tastant that took the longest to detect, reaction time was still under 2s). However the 3 ml of test solution that these authors used in each experiment was administered at a flow rate of approximately 10ml/sec. The slower flow rate used by Dynataste (14ml/min) would result in a slower increase in mouth loading and hence quantity of tastant in mouth, which is most likely causing the detection of the taste to be longer.

Average reaction times to the second tastant, irrespective of identity of the first tastant, were calculated (see Figure 3-2A). A one factor ANOVA test carried out on the data showed that there was no significant difference ($p>0.08$), suggesting that regardless of the identity of the taste administered first, reaction times to the second taste were similar. Salt had a slightly lower, but not significantly lower, average value for reaction time compared the other tastes, which is consistent the literature (Amerine, Pangborn et al. 1965). While the mechanism of transduction for salt detection could be responsible for its quicker reaction time, it is also possible that subjects tended to hold the tube above the part of the tongue that showed a heightened sensitivity to salt compared to the other tastes (if this was their most comfortable position), which could also explain its faster detection time compared to the other tastes. Umami had a slightly longer reaction time compared to the other tastes, which could be due to its mechanism of transduction, the position of the tube above the tongue, or because subjects were less familiar with the umami taste. Interestingly, when a one-factor univariate ANOVA was carried out on the data for reaction times to the second tastant irrespective of its actual identity, and taking into account only the identity of the first tastant, a significant difference ($p<0.01$) was

found in reaction time. An LSD analysis (at 5% significance) showed that when salt was given as the first tastant, reaction time to all the other tastes was significantly quicker (see Figure 3-2B). It is known that the faster the reaction time of a taste, the shorter its persistence (Amerine, Pangborn et al. 1965), so after salt administration, the reaction time to the subsequently administered taste could be quicker due to a lack of persistence of the salty taste.

A)



B)

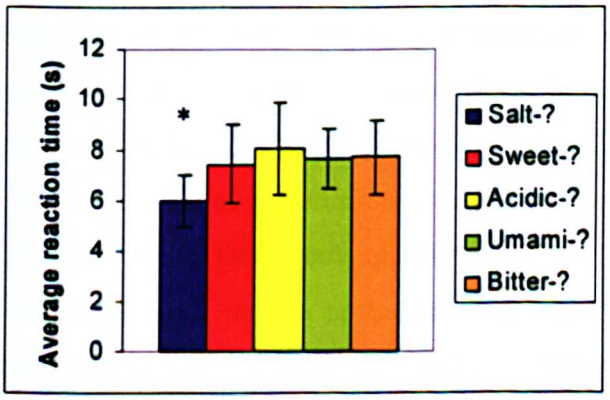


Figure 3-2. Bar chart showing the average reaction time to each of the five tastants A) ignoring the first tastant administered and B) ignoring the identity of the second tastant. Error bars indicate +/- half standard deviation. An asterix above a bar indicates a significant difference in reaction time.

In order to investigate the effect of specific tastant combinations on reaction time more closely, results showing the average reaction time to each tastant in each binary combination was compared to the overall average to that tastant, as shown in Figure 3-3.

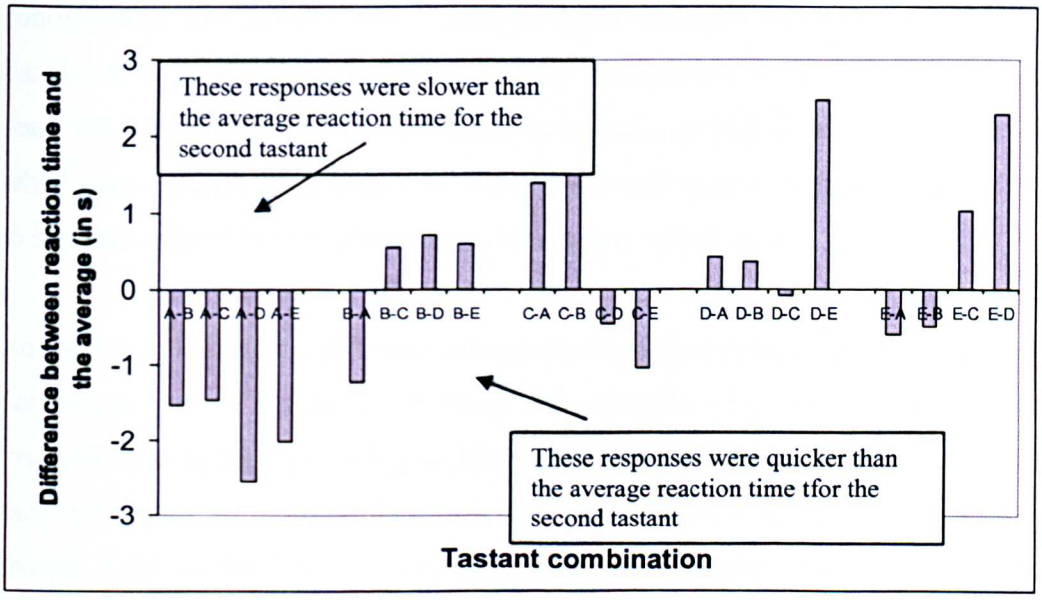


Figure 3-3. Graph to show whether the presence of the first tastant increases or decreases the reaction time to the second, as compared to the average reaction time for the second tastant (A=salt, B=sweet, C=acid, D=umami, E=bitter)

This graph clearly shows that pre-administration of salt shortens reaction time to the subsequent taste administered compared to the average reaction time to that taste, regardless of the identity of the second tastant, although reaction time to umami was especially quick after salt administration. Pre-administration of sugar shortened the reaction time for salt compared to its average, but lengthened reaction times to the other tastes. Pre-administration to acid lengthened subsequent reaction time to the salt and sweet taste, but quickened it for the bitter and umami taste. Pre-administration of umami prolonged reaction time to all the tastes except acid, and had a particularly pronounced effect on lengthening reaction time to bitter. Pre-administration to bitter quickened reaction time to salt and sweet, but slowed reaction time to acid and umami.

The shortening of reaction time to sucrose after adaptation to salt was reported by Bujas,(Bujas, Szabo et al. 1991) which is consistent with our findings, however these authors also observed that pre-administration of salt lengthened reaction time to quinine, which is opposite to what we found. They also reported, as we found, that adaptation to sucrose significantly quickened subsequent reaction time to salt, but they also observed this to a lesser extent for quinine, which we did not. The differences in some of these results could be because solutions were administered one after the other (i.e. discretely) by Bujas, Szabo et al (1991) in their study, and not

continuously, like in our study. Furthermore, although the solutions to which Bujas, Sazabo et al. (1991) measured reaction time were similar to the concentrations used here, the adapting solutions were much more concentrated than the ones used here, which may explain the discrepancy. Finally, the acid used in their study was different to the acid used here to elicit sourness, which may affect results.

According to Kuznicki and Turner (1986), reaction time data allows the possibility of deviations from a normal distribution. For example, if subjects have a tendency towards anticipating the stimulus quality or take inordinate amounts of time to ensure their responses are correct, data may be positively or negatively skewed. A one-sample Kolmogorov-Smirnov test was therefore performed on the data, and results showed that the distribution was not normal, suggesting that some subjects were reacting differently to others (data not shown).

When the average reaction times obtained using Dynataste were converted into *volumes* needed to allow the detection of each taste, it is obvious that very small volumes are sufficient to elicit each taste (see Table 3-3), and the maximum volume needed to ensure detection of the second tastant in the combination with the longest overall reaction time (bitter to umami) was still only 2.2ml.

Table 3-3. Average volumes of each tastant solution need to evoke a taste when administered at 14ml/min.

Taste stimuli	Volume needed to evoke taste sensation (ml)
Salt	1.6
Sweet	1.6
Acidic	1.8
Umami	1.9
Bitter	1.7

In order to see how this volume compared with the volume needed to ensure swallowing, an investigation into drinking and swallowing patterns was carried out.

3.5.2. Investigations into drinking and swallowing patterns

3.5.2.1. Effect of straw dimensions on drinking rate

Results showing the effect of straw dimensions on drinking rate are shown in Figure 3-4 below:

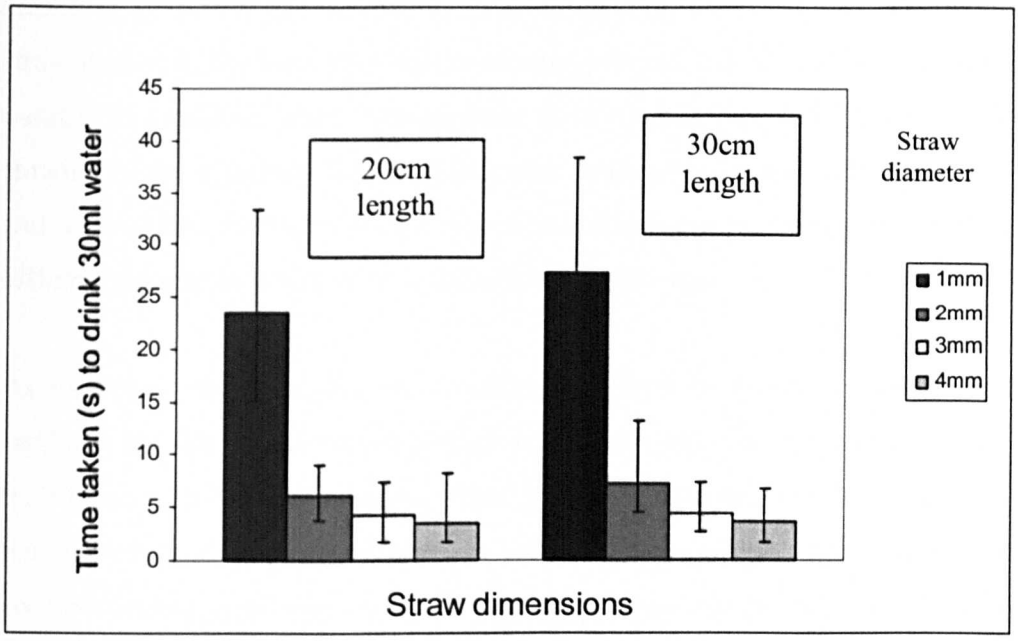


Figure 3-4. Bar chart showing the time taken to consume 30ml water with eight straws of different dimensions. Fifteen subjects were used, and average times are shown here. Error bars indicate the fastest and slowest time observed across the 15 subjects.

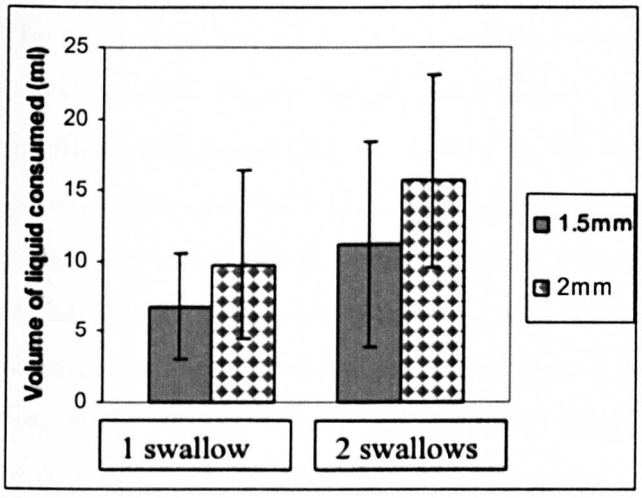
A one-factor ANOVA test carried out on the data showed that when straw diameter was considered as the factor, there was a significant difference in drinking rate ($p=<0.01$), whereas when an ANOVA test was carried out considering straw length as the factor, there was no significant difference ($p=0.45$). An LSD analysis showed that drinking rates with a 1mm diameter straw were significantly slower than with straws of all other diameters, and drinking rates with a 2mm straw were significantly different when compared with all other straw diameters. Drinking rates with straws of 3mm and 4mm diameter, however, did not differ from each other, but did differ from drinking rates with a 1mm or 2mm diameter straw. Since it is desirable for this application that small volumes of liquid were used in the final drink, these results suggest that for the final application, straws of thin diameters should be used, however informal discussion with the subjects indicated that they found it too difficult to drink with the 1mm diameter straw, suggesting that a slightly wider straw would be more desirable.

3.5.2.2. Effect of straw dimensions on swallowing volumes, both during isolated and continuous swallowing

A two factor univariate ANOVA test carried out on the data showed that both straw diameter and subject had a significant effect on the volume consumed in the first swallow, the volume swallowed in the second swallow, and in the total number of swallows ($p<0.01$), but no subject straw interaction was observed. The smaller the straw diameter, the less water was consumed per isolated swallow, and the larger the number of swallows taken in continuous drinking (see Figure 3-5A and B), due to a slowing of drinking rate. This suggests that swallowing occurs as a function of time, and not volume. Furthermore, the significant difference between subjects shows that different people naturally vary in their drinking patterns.

As expected, more volume was consumed in two swallows compared to in one swallow, but not twice as much, which is probably because the subject is quenching their thirst with the first swallow. Since 30ml was consumed in total, results of total number of swallows can be used to estimate the average volume consumed per swallow during continuous swallowing - 3.6ml (maximum volume 8.6ml) when a 1.5mm diameter straw was used, and 8.8ml (maximum volume 15ml) with a 2mm diameter straw. Based on this calculation, when people drink liquid continuously, they consume a smaller volume per swallow than when they just take one isolated swallow (however, this could be due to a gradual decrease in sip volume with successive sips due to the quenching of thirst, since this would also produce a smaller average volume per sip than when just one or two sips were taken).

A)



B)

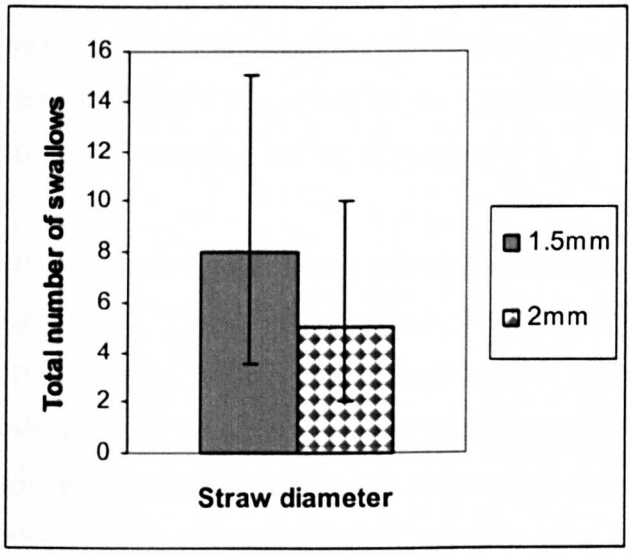


Figure 3-5A and B. A) Bar chart showing the volume consumed per swallow as a function of straw diameter. B) Bar chart to show the average number of swallows taken during the consumption of 30ml of Evian water, as a function of straw diameter. Values given are the average over the 12 subjects, and error bars represent maximum and minimum values.

Taking all these results into consideration, when a straw diameter of 1.5mm was used, the maximum volume consumed per swallow when drinking continuously was 8.6ml, however the maximum volume consumed with one isolated swallow was 10.5ml. Since people may take isolated swallows when drinking the final drink compared to continuous swallows, in order for the concept of dynamically changing flavour to work, each differently flavoured solution should be present at this larger volume of 10ml, to ensure that swallowing, and therefore flavour perception, occurs within each layer. The concept would still be effective for subjects who consumed smaller volumes per swallow, but they will just experience the same flavour for several swallows before they become aware that the flavour profile changed.

3.6. Conclusions

The work described here involved measuring the reaction times to the five tastes in every possible binary tastant combination. Results showed that there was no significant difference in reaction time to the tastant when only the identity of the second tastant was considered (but in general reaction time to salt was quicker than to the other tastes, which is consistent with the literature). Interestingly, reaction time to the second tastant, irrespective of its actual identity, was significantly quicker when salt was administered as the first tastant. It is thought that the quick reaction time to salt reduces its persistence, thus allowing subsequently administered tastes to be detected more quickly. Furthermore, results from these studies showed not only that different subjects varied greatly in their sensitivity to the different tastes (as has been previously and extensively reported in the literature), but that there was a large variation in intensity scores by the same subject for the same solution, highlighting the natural variation within individuals.

Investigations into drinking and swallowing patterns showed that drinking rate was significantly longer with straws of thinner diameter, yet straw length did not affect drinking rate. When the effect of straw diameter on swallowing volume was calculated, it was observed that a smaller straw diameter significantly reduced the volume consumed in the first and second swallow, and in addition increased the total number of swallows needed to consume 30ml of water. These results suggest that straws with small diameters (such as 1.5mm) would be more suitable for this application than straws of larger diameters, since by slowing down drinking rate, thinner straws allow smaller volumes of liquid to be consumed before swallowing. This allows smaller volumes of each flavour to be used in the final drink, making the overall effect more emphatic. This work has shown that during continuous swallowing with a 1.5mm straw, the maximum volume consumed in one swallow was 8.6ml (however during isolated swallowing, which may occur, a maximum of 10.5ml was needed ensure swallowing).

Therefore, at least 10ml of each differently flavoured tomato solution would need to be present in the final drink (since this represents a sufficient quantity to cover the maximum swallow volume and thus allow flavour perception), and this is thought to

represent a much greater volume than would be needed to ensure taste detection at the concentrations used here, regardless of the tastant administration order - even for the binary taste combination with the slowest reaction time (bitter to umami), only 2.2ml of the second tastant was required to ensure detection.

Although only binary, rather than quinary, taste combinations were investigated here, these results suggest that the volume needed to ensure swallowing when drinking through a straw is much more important than the volume needed to ensure the detection of each taste at the concentrations used here (regardless of administration order) in order to allow the overall changing flavour experience to be appreciated. Therefore, it was thought that the five tastants could be administered in an order that was perceived as being the most pleasant, without it having an effect on overall detection.

3.7. Technology transfer

In order to use the results described in this chapter to develop the drink, a way to prepare the 5-layered drink that prevented excessive mixing of the layers needed to be found.

3.7.1. Ways to layer up the different solutions to prevent mixing

A simple way to do this would be to serve this drink with each tastant solution in a different shot glass, so that the consumer could swallow each solution, and therefore perceive its flavour, before moving onto the next one. However, it was desirable that the drink be served as one continuous phase to provide a more attractive product with a hint of mystery and surprise.

Tomato juice is fairly viscous, and it was thought that its inherent viscosity/suspension properties would aid both the layering of the drink, as well as the slowing down of mixing after preparation, if it was used. In order to investigate the effectiveness of different preparation systems, tomato juice was purchased, and half was coloured using a few drops of black food dye (Supercook), to allow visual monitoring of the mixing of layers during and after preparation. Different methods of preparing the drink were tried and photographed.

3.7.1.1. Attempt 1 – directly pouring the different layers in

The first attempt to prepare the drink involved simply pouring the black layer on top of the red layer. However, the force of pouring one layer onto the other caused distortion of layers as shown in the photo below (see Figure 3-6A), making it unsuitable for the application. However, although distorted, the red and black parts stayed stable for hours, without further mixing of the layers.

3.7.1.2. Attempt 2 – using a pipette

In order to eliminate this downward force, attempts were made to pipette the second black layer on top of the red layer. Pipetting with a large Gilson pipette still created enough of a force to cause the distortion of layers, however use of a small plastic pipette produced less distortion, since the force applied was less. However when this method was extended to 4 layers, some distortion was visible. To visualise distortion,

the mixture was frozen and cut in half – photos showing the results are shown in Figure 3-6 B and C.

A) B) C)

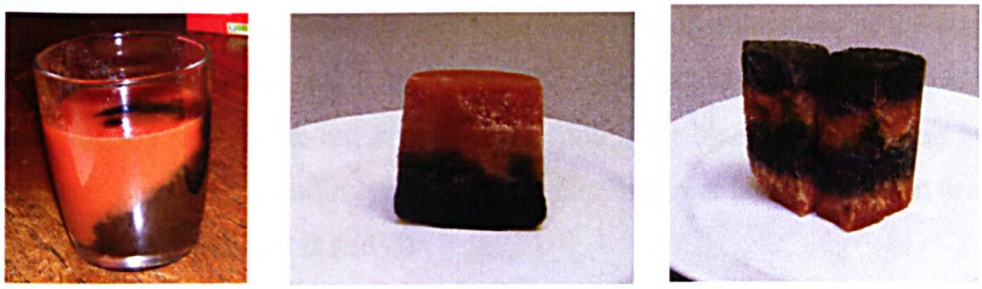


Figure 3-6. A) Images showing the mixing of two different layers of tomato juice when one layer (the black layer) is simply poured on top of the red layer. B) Photo showing the level of internal mixing of differently coloured layers of tomato juice, when subsequent layers were slowly pipette-ed onto previous layers using a small plastic pipette. C) The photo illustrates mixing when 4 layers of alternately coloured tomato juice were prepared using the method of slow pipetting with a small plastic pipette. Drinks have been frozen and photographed upside down to how they were prepared.

In order to prevent any downward force at all, attempts were made to inject the black layer into the red layer via a hole in the side of the container (as shown in Figure 3-7), thus producing a horizontal or projectile force, rather than a vertical or downwards force, which was thought may cause less distortion.

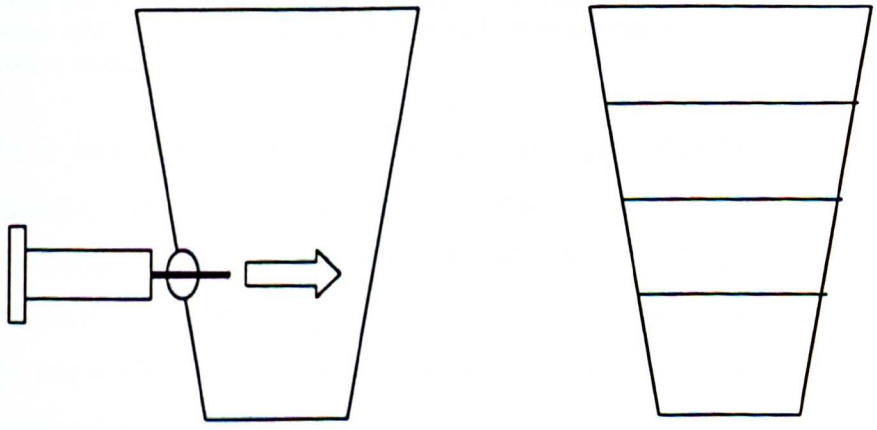


Figure 3-7. Diagram to show how injecting black coloured tomato juice into the side of a container containing red tomato juice could result in a 3-layered system

Although no black colouring could be seen on the surface of the drink, suggesting the black layer had been successfully injected in between the two red layers, inspection of the inside of the drink after freezing and cutting in half showed that this had not occurred, but instead the black tomato juice had accumulated by the hole through which it had been injected (see Figure 3-8A).

3.7.1.3. Attempt 3 – freezing the bottom layer

Attempts were then made to freeze the red layer, and then immediately pour the black layer over the top. After conventional freezing, the red tomato juice defrosted slightly unevenly, causing the black layer to move down the sides of the container as the red layer defrosted, causing mixing. Therefore, liquid nitrogen was used to freeze only the top part of the red layer, before adding the black juice. Mixing was much reduced, however extending the process to four different layers produced pronounced mixing (see Figure 3-8 B and C).

A) B) C)

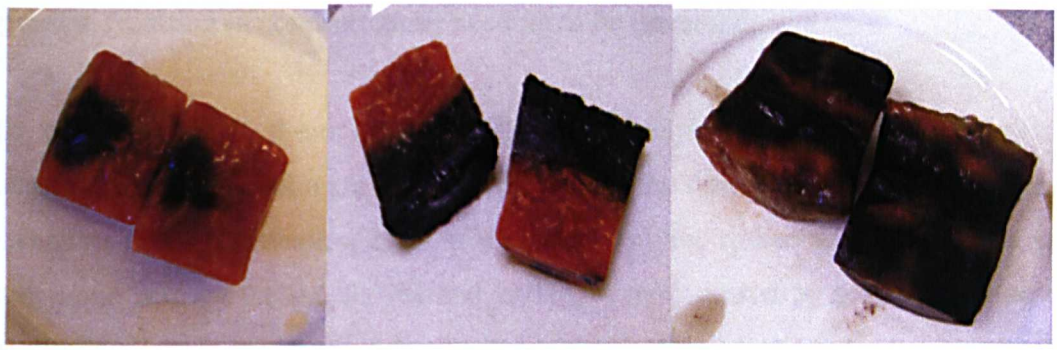


Figure 3-8A, B and C. Photos are taken of the drink after freezing and cutting in half. A) Photo showing the mixing of two differently coloured layers of tomato juice, where the black layer is injected into the red layer via a hole in the side of the container. B) Photo showing the level of internal mixing of differently coloured layers of tomato juice, when the surface of the previous layer was frozen with liquid nitrogen before adding the next layer. C) Photo illustrates mixing when 4 layers were prepared using the method of freezing a previous layer with liquid nitrogen before adding the next layer.

From these different attempts tried here, it seemed that the method involving slowly pipetting the successive layers produced the least mixing between layers. Furthermore, because the layers stayed separate for hours due to the inherent thickness of the tomato juice, these drinks could be prepared in advance of service, an important factor to consider in the development of such a dish for a restaurant application.

3.7.2. Preparing the prototype

Knowledge of the minimum volumes needed to allow perception (by ensuring swallowing), and having found the most effective way to administer the different layers, a prototype of the drink could be developed. Initially, concentrations of tastants were added to the tomato juice at those concentrations used for the experiment investigating reaction times, but informal testing revealed that the

concentrations needed to be increased in order to be perceived. It is well known that the presence of some tastes can either enhance or suppress the perception of other tastes when administered together (Shallenberger 1993; Keast and Breslin 2003), so this may be playing a role here. For example, it is generally agreed that when present together, MSG suppress bitterness (Woskow 1969; Kemp and Beauchamp 1994), bitterness is suppressed by salt (Keast and Breslin 2003), and at medium or high concentrations sweetness was generally suppressive of the other tastes (Keast and Breslin 2003). Since tomato juice naturally contained all the five tastants, it was thought that perception of the added tastes may be suppressed by the tastes naturally present, resulting in concentrations needing to be increased.

In order to see if these layers of different flavours could indeed be detected separately during drinking, a simple prototype of the drink was prepared. Measuring cylinders (50ml) containing 20ml of each tomato juice spiked with the tastants salt, sugar and acid (at 0.05M, 0.09M and 0.01M) were prepared by gently pipetting the successive layers in. (20ml rather than 10ml of volume was used since straws of a larger diameter than 1.5mm were used to consume the drink). When these drinks were drunk with a straw during informal testing with members of the Division of Food Sciences (University of Nottingham) and with the sponsor Heston Blumenthal, the flavour was indeed perceived by many to be changing during the consumption process. As the work described here has shown, In order to reduce the final volume served in the drink, straws of smaller diameter could be used.

Furthermore, since the work described in this chapter has shown that the volume required of each tastant solution to ensure swallowing was much greater than that needed to ensure taste detection, regardless of administration order, for the final drink the restaurant can chose the order of tastant administration that gives the most pleasant experience. Informal discussion with the subjects after the test did indicate that subjects found some binary taste combinations (such as the transition from sweet to umami) unpleasant, so this transition should be avoided.

3.7.3. Other administration systems

When recruiting subjects to try this prototype, it was interesting to observe that the majority of people do not actually like tomato juice, due to its similarity in texture (but difference in temperature to) tomato soup, which may have important implications if tomato juice is to be used for the final application. An alterative to using tomato juice could be to use tomato water. Tomato water can be prepared by centrifuging large quantities of chopped tomatoes. Tomato water with a flavour of cooked tomatoes can also be prepared by boiling or roasting the tomatoes, before centrifugation. However, tomato water is non-viscous, so does not have the natural thickness that tomato juice has in allowing preparation by successive pipetting of different layers, and also in preventing the subsequent mixing of layers. Therefore, if tomato water were to be used for the final application, its texture would need to be modified – for example, gelling the different solutions in weak fluid gels would allow the solutions to be layered horizontally, and also prevent any subsequent mixing. Alternatively the drink could be pumped into the consumers mouth using continuous flow via Dynataste, however this administration system was seen as slightly impractical, expensive, and almost medical, and therefore inappropriate for use in one of the best restaurants in the world. A solid delivery system, the frozen tomato popsicle, was also tried. Tomato juice (20ml), heightened with a specific tastant, was poured into plastic tubing and frozen. Once this layer had frozen, the next layer, heightened with a different tastant, was poured in and so on, thus preventing mixing. The final product could then be eaten as a lolly, and each part of the lolly would be heightened in a specific taste. However, use of a solid matrix did not produce an experience that surprised the consumer in the same way as the liquid system did, because the consumer would be able to understand more easily how it had been created.

3.8. Further work

Using food dye to monitor the mixing of the different layers in the final drink, while providing a quick and easily visible model, may not have been a correct reflection of the relative amount of tastant mixing, because a food dye molecule is much larger, and therefore less able to migrate, than a small tastant molecule. Therefore, it would be useful to compliment this study with some data on the actual level of mixing of the different tastant molecules between the different layers in the final drink. Although the sampling of the prototype with the sponsor indicated that the different flavours were perceived sequentially, suggesting minimal mixing of tastants, measurements of the salt, acid and sugar content of the drink at different levels after preparation (using equipment such as pH meters and refractometers) would give a more exact indication of the level of mixing that was occurring.

Furthermore, sensory data obtained from subjects who can measure the change in tastant intensity while actually drinking the tomato flavoured beverage (using techniques such as Time Intensity) may provide valuable information on the level of mixing that may be occurring between different tastant layers, as well as potential taste suppression and enhancement that may be occurring due to the presence of tastants naturally found in tomato juice, which would provide helpful information in further optimising the drink for restaurant use.

4. The ripening banana product

4.1. Aim

To use scientific knowledge to aid the development of a banana flavoured product that changed flavour dynamically, from an unripe to a ripe flavour, as it was consumed.

4.2. Introduction

4.2.1. Finding a suitable banana base flavour

Ideally, real bananas would be used for the application – bananas of varying ripeness would be selected and processed in some way to produce the final drink. Initially, a selection of both unripe and ripe bananas (based on their peel colour) were chosen, peeled, mashed and centrifuged to obtain a supernatant that it was thought could be used for the final drink. However, the supernatant obtained from bananas of both ripeness levels was very thick (thought to be due to the presence of sugar and/or starch), alcoholic in flavour (thought to be due to the rapid breakdown of certain compounds into small alcoholic ones), and was also described as "sickly", "tongue tingly", and "synthetic" – in short, the final flavour of the processed banana was very different from that of the banana when consumed directly from its skin. As soon as a banana is removed from its skin and subject to processing (i.e. slicing or mashing), cells and the subcellular compartments contained within them break open, allowing substrates and enzymes to contact, and react with, each other. These reactions cause the blackening of bananas due to the oxidation of phenols in the banana cells (Hutton 2004), as well as significant changes to the flavour of the banana (Fisher 1997). This gives the banana a different flavour profile to the one it had when contained in its skin, and furthermore, one that is continuously changing with time. This is one of the major limitations of using real bananas for the application. Furthermore, the natural variation in composition that occurs with natural products like fruits would make it difficult to obtain reproducible products - different bananas would be used each day

to make the drink, causing heterogeneity in the final product. Finally, the ripeness stage of a banana is usually assessed by peel colour (Poland, Manion et al. 1938; Wyman and Palmer 1964; Goldstein 1969), which changes from green to yellow to brown during the ripening process (Harris 1937). However, a banana may have brown spots on its skin due to bruising rather than ripening, so two different bananas judged to be of equal ripeness based on the colour of their skin may differ in the ripeness of their flavour. Other more reliable methods exist for measuring ripeness, such as monitoring CO_2 or ethylene production (Wyman and Palmer 1964), both of which increase during the ripening process. However, these methods take time, and if this drink was ultimately to be served in a restaurant situation, a faster way of screening ripeness was needed.

For all these reasons, it was decided to *develop* synthetic banana flavours of differing ripeness, based on the natural composition of bananas at the different stages of ripeness, rather than use actual bananas for the final application. Flavourists spend much time achieving this - they use knowledge of the composition of aromas that have been detected in natural products by analytical means to create synthetic analogues. These flavours are then added, along with suitable tastants, to relevant matrices in order to mimic the flavour perceived from the original food.

Extensive work on the volatile fraction of banana has resulted in the detection of 350 (according to Marriot 1980) and the identification of 250 (according to Mayr, Mark et al. 2003) volatile compounds. Esters, alcohols, acids and carbonyls are the four main classes of compounds that give strength and character to banana aroma, while amines and phenols also contribute. These have been well reviewed (Mayr, Mark et al. 2003); (Salmon and Martin 1996; Wang, Chen at al. 2007)) and are outlined in Table 4-1.

Table 4-1. Table outlining the major classes of aromatic compounds found in bananas (Marriott 1980; Mayr, Mark et al. 2003; Wang, Chen at al. 2007).

Class	Compounds identified
Esters (particularly the acetates)	More than 100 esters have been identified Acetates predominate, followed by butyrates, and together they account for about 70% of the volatile components They have low odour thresholds, and are present in high concentrations – characteristic flavour compounds of banana Isopentyl acetate and isobutyl acetate are two of the most important, (quantified at 75 and 47ppm respectively) Other important aromas that have been identified include pentan-2-one, ethyl acetate, 2-pentyl acetate, butyl acetate, hexylacetate, ethyl butanoate, isobutyl butyrate, isoamyl butyrate, and isoamyl isovalerate
Alcohols	These are second most important group – 57 saturated and unsaturated alcohols have been identified The most abundant are isopentanol, 2-pentanol, isobutanol, hexanol
Acids	Bananas contain many volatile organic acids – 35 have been reported, including acetic acid, 1-butyric, 2-methyl propionic, and 3-methylbutyric acids
Carbonyls	Approximately 30 compounds have been identified (both aldehydes and ketones) Two aldehydes are present at high concentrations – 2E-hexenal (18-32ppm) and hexanal (5ppm and 22ppm)
Phenol derivatives	Less than 10 compounds have been identified, a major one of which is eugenol

While all of these aromas contribute to banana flavour, for simplicity flavourists usually only add those aromas perceived as being the most important when developing flavours. Salmon and Martin (1996) identified the composition of four commercial banana flavours and found that each flavour contained only about 6 aromas that could be identified. Furthermore, each flavour contained different aromas, indicating that different flavourists differ in which aromas they think are most important in generating banana flavour.

Creating realistic flavours based on a biochemical analysis of the actual food is *not* an easy process. Fruit aroma compounds are normally identified from extracts of the fruit, which complicates matters - flavour molecules are susceptible to a large number of different chemical changes once they are removed from their intact form

(as the preliminary work on centrifuging bananas described earlier showed) - for example, aldehydes are easily oxidised to acids, terpenes may rearrange and isomerise in the presence of acid, exposure to light may cause photo-oxidation or rearrangements, and unsaturated compounds may polymerise. Heat may also cause changes in the flavour components. These transformations are a real concern during the collection and concentration of foods for flavour determination, since they produce new flavour compounds not previously present in the food (Fisher 1997).

Due to the difficulties in developing realistic flavours, a number of well known flavour companies were contacted and asked to supply their most natural banana flavour. Gas Chromatography Mass Spectrometry (GC-MS) was used in this study to identify the key aromatic components of the chosen flavour, to elucidate which aromas were key to generating this natural flavour. This information would allow us to reproduce our own natural generic banana flavour if needed, although for the investigation under study here it was thought that it would be more consistent to use this pre-prepared flavour directly.

Flavour companies do not normally offer flavours that cover a spectrum of ripeness levels, so for the application here this banana flavour needed to be modified to create flavours mimicking a range of different ripeness levels. The ripening of bananas is characterised by changes both in the tastant and aroma profile, as well as the colour and the texture. In order to create banana flavour analogues of differing ripeness that were as realistic as possible to their natural counterparts, information on the changes in banana composition that occur during the ripening process was required, since this could be used to tweak the generic banana flavour so that it was perceived to differ in ripeness. Since taste and aroma are the major two elements driving flavour perception, these will be considered in detail.

4.2.2. The ripening of the banana fruit

4.2.2.1. Changes in tastant levels during the ripening of bananas

Sugar levels

According to Poland, Manion et al (1938), previous work has shown that glucose, fructose, sucrose, as well as small amounts of maltose, were all present in ripe banana pulp, but this finding was disputed by other authors who found that the

quantification of glucose and fructose alone accounted for all the reducing sugars present in banana pulp, and it is now accepted that bananas contain only sucrose, fructose and glucose (Marriott 1980; Robinson 1996). Sugar levels are low in the unripe fruit, but increase during ripening due to the hydrolysis of starch. The main increase in sucrose concentration occurred early in the ripening process, and reached a maximum towards the end of the ripening, after which sucrose concentration actually started to decrease. Glucose and fructose, however, both started to increase from about half way through the ripening process, with glucose levels always slightly exceeding those of fructose (Tarkosova and Copikova 2000). Actual concentrations were estimated from the figures in the publication by Tarkosova and Copikova (2000) and are shown in the table below (Table 4-2). These values were similar to those found by Poland, Manion et al (1938) except that this latter group found slightly higher levels for the final concentrations of glucose and fructose (at 4.2% and 3.3% respectively).

Table 4-2. Changes in sugar content during ripening. Values calculated from figure 1 in (Tarkosova and Copikova 2000)

Stage of ripeness, as determined by peel colour	Sucrose (%w/w)	Glucose (%w/w)	Fructose (%w/w)
1 (green)	0.8		
2 (more green than yellow)	1.7	0.2	
3 (more yellow than green)	6.0	0.4	0.2
4 (yellow with a green tip)	9.0	1.0	0.8
5 (yellow)	11.5	1.0	0.9
6 (full yellow)	12.0	1.2	0.9
7 (light brown spots)	11.5	3.0	2.5
8 (increasing brown areas)	11.0	3.0	2.6

Acidity levels

Normally acid levels decrease during the ripening of fruits, however banana acidity actually increases during the ripening process (Wyman and Palmer 1964). According to McGee (2004), this increase in acidity helps the banana flavour become fuller in several dimensions, presumably by increasing and extending the overall flavour profile. However, compared to sugar content, the acid content contributes a fairly

minor amount to the banana total mass, and only ever comprises about 0.3% of the banana total weight (McGee 2004).

Many authors have qualified and quantified the acids present in the banana, and all agree on malic acid being the principal acidic constituent. Hartman and Hillig (1934, as reported by Harris and Poland (1937)) studied 58 different fruits and vegetables and quantified the acid content of bananas at 0.37 - 0.50% malic acid, and 0.15% - 0.32% citric acid. These values were similar to those reported by Ballot, Baynes et al (1987), who quantified malic and citric acid levels at 0.5 and 0.15% respectively. Other acids have also been detected, such as low level of ascorbic and oxalic acid (Ballot, Baynes et al. 1987) as well as tartaric acid in earlier reports (according to Wyman and Palmer 1964), but it is presently accepted that the main organic acids are malic, citric and oxalic acid (Robinson 1996).

Work by Harris and Poland (1937) showed that during ripening, malic acid content increases to a peak, and then gradually decreases as the fruit matures. The range of malic acid content over the ripening period ranged from 0.053 to 0.373%, and is shown in detail in the table below (Table 4-3).

Table 4-3. The change in malic acid concentration over the ripening process (taken from (Harris 1937).

Stage of ripeness	Average of values determined for malic acid (%)
1 (all green)	0.053
2 (all green)	0.092
3 (green, traces of yellow)	0.373
4 (more yellow than green)	0.358
5 (yellow, green tip)	0.352
6 (full yellow colour)	0.333
7 (yellow flecked with brown)	0.314
8 (black-coloured skin indicating dead ripeness of fruit)	0.301

Wyman and Palmer (1964) quantified changes in levels of all the major organic acids in banana fruits at three different stages of ripeness, as shown in Table 4-4. Their results therefore give an indication of the relative proportions of the different acids, and how these change during ripening.

Table 4-4. Changes in the organic acid content of bananas during ripening. The "citric peak" acidity corresponds to citric acid plus certain phosphates. Taken from Wyman and Palmer (1964).

	Stage of ripening, ass assessed by peel colour		
Mean acid level (in meq/100g)	**Green**	**Yellow-green through yellow with green tips**	**Fully yellow through yellow flecked with brown**
Malic	1.36	5.37	6.20
"Citric peak"	0.68	1.70	2.17
Oxalic	2.33	1.32	1.37

From these results it appears that oxalic acid is the dominant acid in the unripe banana, and during ripening both malic and "citric peak" (which corresponds to citric acid plus certain phosphates) acidity increase 3-4 fold, while oxalic acid drops to about 60% of its original value (Wyman and Palmer 1964). Therefore changes in the levels of these three acids was thought to be important to the perception of the ripening banana flavour.

Changes in astringency

It is generally agreed that the astringency in bananas and many other edible fruits is associated with the presence of particular oligomeric forms of flavanols, the so-called condensed tannins of intermediate size. One of the major changes that occurs during the ripening of green bananas is the loss of their characteristic astringency (Ramirez-Martinez , Levi at al. 1977).

4.2.2.2. Changes in aroma levels during the ripening of bananas

During the process of ripening, overall aromatic content, like sugar and acid content, increases – whilst the unripe banana is lacking in aroma and flavour, a ripe banana is usually associated with a large array of aromas (Goldstein 1969; McGee 2004). Mayr, Mark et al (2003) investigated the changes in aromatic profile of bananas during ripening, and found that when the headspace composition of the ripe banana and the unripe banana was compared, the following differences were observed - overall they found lower concentrations of aromas in the unripe banana relative to riper equivalent, and they also found that the ripe banana had a higher fraction of

high molecular weight volatile organic compounds. Specifically, they detected a high abundance of isopentyl and isobutyl acetate in the ripe banana, while in the unripe banana 2-E-hexenal and hexanal, both "green" compounds, predominated. Nosespace measurements using APCI-MS on subjects eating unripe bananas showed that neither isopentyl or isoamyl acetate could be detected nor were 2-E-hexenal or hexanal detected in the ripe banana. Therefore, changes in levels of these four aromas was thought to be key to the perception of banana ripeness.

4.2.3. Developing banana flavours of varying ripeness

4.2.3.1. Creating unripe and ripe banana flavours

Since tastants and aromas are key to driving flavour perception, adding levels of these (that have been previously determined at each stage of ripening according to the literature) to a matrix, along with a generic banana flavour, was thought to be key in generating banana flavours that were perceived to increase in ripeness. Initially, aqueous solutions representing the two extremes of ripeness were prepared based on the concentrations at which these aromas and tastants are naturally present in very unripe and very ripe bananas, according to the literature (see Table 4-5). Water was used since it is the easiest matrix to use for flavour development. Bench testing was used to guide development and modify flavours in order to make them as realistic and representative as possible to both a very unripe and a very ripe banana flavour.

Table 4-5. Initial concentrations used for the most unripe and ripe banana flavour, based on concentrations previously quantified in the literature for unripe and ripe bananas.

	Levels in the unripe banana according to the literature	Concentrations to be added to the most unripe banana flavour	Levels in the ripe banana according to the literature	Concentrations to be added to the most ripe banana flavour
Sugar levels	An unripe banana contains only sucrose, at a concentration of ~ 0.8% (Tarkosova and Copikova 2000)	1%	A ripe banana contains 11% sucrose, but reaches 12% at stage 6 of ripening (Tarkosova and Copikova 2000)	12 %
			3% glucose (Tarkosova and Copikova 2000) or 4.2% glucose (Poland, Manion et al. 1938) have previously been quantified in ripe bananas	3.5 %
			2.6% fructose (Tarkosova and Copikova 2000) or 3.3% fructose (Poland, Manion et al. 1938) have previously been quantified in ripe bananas	3 %
Acid levels	Malic acid has been quantified in the unripe banana at 0.05% (Harris 1937)	0.05%	Malic acid has been quantified at 0.3% in ripe bananas (Harris 1937), whereas other authors reported levels as high as 0.5% in the banana (Harris 1937; Ballot 1987), so 0.4% was chosen initially	0.4 %

	"Citric peak" content has been quantified at half of malic acid levels (Wyman 1963) in the unripe banana	0.025%	Wyman and Palmer (1964) reported the "citric peak" content to be approximately one third of the malic acid content in the yellow flecked with brown banana.	0.1 %
Aroma levels	Hexenal has been quantified at 30ppm (Mayr, Mark et al. 2003) in the unripe banana	0.003%	Isopentyl acetate has been quantified at 75ppm (Mayr, Mark et al. 2003) in the ripe banana	0.0075%
	Hexanal has been quantified at 20ppm (Mayr, Mark et al. 2003) in the unripe banana	0.002%	Isobutyl acetate has been quantified at 50ppm (Mayr, Mark et al. 2003) in the ripe banana	0.005%

The generic banana flavour was included in the samples at the concentration suggested by the manufacturer, but a higher level was added to the ripe banana sample compared to the unripe sample, since it is known that banana flavour increases overall during the ripening process (McGee 2004).

Although oxalic acid is naturally found in both banana and other plant tissues (including rhubarb, spinach and beet), and occasional consumption of high oxalate foods as part of a nutritious diet does not pose a particular problem (Noonan and Savage 1999), oxalic acid binds calcium and other minerals, and was therefore excluded from these studies for health and safety reasons. Similarly, it is not recommended that compounds which elicit pure astringency should be swallowed, so it was unlikely that the use of such compounds would be approved by the University Ethics Committee. While natural compounds that elicit astringency do exist (such as tea), these compounds also impart their own flavour, so were seen as unsuitable for use here.

4.2.3.2.Creating banana flavours of intermediate ripeness

The final concentrations for the unripe and ripe flavour extreme that had been agreed from bench testing were used as a basis to create three intermediate samples of increasing ripeness. Bench testing was again used to guide flavour development and final concentrations that were perceived by bench testing to represent five different stages of ripeness were developed in this way. In order to see if these final five flavours were indeed being perceived as significantly different in terms of ripeness, a sensory test combining ranking and rating was carried out, to measure the perceived ripeness of each sample

From now on, these banana flavours of different ripeness will be referred to as I to V (where I represents the most unripe flavour, V the most ripe flavour, and II, III, and IV represent flavours of intermediate increasing ripeness respectively).

Prior to presentation of the final flavoured samples to the sponsor, a flavourist was asked to sample the five flavours and give advice on how they could be improved to make them more authentic and realistic. These suggestions are shown in Table 6.6 in the appendix, and it is thought they may be useful for the restaurant when developing the concept for the final dish. However, for the purposes of this study, these modifications were not included.

4.2.4. The importance of the matrix

As well as the important role that aromas and tastants play in determining flavour, texture also has an effect. According to a review by Delwiche (2004), it was assumed for some time that the role that texture was playing in flavour perception was in controlling the accessibility of tastants and aromas in a food to the receptors (i.e. it was acting at a physical level), however recent work has suggested that somatosensory tactile stimuli can also interact with taste and aroma, modulating their perception (i.e. that texture was acting at a cognitive level). Bananas are associated with a characteristic texture and mouth-feel which is likely to contribute to flavour perception at the cognitive level. When water was used as the matrix, it was thought that its lack of texture may be affecting the perceived flavour – banana flavours are rarely experienced in an aqueous matrix. Although water is the simplest matrix to use for flavour development, it was thought that matrices that had textures and mouth-

feels more similar to that of an actual banana would be important in generating the full flavour experience, and would therefore be more appropriate for the application. It was initially assumed that for this application these dynamically changing flavour experiences should be served in the form of a drink, since this would provide the most impressive flavour experience. However, it was also thought that using a matrix with a texture congruous to the banana would be important, so both a liquid matrix and a solid matrix were investigated as possibilities for the final application, to see to what extent texture was affecting the ripening banana flavour experience. Not only will the different matrices have textures that may affect banana flavour perception at the cognitive level, the different matrices may have an effect on flavour release, and therefore flavour perception, at a physical level, so this needed to be tested.

4.2.4.1. The effect of the matrix on flavour perception at the physical level

The texture of a food affects the rate and intensity of flavour release (and therefore flavour perception) in two ways. Firstly, the matrix itself may physically entrap flavour molecules in a general way (this is known as the texture-specific effect). Secondly, flavour molecules themselves may be specifically bound by thickening or gelling agents (this is known as the agent-specific effect) (Guinard 1995). As a result of this, flavourists often need to make different flavour formulations for different types of food, in order to produce similar levels of flavour perception overall (Taylor 2002). Therefore, the effect that these matrices will have on the perception of banana flavours under development here will need to be investigated.

<u>The texture specific effect</u>

For solid foods, flavour release is normally reduced by increasing the mechanical strength of foods – for example it has been shown that softer gelatine gels release larger concentrations of volatile compounds than harder gelatine gels (Koliandris 2007). For liquid foods, flavour release is affected by their viscosity. It is generally accepted that increasing viscosity through the addition of thickeners results in a decrease in perceived intensity of volatile and non-volatile components (Hollowood 2002). Therefore, higher flavour levels will be required for optimum perception in high viscosity foods compared to low viscosity foods. Furthermore, the decrease can depend on thickener type. It is currently believed that the thickener type affects the efficiency of mixing between the liquid food and the saliva. When mixing with saliva

is poor, tastants will be retained in the food rather than distributed to receptors, reducing overall flavour perception (Ferry, Hort et al. 2006).

The agent-specific effect

Specific interactions have been reported between flavour compounds and both carbohydrates and proteins (Fischer 1997; Godshall 1997), as well as lipids and phenols (Guinard 1995). Sugars and salts present in the food matrix may also influence the movement of volatile aroma compounds from the saliva to the air, and this affects subsequent perception in the nasal cavity (Taylor 2002). In particular, more hydrophilic compounds are retained and more hydrophobic compounds released in model systems as sucrose concentrations are increased (Marsh, Friel et al. 2006). The fat content of a food also plays a role in flavour perception – fat also has a significant effect on the partition of volatile compounds between the food and the air phases, with lipophilic aroma compounds being the most affected. If fat content is reduced, the amount of lipophilic aromas in the flavour formulation also needs to be reduced to maintain the same profile of aroma release from the product (Bayarri 2006).

4.2.4.2. The matrices to test

As explained previously, matrices were chosen that were thought may act congruently to the perception of banana flavour. Both a liquid matrix (milk), and a solid matrix (gelatine), were tested. These two matrices were chosen for the following reasons: - a banana *per se* is actually solid, so using a solid matrix like gelatine seemed obvious. Furthermore, gelatine gels melt in the mouth in a way that may mimic the mastication of a banana. However, milk will also be used as a possible matrix, because it is thought that it may also provide a texture and mouth-feel congruous to the banana flavour - most of the banana flavoured products available on the market are milk-based. For example, both Sainsburys and Asda offer a wide range of banana flavoured milkshakes and milk drinks; banana flavoured Nesquik and porridge (both of which are prepared using milk); and a range of other banana flavoured dairy products (such as banana flavoured yoghurts and ice-cream). Therefore, because people will be familiar with tasting banana flavours in milk, it is thought that it may provide a good matrix by enhancing the overall flavour experience in a congruent way.

4.3. Objectives

- To develop five different banana flavours, based on concentrations stated in the literature, that were perceived by sensory analysis to be increasing in ripeness from unripe to ripe.
- To test the suitability of different matrices for the final application, to see which is most congruent to the banana flavour experience, and to investigate how these different matrices affect the release and perception of the different banana flavours.

4.4. Materials and methods

4.4.1. Preparing the banana flavours of varying ripeness

4.4.1.1. Materials

All materials used for testing were food-grade. Sucrose (Tate and Lyle, Warrington, UK), D-fructose (98%, Acros Organics, USA), D-glucose (99%, Acros Organics), malic acid (FiskeFoods, Milton Keynes, UK), citric acid (Fluka, Sigma Aldrich, Fluka), trans-2-hexenal, >=95% (Sigma Aldrich, Gillingham, UK), hexanal, 97+% (Sigma Aldrich), isoamylacetate (IAA), also known as isopentylacetate (IPA) 97+% (Sigma Aldrich), isobutylacetate (IBA), 97+% (Sigma Aldrich).

All aromas were pre-dissolved at 10% w/w in propylene glycol, (99.5+%, Sigma Aldrich) and placed on the roller bed before use to ensure thorough mixing.

The following companies supplied banana flavours for testing: Firmenich (51473, 54330), Symrise, Quest, Clarement, Givaudin (137808, 137807 and 137803), MSK (11722, 12084), Aromco (NF2631, NF3425). All these flavours were pre-dissolved (1% w/w) in propylene glycol (99.5+%, Sigma Aldrich) and tested in sweetened water, at a final flavour concentration of 0.01%. The more successful flavours were also tried at 0.02%, as well as in solutions containing the characteristic "unripe" and "ripe" aroma and tastant levels.

4.4.1.2. Bench testing

Bench testing indicates informal testing which was used to give direction for flavour development. Subjects, all with experience in sensory testing, were recruited from the Division of Food Sciences, University of Nottingham, based on their availability at the time of testing and their willingness to participate. Numbers varied between 3 and 10, depending on subject availability at the time of testing. Sessions were carried out in the training room of the sensory science centre. Data obtained from this testing was not analysed statistically, unlike the data obtained from sensory testing. Bench testing was used for the following reasons:

Choosing the most appropriate generic banana flavour:

The different banana flavours supplied were assessed for the attributes "naturalness" and "neutralness" (i.e. mid-ripeness). The most appropriate flavour was chosen accordingly.

Guidance in flavour development:

Subjects tried the flavour compositions under development, and made suggestions on how they could be improved to make them more authentic and realistic. Solution compositions were modified accordingly, and retested. This process was repeated until subjects thought that the flavours were truly representative of bananas of differing ripeness.

4.4.2. GC-MS of the chosen generic banana flavour

Headspace sampling: The banana flavour was prepared in distilled water at 0.027% (since at this concentration the flavour was readily soluble in water (Shane Avison, Firmenich, private communication)), transferred to a 250mL glass bottle (Fisher Scientific, Loughborough, UK), and sealed with a screw-cap. Solid phase microextraction (SPME) of the headspace volatiles from the banana flavour was carried out using a 2cm stable flex fibre, coated with 50/30μm poly(divinylbenzene) (DVB)/carboxen/poly(dimethylsiloxane) (PDMS) (Supelco, Bellefonte, PA) and conditions as recommended by the manufacturer. The SPME fibre was exposed to the headspace of the flavour solution through a small hole in the bottle screw cap for 5min at room temperature and transferred to the GC injector.

Analysis of flavour headspace to identify volatile components: Volatile components from the SPME fiber were analysed by GC (Thermo Ultra – DSQ, Thermo Fisher Scientific, USA) fitted with a ZB-5 column (Phenomenex, Cheshire, UK); 30m x 0.25mm i.d.; film thickness, 1.0μm). The injector was operated in splitless mode (250°C, 1.5min) with helium as the carrier gas (1.0mL/min or "head pressure, 20psi"). The oven temperature programme was as follows: 40°C for 2 min, 10°C/min to 230°C, and hold for 2 min. The EI-MS was operated in full scan mode over the m/z range of 40-250 (scan time, 0.45s; interscan delay, 0.05s). The SPME fibre was chosen because it is multi-coated, and will therefore absorb (and thus allow identification of) compounds of different polarities and functional groups.

4.4.3. The perceived ripeness of the banana flavours

Solutions of flavours I to V were prepared at the following concentrations (see Table 4-6), using bottled water (Evian).

Table 4-6. Quantities of tastants and aromas that were present in the final samples used for sensory analysis after modification by bench testing. All aromas were pre-dissolved in 1% propylene glycol, but concentrations given represent concentrations present in the final drink.

Compound added	Concentrations added (in g/L)				
	Banana flavours of increasing ripeness				
	I	II	III	IV	V
Sucrose	10	20	35.5	45	50
Glucose	0	1.5	2.5	7.5	15
Fructose	0	1	2	7.5	12
Malic acid	0.4	0.5	0.6	0.7	0.8
Citric acid	0.2	0.2	0.2	0.2	0.2
IBA	0	0	0	0.025	0.05
IAA	0	0	0	0.025	0.05
Hexenal	0.0315	0.016	0	0	0
Hexanal	0.021	0.01	0	0	0
Generic banana flavour (Firmenich Flavour 51474)	0.05	0.06	0.07	0.08	0.09

4.4.3.1. Subjects

44 subjects (27 female; approximate age range 20-50) were recruited from the Division of Food Sciences, University of Nottingham, based on their willingness to participate, and their enjoyment of banana flavour.

4.4.3.2. Presentation of samples

Tests were carried out in sensory booths illuminated with northern lighting and set to positive air pressure, designed according to the British Standards (Guide to design of test rooms for sensory analysis of food. BS 7183:1989). 15ml of each solution I-V was served to each subject in small 30ml plastic tasting cups. Samples were all labelled with randomly generated 3-digit codes, and served to subjects in a balanced order that was randomised across subjects (Williams Latin Square design).

4.4.3.3. Method of rank-rating

This technique of rank rating was chosen because it has been shown to reduce the errors caused by memory loss that can occur when using the traditional approach to intensity scaling. The method forces subjects to retest samples whose flavours they had forgotten and accordingly reduces discrimination errors, thus increasing ability to discriminate (Kim 1998). Furthermore, this technique was chosen because results not only gave an idea of how the samples were ordered, but also how evenly spaced they were in terms of perceived ripeness.

Subjects were asked to rate the ripeness of the samples on a 9-point category scale. They were provided with a laminated cardboard strip (264mm x 51mm) labelled "unripe" at one end and "ripe" at the other, and divided in-between into 9 sections, numbered 1-9. (An example of the strip used is shown in Figure 4-1) Subjects tasted the samples in the order supplied. After tasting the first sample, the subject responded by placing it in front of the appropriate number on the cardboard strip scale. The subject did the same for successive samples. Previously tasted samples could be retested as often as desired to ensure accurate placement on the scale, but subjects were asked to consume approximately the same amount of sample each time. Containers were refilled with more sample when necessary. Subjects were told that they did not have to place samples at each end if they did not feel that they represented either extreme.

1 Unripe	2	3	4	5	6	7	8	9 Ripe

Figure 4-1. An example of the scale used to assess samples for ripeness.

Once subjects were happy with their decision, they were provided with a paper score sheet and asked to place their responses on the answer sheet. The use of palette cleansers (bottled water and crackers) was encouraged to cleanse the palate between sampling.

4.4.3.4. Analysis

Values of perceived ripeness for each subject for each sample obtained from this method were inputted into SPSS Statistics and analysed. This software was used to obtain descriptive statistics of the data (including modes, medians and means). The Friedmans ranked analysis of variance was used to calculate whether median values

for the five samples were significantly different. Where an overall difference existed, rank sum totals were calculated from the mean rank sums provided by the Friedmans output by multiplying them by the total number of observations. Samples were significantly different if the difference between their rank sum totals was equal to, or greater than, the test statistic, which was calculated as follows:

$$\text{Test statistic} = 1.96 \times \sqrt{(J \times P \times (P+1) / 6)}$$

where J=number of subjects, P=number of products

Equation 4-1. Equation to calculate the test statistic.

4.4.4. The effect of the matrix on the perceived ripeness of the flavours

4.4.4.1. Using milk as the matrix

Samples were prepared at concentrations detailed above (Table 4-6) using full fat milk (pasteurised, 3.6% fat) instead of water. Samples were stored at 4°C prior to the test. Only subjects that had participated in the previous test, and liked milk, were used for this test. 26 subjects were used in total (15 female; approximate age range 20-50). The method of rank rating was used to investigate the effect of this matrix on the perceived ripeness of the five banana flavours, as described in see Section 4.4.3.3.

4.4.4.2. Using gelatine as the matrix

To prepare the differently flavoured gel samples, the acids and sugars needed for each flavour were first dissolved in bottled water, according to the quantities outlined in Table 4-6, and heated in a water bath to 70°C. Powdered gelatine (Supercook, Leeds, UK) was added at a final concentration of 2.75% to the heated water and the mixture shaken. The aromas were added once the solution had cooled to 40°C, to minimise aroma evaporation. Solution was then poured out into plastic weighing boats (100mm^2, Fisher Scientific, Loughborough, UK) and left to set in the fridge (average temperature - 4°C). Samples were stored at 4°C prior to the test.

Three small cubes (approximately 0.5cm^3) of gel were cut out and served to subjects in small plastic tasting pots. Only subjects that had previously participated in the first test, and consumed gelatine, were used for this test. 25 subjects were used (15 female; approximate age range 20-50). The method of rank rating was used to

investigate the effect of this matrix on the perceived ripeness of the five banana flavours, as described in see Section 4.4.3.3.

4.5. Results and discussion

4.5.1. Preparing the banana flavours of various ripeness

4.5.1.1. Choosing a generic banana flavour

Results from bench testing indicated that Flavour 51473A from Firmenich was the most natural and neutral tasting banana flavour. The chromatogram produced from the GC analysis, shown in Figure 4-2, detected the presence of 7 major compounds in this flavour. Interestingly, while these compounds were similar, they were not identical to the compounds specified in the data safety sheet for this flavour. Compounds that had been identified both in the data safety sheet and by GC analysis are named on the chromatogram below along with their concentrations as stated in the data safety sheet. Compounds identified only by GC analysis, and not listed in the data safety sheet, are just named.

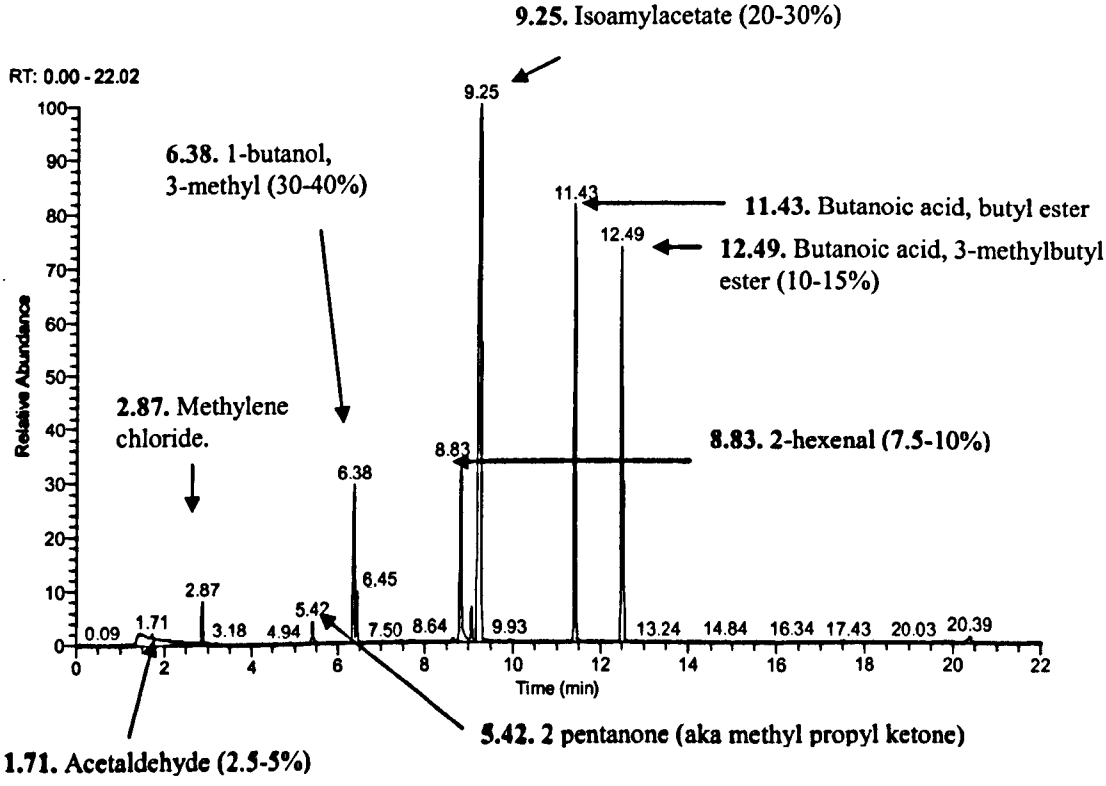


Figure 4-2. Chromatogram of major aromatic compounds in flavour 51473A (Firmenich). Concentrations correspond to those listed in the data safety sheet.

Among the compounds identified in the chromatogram but not in the safety sheet:

- *methylene chloride* is a common solvent and probably just an artefact of the method
- *2-pentanone* has previously been identified by Mayr, Mark et al (2003) in the nosespace of bananas, and is described as a compound naturally found in a banana (Flavour and Fragrances, Sigma Aldrich). The peak on the chromatogram is fairly small, which may explain why the flavour company is not be obliged to list it in the data safety sheet.
- *butanoic acid, butyl ester* has previously been reported in banana, but *only* in freeze dry banana products (Wang, Chen at al. 2007).

Interestingly, the safety data sheet also lists *propanal, 2-methyl* as an ingredient at 1-2.5%, but this compound was not detected on the chromatogram. It is possible that at this low concentration the compound could not be detected (acetaldehyde, which is present at the higher concentration of 2.5-5%, was only just detected in the spectrum). Furthermore aldehydes such as propanal 2-methyl and acetaldehyde react with alcohols, so may be reacting with the propylene glycol or other acids present here to form acetals, which would further reduce their concentration and make detection by GC-MS even more difficult.

As this chromatogram shows, flavour 51473A contains both the characteristic unripe banana aroma 2-hexenal, as well as the characteristic ripe banana aroma isoamyl acetate, which may explain why it was seen in bench testing as fairly neutral (i.e. mid-ripe) in terms of perceived ripeness.

Results from this analysis shows that these aroma compounds are important in generating a neutral and natural banana flavour, so these aromas could be added instead of flavour 51473A to our final samples. However, GC-MS provides limited information into the relative quantities of these different aromas, so in order to create an identical flavour, trial and error testing would be needed to re-create the final flavour. Therefore, flavour 51473A was used directly in the samples to simplify development.

1.5.1.2. Preparing the most unripe and ripe flavours

As explained in the introduction, solutions of the most unripe and ripe banana flavour were initially prepared based on the concentrations outlined in the literature

(as shown in Table 4-5), but bench testing on these initial flavours indicated that at these concentrations, the solutions did not elicit flavours reminiscent of an unripe and ripe banana flavour. While the most unripe flavour was perceived as "astringent", it was "too acidic to be reminiscent of a real unripe banana", so it was clear that acid levels needed to be reduced. The sample was also perceived as a bit bland, indicating that aroma levels needed to be increased. It is interesting that the sample was described as astringent, even though there were no astringent compounds actually present. It is thought that astringency was either caused directly (by the aldehydes, which contain double bonds and so are often perceived as quite sharp); or indirectly (an actual unripe banana is associated with astringency, so if the tastant and aroma content was reminiscent of the flavour of an unripe banana, astringency was perceived by psychological association). The most ripe banana flavour was perceived as "far too sweet and acidic", so both sugar levels and acid levels needed to be reduced. It was also perceived as "having a strong flavour of isoamylacetate", suggesting that levels of this aroma should be reduced. Concentrations used for the most unripe banana flavour, and the most ripe banana flavour, were modified based on these comments, as shown in Table 4-7.

Table 4-7. Concentrations of tastants and aromas, based on concentrations previously quantified in the literature for unripe and ripe bananas, as well as final concentrations, after modifications by bench testing

Compound	Concentrations added to unripe flavoured extreme		Compound	Concentrations added to unripe flavoured extreme	
	Initially (based on the literature) – as shown in Table 4-5.	Finally (after bench testing modifications)		Initially (based on the literature) – as shown in Table 4-5.	Finally (after bench testing modifications)
Sugar levels			**Sugar levels**		
			Sucrose	12 %	5.4%
			Glucose	3.5 %	1.62%
Sucrose	1%	1%	Fructose	3 %	1.35%
Acid levels			**Acid levels**		
Malic acid	0.05%	0.04%	Malic acid	0.4 %	0.08%
Citric acid	0.025%	0.02%	Citric acid	0.1 %	0.02%
Aroma levels			**Aroma levels**		
			Isopentyl acetate		
Hexenal	0.003%	0.00315%		0.0075%	0.005%
Hexanal	0.002%	0.0021%	Isobutyl acetate	0.005%	0.005%
Generic banana flavour	n/a	0.005%	**Generic banana flavour**	n/a	0.009%

These findings suggest that the levels of tastant and aroma needed in aqueous solutions were less than the concentrations found in real bananas, since when tastants and aromas were added to aqueous solutions at the concentrations that they have been detected in bananas, the flavours were far stronger than those perceived when actually consuming bananas. As explained in section 4.2.4.1, higher levels of flavours will generally be required for optimum taste perception in high viscosity

foods (like a banana) compared to a low viscosity food (like water). Also, a banana contains starch. As a general rule, carbohydrates decrease the volatility of compounds relative to water by a small or moderate amount, and furthermore the amylose chain of starch, and to a lesser extent the amylopectin chain, forms inclusion complexes with many compounds (Godshall 1997). The presence of starch in an actual banana may therefore be reducing the perception of flavour by these two ways, which did not occur when water was used as the matrix.

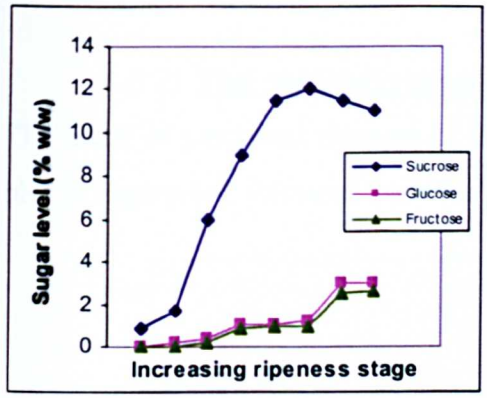
4.5.1.3. Developing flavours of intermediate banana ripeness

Banana flavours of intermediate banana ripeness were deduced based on the final concentrations decided for the unripe and ripe banana flavours (as a result of bench testing), as shown in Table 4-7. Reasons explaining how compositions of the intermediate flavours were deduced, based on these final concentrations, is described in Table 4-8 for the acid and aroma levels, and in Figure 4-3 for the sugar levels.

Table 4-8. Table showing the principles used in preparing flavour composition for the three banana flavours of increasing ripeness.

Compound	Composition in the flavoured solution of intermediate ripeness, based on the concentrations agreed on for the unripe and ripe extremes (see Table 4-7Error! Reference source not found.)
Citric acid	The levels of citric acid used in the final unripe and ripe extreme did not change, so citric acid concentration was kept constant over the five solutions
Malic acid	The levels of malic acid increased two-fold from the final unripe to the ripe extreme, so levels were increased linearly over the five solutions.
Generic banana flavour	The level of generic banana flavour, which almost doubled from the final unripe to ripe extreme, was therefore increased linearly over the five solutions.
Aromas characteristic of the unripe banana flavour	Hexenal and hexanal were added at lower levels to solution II, the second most unripe sample, than they were in the unripe extreme (sample I).
Aromas characteristic of the ripe banana flavour	Isoamyl acetate and isobutyl acetate were added to solution IV at lower levels than they had been added to the ripe extreme (i.e. sample V).
Aroma profile of the mid-ripe banana flavour	No aromas (except the generic banana flavour) were added to sample III, the midripe solution, so that it had neither a characteristic unripe, or ripe, flavour

A)



B)

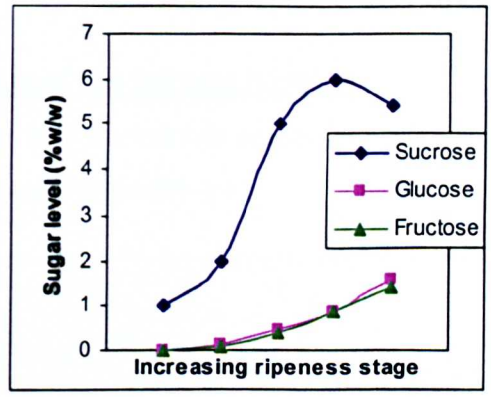


Figure 4-3A and B. Changes in sugar levels over the ripening process. A) This graph is based on the figure published by Tarkosova and Copikova 2000, which represents actual changes in the sugar levels during ripening. B) This graph represents the levels used to prepare the intermediate samples, based on the concentrations agreed on for the unripe and ripe extreme (see Table 4-7 Error! Reference source not found.**), and modified according to the trend published by Tarkosova and Copikova 2000.**

Solutions of the three intermediate flavours were prepared accordingly and sampled in bench testing along with the unripe and ripe flavour extremes. Comments from bench testing on these flavours and the resulting flavour modifications are detailed in Table 6-5 in the appendix. The main comments were that although the unripe samples (i.e. flavours I and II) were fairly reminiscent of an unripe banana flavour, the ripe banana flavours (i.e. flavours IV and V) were perceived as too sweet. Furthermore, ripeness did not increase linearly between the five samples – a much larger difference in perceived ripeness was observed between samples II and III than between the last three samples – samples IV and V were actually perceived to be of similar ripeness. Interestingly, the pattern of perceived ripeness correlated directly with actual sugar contents (the four samples II, III, IV and V contained 20g, 50g, 60g and 54 g/L respectively). These results suggest that sugar is a key factor in driving the perception of ripeness in an aqueous medium, and for the five samples to be perceived as increasing in ripeness in an equal fashion, sugar content needed to be increased linearly, even if this is not what occurs during the actual ripening process. This phenomenon has previously been shown – a drop in perceived banana flavour was evident among naïve subjects and the majority of experienced subjects when sucrose was removed or decreased, even though the ripe banana aroma levels stayed constant (Hort 2004). These findings further exemplify how the matrix of a food or drink is a key factor affecting flavour perception, and how different matrices need different flavour profiles in order to be perceived as the same when consumed.

4.5.2. The perceived ripeness of the banana flavours

The order of perceived ripeness of the five flavours in water, as shown by the box plot in Figure 4-4, followed the order I→II→III→IV→V.

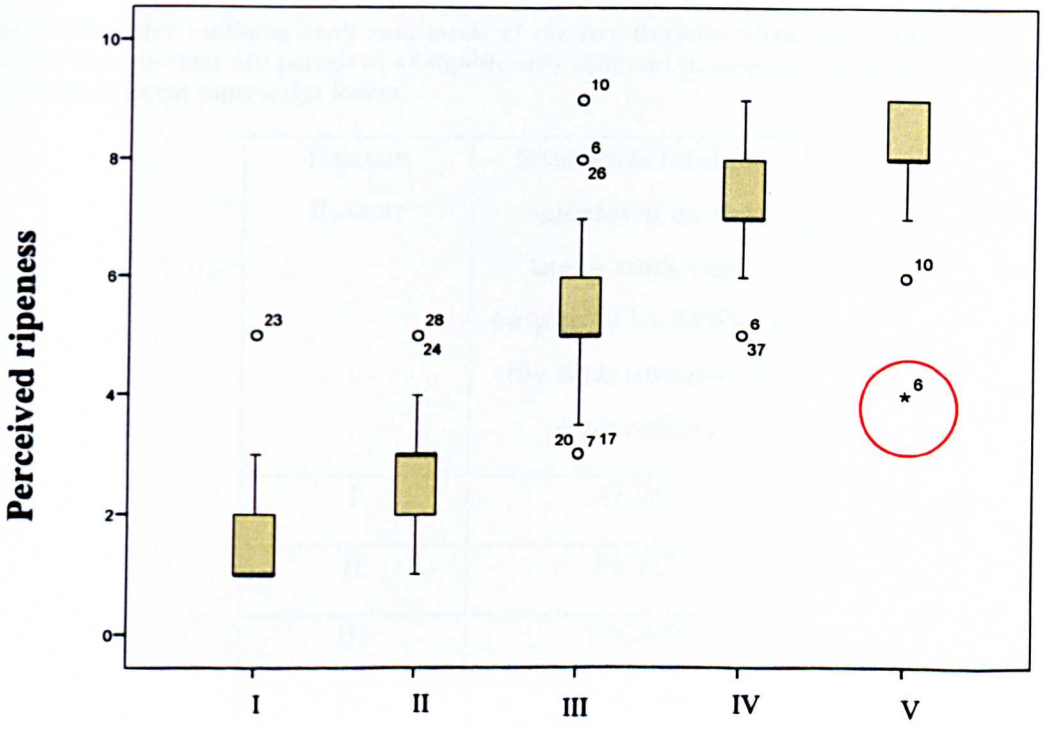


Figure 4-4. Box plot showing values for perceived ripeness of the five banana flavours in water. Thick black lines indicate the median value. The boxes represent the middle 50% of responses. "Whiskers" extending above and below the box represent the highest and lowest values that are not outliers.

Results from subject 6 were removed from the analysis, since the box plot revealed that this subject was an extreme outlier, as shown in Figure 4-4. An extreme outlier is one whose value sits more than 3 box plots from the median value. A Friedmans Ranked Analysis of Variance test carried out on the remaining data showed that the samples differed significantly in terms of perceived ripeness ($p < 0.01$), and that they followed the expected order. Rank sum totals were calculated by multiplying mean sum ranks (as calculated by SPSS) by the total number of observations, as previously explained (see Section 4.4.3.4) and these rank sum totals are shown in Table 4-9. The difference between the rank sum totals of the samples was also calculated, and compared to the test statistic to see if significant differences between individual

samples existed. The test statistic was calculated as follows (see Equation 4-2, according to the equation described in section 4.4.3.4):

$$\text{Test value} = 1.96 \times \sqrt{(43 \times 5 \times 6 / 6)} = 28.74$$

Equation 4-2. Test statistic calculated for when banana flavours were sampled in water.

Table 4-9. Table outlining rank sum totals of the five flavours when water was used as the matrix. Samples that are perceived as significantly different in terms of perceived ripeness are allocated different superscript letters.

Banana flavour	Rank sum total (as calculated by the mean rank sum outputted by SPSS by the total number of observations)
I	47.96^{a}
II	84.48^{b}
III	136.84^{c}
IV	181.72^{d}
V	210.78^{e}

As shown in Table 4-9, samples I to V were all perceived as significantly different from each other in terms of ripeness, since the difference in rank sum totals between all consecutive samples exceeded the test statistic. This shows that these five flavours did indeed increase sequentially in perceived ripeness, from flavour I to flavour V, suggesting that the flavours were successfully demonstrating a perceptual ripening effect.

The effect of the matrices gelatine and milk on the perceived ripeness of these five flavours was then investigated.

4.5.3. The effect of the matrix on perceived ripeness

4.5.3.1. Using gelatine as the matrix

The order of perceived ripeness of the five flavours in gelatine, also followed the order I→II→III→IV→V, as shown in Figure 4-5 below:

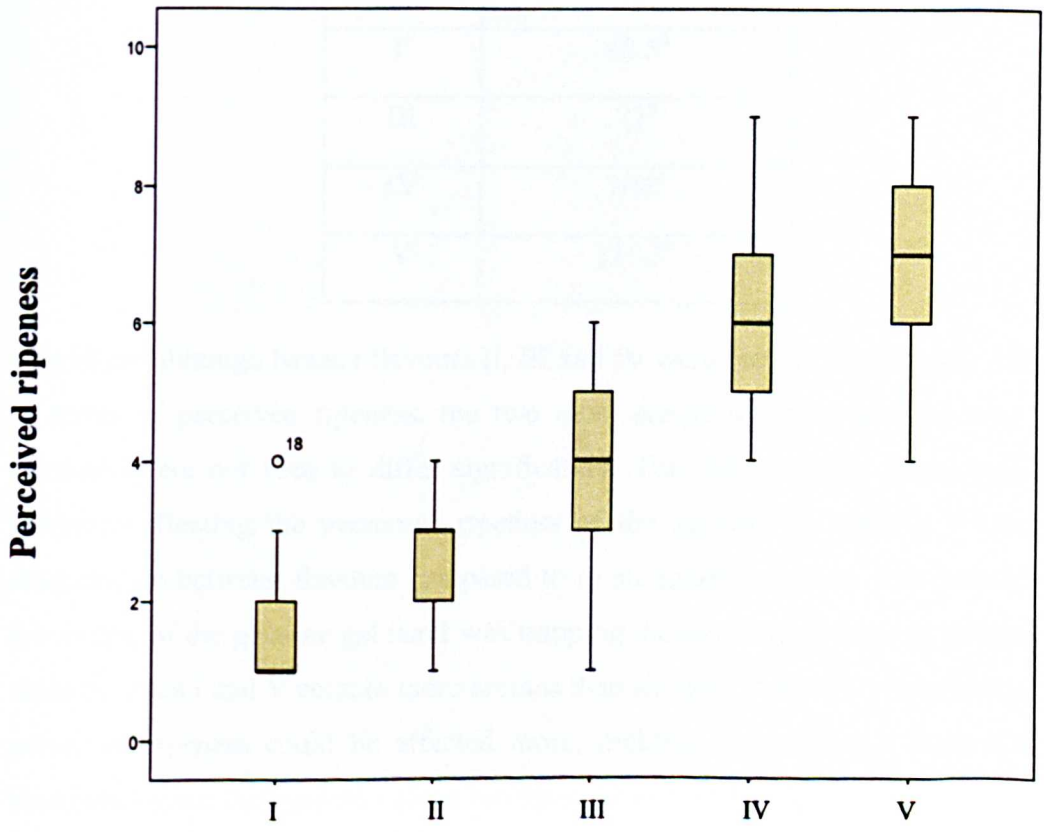


Figure 4-5. Box plot showing values for perceived ripeness of the five banana flavours in gelatine. Thick black lines indicate the median value. The boxes represent the middle 50% of responses. "Whiskers" extending above and below the box represent the highest and lowest values that are not outliers.

A Friedmans Randed Analysis of Variance test carried out on the data showed that the samples differed significantly in their perceived ripeness ($p<0.01$). Rank sum totals were calculated as previously explained, and are shown in Table 4-10. The difference between the rank sum totals of the samples was also calculated, and compared to the test statistic. The test statistic was calculated as follows (see Equation 4-3):

$$\text{Test value} = 1.96 \times \sqrt{(25 \times 5 \times 6 / 6)} = 21.9$$

Equation 4-3. Test statistic calculated for when banana flavours were sampled in gelatine.

Table 4-10. Table outlining rank sum totals for the five flavours when gelatine was used as the matrix. Samples that are perceived as significantly different in terms of perceived ripeness are allocated different superscript letters.

Sample	Rank sum total
I	32^{a}
II	48.5^{a}
III	72^{b}
IV	106^{c}
V	116.5^{c}

Therefore, although banana flavours II, III and IV were seen as significantly different in terms of perceived ripeness, the two most unripe samples, and the two ripest samples, were not seen to differ significantly. The matrix of the gelatine gel was therefore affecting the perceived ripeness of the samples by making it harder to discriminate between flavours compared to in an aqueous system. It is possible that the matrix of the gelatine gel itself was trapping the aroma molecules in general, and since samples I and V contain more aromas than samples II and IV respectively, their perceived ripeness could be affected more, making discrimination more difficult. However, when the median values for ripeness of the five flavours were compared for the two matrices, as shown in Figure 4-6, it was apparent that while the ripe flavours in gelatine were perceived as less ripe than their equivalent samples in water, the unripe samples had equal median values for perceived ripeness whether water or gelatine was used. This suggests that a general suppression of aromas is not be occurring, or the median values for the perceived ripeness level of the unripe flavours in gelatine would be expected to differ from that in water, which they do not. (However, subjects did not sample the flavours in the two matrices simultaneously during testing, so comparisons were not made directly).

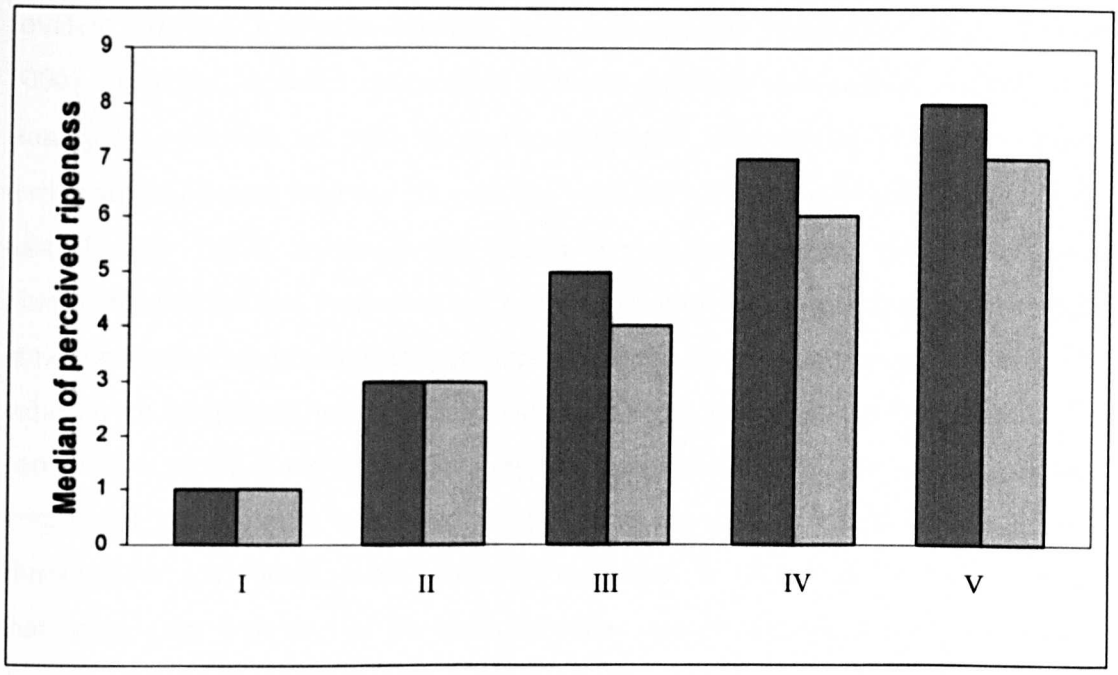


Figure 4-6. Bar chart comparing median values for perceived ripeness obtained when either water or gelatine gel was used as the matrix. Dark grey indicates median values when water was used as the matrix. Light grey indicates when gelatine was used as the matrix.

Possible reasons why the gelatine matrix may be specifically affecting the perceived ripeness of the riper samples were therefore considered. The reduction in perceived ripeness of the riper samples could be due to the tastant release being affected, since tastants are key to the perception of fruity flavours (Hort and Hollowood 2004) and the ripe flavours do contain higher levels of sugars and acids than the unripe flavours, and would therefore be more affected. Although gelatine gels melt at mouth temperature and it has previously been shown that gelatine solutions mix well with saliva and show good tastant release (Koliandris 2007), the melting temperature of gelatine gels has been shown to increase with increasing concentrations of sugars (Gekko 1992), so it is possible that for the riper flavoured samples the subject may have consumed the gel before it had melted, limiting tastant release and thus flavour perception in this way. However, it is also possible that the presence of the gelatine was affecting the release of ripe aromas – perhaps isoamyl acetate and isoamyl butyrate are being specifically bound by the gelling agent, and therefore not released. Specific binding of aromas to proteins has previously been reported (which is of little surprise since it is the specific binding of aromas to protein receptors that causes aroma perception) - according to Juteau, Cayot et al (2004), interactions between

proteins and aromas have been the subject of numerous studies, and have shown that covalent binding, hydrogen binding, and hydrophobic interactions exist (Juteau 2004). However, specific interactions between gelatine and isoamyl acetate and isoamyl butyrate does not seem to have been reported, although the interaction of the unripe aroma hexanal with soy proteins has been the subject of a detailed study in the past (Fischer 1997). Although the results shown here suggest that the specific binding of gelatine and hexanal is not occurring (since the perceived ripeness of the unripe samples does not appear specifically affected by the matrix when the median values were compared) informal discussion with subjects did indicate that despite the perceived order of ripeness not changing, the unripe flavours in the gelatine gel did seem quite muted when compared to the same flavours in water, and lost their characteristic astringency, which could be explained by such binding. It is possible that using only 9 points on the ripeness scale was preventing these more subtle differences from being observed.

Alternatively, the gel preparation process could be responsible for the reduced flavour perception in the ripe banana flavoured samples – unlike when water was used as the matrix, the water used to prepare the gelatine samples needed to be heated first. When a solution of sucrose is heated in the presence of some acid, it breaks apart into its two component monosaccharides (McGee 2004). Since samples III, IV and V all contained much more sugar than the unripe samples, this may explain why the perceived flavour of the riper samples was more affected than the unripe samples (although one would expect these samples to actually be perceived as more ripe if the sucrose was breaking down into monosaccharides, due to an overall increase in sweetness, which is contrary to what was observed). Alternatively, the ripe aromas may evaporate more readily when added to warm water than the unripe aromas, however inspection of the data sheets indicate that both isoamylacetate and trans-2-hexenal have similar boiling points (at 141°C and 147.5 °C respectively), both of which are higher than the boiling points of hexanal and isobutyl acetate (at 121.5°C and 117°C respectively). If aroma evaporation *was* occurring during the preparation process, one would expect all the samples to be affected equally.

In order to investigate this further, the aroma release during consumption of both the aqueous and gel based samples could be monitored using APCI-MS, to see if there is

a difference in the levels of aromas being released, or if flavour is more affected at the cognitive level.

4.5.3.2. Using milk as the matrix

The order of perceived ripeness of the five samples when milk was used as the matrix also followed the order I→II→III→IV→V, as is shown by the box plot in Figure 4-7.

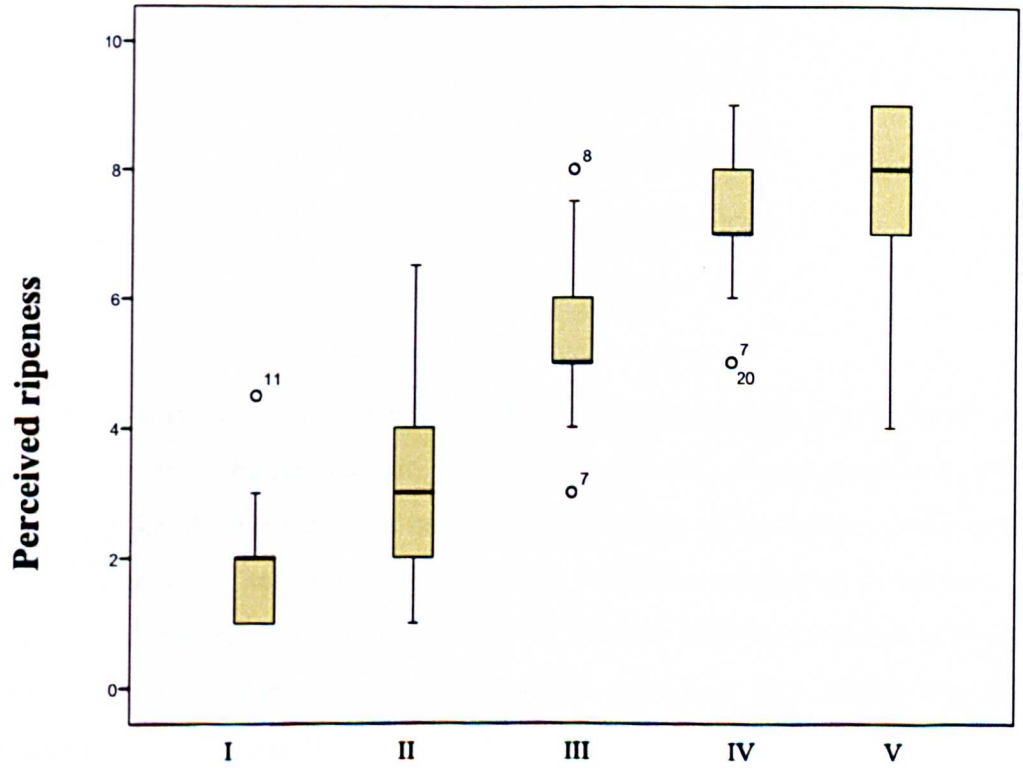


Figure 4-7. Box plot showing values for perceived ripeness of the five banana flavours in milk. Thick black lines indicate the median value. The boxes represent the middle 50% of responses. "Whiskers" extending above and below the box represent the highest and lowest values that are not outliers.

A Friedmans Ranked Analysis of Variance test carried out on the data showed that the samples differed significantly in terms of perceived ripeness ($p<0.01$). In order to understand which samples differed significantly from each other, differences between the rank sum totals, as shown in Table 4-11, were compared to the test statistic. The test statistic was calculated as shown below (see Equation 4-4):

Test value = $1.96 \times \sqrt{(25 \times 5 \times 6 / 6)} = 21.9$.

Equation 4-4. Test statistic calculated for when banana flavours were sampled in milk.

Table 4-11. Table outlining rank sum totals of the flavoured samples when milk was used as the matrix. Samples that are perceived as significantly different in terms of perceived ripeness are allocated different superscript letters.

Sample	Rank sum total
I	28^{a}
II	48^{a}
III	78^{b}
IV	106.5^{c}
V	114.5^{c}

When milk was used as the matrix, as when gelatine was used, the two most unripe and the two most ripe flavours did not differ significantly in their perceived ripeness, suggesting that discrimination is harder when matrices more complex than water are used. It is generally accepted that a reduction in flavour perception of liquids occurs with an increase in viscosity (Baines 1987), however milk has a similar viscosity to water, so it is unlikely that the reduction in discriminating ability was due to viscosity. It is possible that, as is hypothesised for gelatine, the presence of the matrix in general is causing the aromas to be bound, which is why flavours I and V, which contain higher levels of aromas than their counterpart flavours II and IV, are affected more in terms of their perceived ripeness, making discrimination more difficult.

When the median values for perceived ripeness were compared directly for the two matrices, only the perceived ripeness of the most unripe sample differed - the most unripe sample was actually perceived as less unripe (i.e. riper) than when water was used (see Figure 4-8), but again subjects did not sample the flavours in both the matrices simultaneously during sensory testing, so comparisons were not made directly.

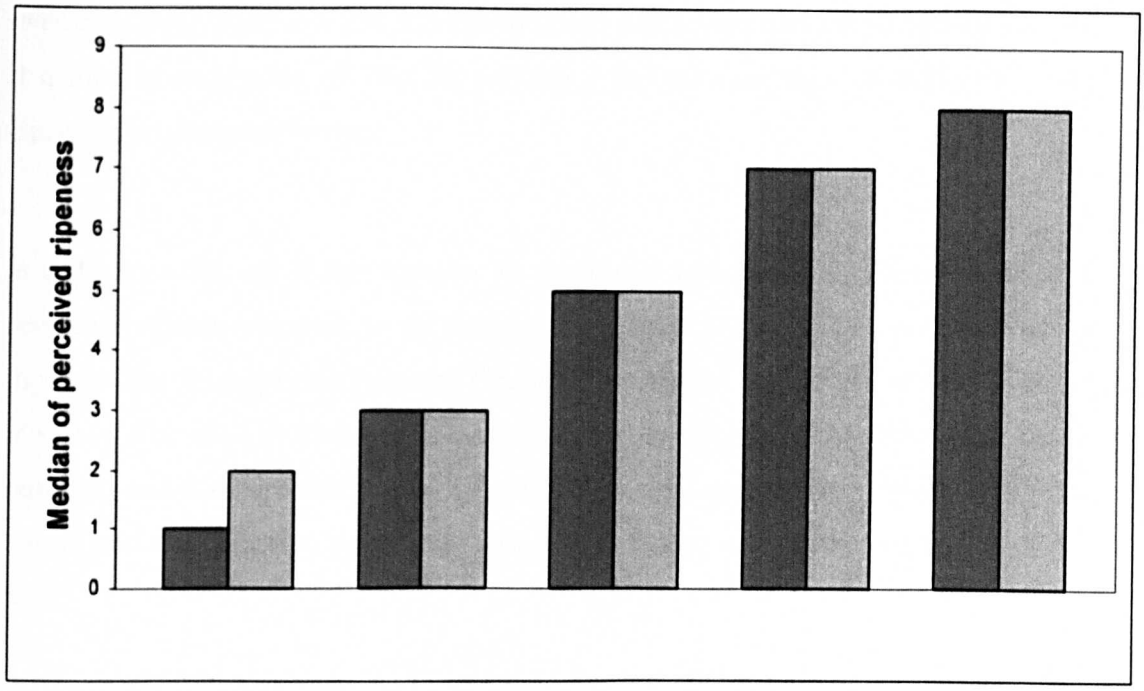


Figure 4-8. Bar chart comparing median values obtained when either water or milk was used as the matrix Dark grey indicates median values when water was used as the matrix. Light grey indicates when milk was used as the matrix.

Possible explanations for why the most unripe flavour could be perceived as less unripe when milk was used as the matrix are proposed. Firstly, the presence of fat in the milk could be affecting the release of aroma. As explained in section 4.2.4.1, fat has a significant effect on the partition of volatile compounds between the food and the air phases, and formulations containing fat usually need higher levels of lipophilic compounds to maintain the same profile of aroma release from the product. If the partition of the unripe aromas was specifically being affected by the presence of the fat, this may explain why the most unripe flavour was being perceived as less unripe when milk is used as the matrix. Secondly, milk contains the protein β-lactoglobulin – the presence of β-lactoglobulin in aqueous solutions induces a decrease in the volatility of hydrophobic flavour compounds, mainly due to hydrophobic interactions into the central cavity of the protein (Guichard 2000). According to Seuvre, Turci et al (2008), non-reversible covalent bonding of t-2-hexanal with dairy proteins has previously been reported, which may explain why the unripe flavour was perceived as less unripe in milk. Thirdly, milk contains the sugar lactose, so it is possible that the presence of lactose in the milk increases overall sweetness. Lactose is only 40% as sweet as sucrose (McGee 2004) and milk only contains 5% sugar (assumed to be entirely lactose), so plain milk will have a

sweetness equivalent to a 2% sucrose solution. Since flavour I only contains sucrose at a final concentration of 1%, the additional natural sweetness of milk may make this sample perceptually riper.

In order to work out if this increase in perceived ripeness is specifically due to a decreased release of aroma, or an increase in *perceived* ripeness due to the increased sugar levels, Atmospheric Pressure Chemical Ionisation Mass Spectrometer (APCI-MS) could be used to monitor aroma release during consumption of the milk based samples, and compare this to when the flavours were consumed in water alone. To investigate the effect of sweetness, tongue swabbing, or sensory testing, could be used.

In conclusion, as these results show, depending on the matrix used, flavour formulations may need to be amended slightly from the original formulation (i.e. those that were formulated in water) in order to produce five banana flavours that differed significantly from each other in terms of perceived ripeness.

4.5.4. The enhancement of the banana flavour experience with texture

Gelatine gels are semisolids, like bananas, so it was thought that gelatine would provide a matrix more congruent to the experience of eating a real banana, enhancing flavour perception. However, informal discussion with the subjects indicated that when the flavours were tried in milk, the mouth-feel of the samples was much more reminiscent of the mouth-feel of a real banana compared to when gelatine was used. This is thought to be because the fat in the milk acts to coat the tongue, in a similar way that banana tissue does. Furthermore, as discussed in section 4.2.4.2, banana is a flavour that is commonly associated with milk – banana milkshakes, and banana flavoured milks, are readily available commercially. Some subjects had eaten mashed up bananas with milk as a child, so for them when milk was used, it brought back memories of their childhood, adding to the overall pleasure of the flavour experience.

4.6. Technology Transfer

These findings outlined in this work suggest that out of all the different matrices tested, milk was the best matrix to use for this application. However, because milk is liquid, the same challenge applies to the preparation of this drink as did for the tomato drink. However, milk can easily be thickened, (as milkshakes normally are) which may then allow it to be subsequently layered. The tomato drink in chapter 3 was administered fairly satisfactorily in layers, using the inherent thickness of the tomato juice. To investigate possible thickeners to use for this application, the thickeners used in commercially available banana flavoured milk based drinks were noted (see Table 4-12). (Note – the function of these additives is not specified, so some of them may be present for stabilisation reasons rather than principally for thickening).

Table 4-12. Table listing the thickeners present in some commercially available banana flavoured products

Commercially available drink	Thickeners present
Sainsbury's fruity banana flavoured milk	Carrageenan and xanthan gum
Yazoo Banana flavoured milkshake	Mono- and di-glycerides of fatty acids, carrageenan, guar gum
Big Banana *Forgoodness Shakes*	Carboxy methylcellulose, carrageenan

Although carrageenan, the most popular thickener in these milkshakes, could be used, in order to properly disperse carrageenan in a liquid the liquid needs to be heated - due to the volatile nature of the aromas used in these flavours, it may be better to use a cold active thickening agent. Guar gum and cellulose mentioned above are both cold active thickening agents, (as is alginate, but this isn't listed in this table, and therefore isn't generally used to thicken milkshakes). Thickening the milk base would also allow for changes in texture and colour to be made. Both the texture and colour of a banana change dramatically during the ripening process, and both of these inputs may be important in contributing to the overall perception of a ripening banana. Different colours could be added to the different layers to enhance the perceived ripening banana experience, and texture could be changed by adding different levels of thickener to the sample. However the addition of a thickener,

especially if used at different levels throughout the drink, may change the mouth-feel, mixing and flavour release properties of the final product, so further tests would need to be carried out to investigate this. Furthermore, if the different flavoured layers were modified visually like this, the overall magic of the changing flavour drink may be reduced.

Unlike for the tomato drink, where mixing was not seen as such an important problem because all of the five tastes were naturally present throughout the drink, for the banana drink, any mixing of flavours could ruin the effect – for example the presence of ripe banana aromas in the unripe banana flavour would affect its perception. For this reason, a solid matrix would be more convenient, however, as explained above, gelatine was not suitable for the application in terms of congruency. Other solid matrices were therefore considered, including an unflavoured Angel Delight mix. Angel Delight offers the advantage not only of containing milk, so would offer the benefits that milk had in terms of congruency to flavour perception, it also has a texture that is similar and reminiscent of an actual banana. Furthermore, Angel Delight, like an actual banana, contains starch, so would also provide a starchy mouth-feel associative of bananas.

Therefore, the five flavours were developed in this matrix of Angel Delight, however there were significant limitations to using this matrix. While the texture was optimal, significant adjustments needed to be made to the flavour composition (see Table 6-7 for details of the preparation and flavour development process) mainly due to the high level of natural sugar present in the unflavoured angel delight, as well as the fact that the final preparation needs to be whipped, which dilutes the flavours by incorporating air. Furthermore, the matrix contains modified starch and hydrogenated fat, as well as numerous emulsifiers, gelling agents and other additives, and it was thought that these may either be entrapping flavour molecules within the matrix, or reducing the release of tastants into the saliva phase due to poor mixing with saliva, thus affecting flavour perception. Furthermore, the final angel delight product contained both starch and milk, both of which have previously been reported to trap specific flavour molecules.

4.7. Conclusions

The work presented here describes the process involved in developing a banana flavoured product whose flavour changed dynamically as it was consumed, mimicking the ripening of a banana. Firstly, a suitable generic flavour that was typical of a natural and neutral banana was found, and the flavour was analysed by GC-MS. The following aromas were detected – isoamylacetate, 1-butanol 3-methyl, butanoic acid butyl ester, butanoic acid 3-methyl butyl ester, 2-hexexal and 2-pentanone - suggesting that these aromas are important in generating a neutral and natural banana flavour. Secondly, a thorough investigation of the physical and biochemical changes that accompany the ripening of a banana was carried out, using information reported in the literature. It was found that during ripening, levels of sugars and acids in banana tissue increase, while the aroma profile changes from one that is predominated by the unripe aromas hexenal and hexanal, to one where isoamylacetate and isobutyl acetate predominate.

When concentrations of the aromas and tastants that had been quantified according to the literature in actual banana tissue of increasing ripeness, were sampled in aqueous solutions (i.e. in the absence of banana tissue), flavours were perceived to be very strong and not reminiscent of true banana flavours, suggesting that both the presence of starch in the banana and the matrix itself is suppressing flavour perception. Flavours were therefore modified accordingly, and sensory analysis confirmed that the compositions chosen for the final flavours were perceived to be increasing linearly in ripeness, and furthermore each was perceived as being significantly different in terms of perceived ripeness.

When these flavours were added to different matrices (namely milk and gelatine), it was found that discrimination between the two most ripe and unripe samples was much harder when these matrices were used compared to water, illustrating how different matrices affect flavour perception. In addition, different matrices had different effects on on flavour perception at the cognitive level, and while milk provided a mouth-feel congruent to banana flavour, a starch-based product like angel delight had a texture more similar to an actual banana compared to aqueous systems or gels, and was therefore more congruent in delivering the overall banana experience. Furthermore, the use of a semisolid matrix like angel delight, compared to milk, would allow the different flavours to be delivered more easily in the final

dish, and would prevent mixing of different layers. However, angel delight required significant modifications to the flavour profile in order to deliver a ripening experience that was analogous to when the flavours where present in the other matrices.

Since different matrices required very different flavour formulations, the final matrix to use would need to be decided, so that flavour compositions could be modified in order that each flavour was perceived to differ significantly in ripeness in the final product. Advice from a flavourist may be beneficial at this point to optimise the final flavours.

In general, these findings show the pivotal role that texture plays in flavour perception, both at the physical as well as the cognitive level, which has very important implications for the final delivery of this concept.

4.8. Further work

Further work could include justifying the information reported in the literature on the processes that occur during the ripening of bananas by carrying out APCI-MS and GC-MS measurements of actual bananas representing different stages of the ripening process. This may also provide a possible insight into how the flavours developed in this study could be improved and made more authentic, by identifying prominent flavour compounds that were perhaps not included in the flavours.

Furthermore, further work on tongue swabbing and APCI measurements while consuming the banana flavours in the different matrices could be carried out, since this would help further elucidate the exact effect that the matrix is having on the perception of these flavours, and to understand whether it is occurring at a physical or cognitive level.

5. Overall conclusions

The work described in this thesis shows how science can play a role in developing novel texture and flavour experiences for use in a restaurant setting.

The first concept developed here was a hot gel that melted in the mouth on consumption, in a fashion analogous to gelatine, which it was thought would provide an interesting and pleasurable textural experience for the diner. Since it is well known that the popularity of gelatine is due to a combination of its melt-in-the mouth ability, its desirable mouth-feel, and its good flavour release, a systematic approach was taken to measure the melting behaviour and flavour release characteristics of hot HPMC gels, in order to assess their suitability as a gelatine alternative. Results showed that while gels made from all of Dow's E-type and F-type HPMC range displayed melting temperatures above 37°C, when tested at their minimum gelling concentrations. HPMC solutions of low molecular weights, despite being present at higher concentrations, displayed more desirable mouth-feel attributes, and were predicted to show better flavour release, than those made of the higher molecular weight HPMCs. This shows how a thorough exploration and understanding of the properties of a successful product can be used to test, and predict the efficiency and success of, novel ingredients. Results from these studies also provided a valuable insight into how the different techniques used to monitor melting processes (namely rheology and calorimetry) gave different values for melting temperatures, because the different techniques actually measure different processes that occur during the gel-sol transition of HPMCs. Furthermore, values obtained from rheological studies were greatly affected by entanglement coupling. As a result if this, empirical methods were used in conjunction with these techniques in order to ascertain the "real" melting temperatures, and furthermore provided a simple method that could be used in a restaurant for future characterisation.

The second part of this thesis focussed on developing drinks whose flavours changed during the consumption process, and how a thorough understanding of how the science of flavour perception, as well as the use of sensory science, can be exploited to develop new concepts. The first drink developed was a tomato flavoured drink

whose flavour changed due to a sequential heightening of its tastant profile. Results obtained from a thorough sensory investigation indicated that the major factor determining the quantity of each differently flavoured tastant solution needed in the final drink did not depend on the order in which the tastants were provided, as was initially thought, but was dictated by the volume of each solution needed to ensure swallowing, and thus allow full flavour perception. The second drink developed was a banana flavoured one, where the actual flavour of the drink changed on consumption to mimic the ripening of a banana fruit. The work carried out here highlighted the key aromas needed to produce a realistic banana flavour, and a thorough investigation of the literature helped guide how the aromas and tastants needed to be modified across the five flavours to produce flavours that were perceived to be increasing in ripeness. Sensory analysis was used to guide development, and interestingly the levels of aromas and tastants needed when an aqueous matrix was used to produce flavours perceived to represent a ripening banana were much lower than those actually present in bananas over the ripening process. Furthermore, the work presented here showed not only that using different matrices affected banana flavour perception, presumed to be due to specific and non-specific binding of the aromas and tastants, but that a matrix with a texture congruent to that of banana flavour was needed to enhance banana flavour perception, and that milk and starch bases, compared to aqueous bases, were more successful in this. These findings therefore highlight the key factors that need to be considered when creating novel flavoured products, and how the use of sensory science is crucial to this developmental process.

Therefore, this work has shown how thorough scientific explorations show useful benefits in guiding the development of novel flavour and texture experiences, and help to bridge the gap between the world of the scientist, the chef, and the consumer.

6. Appendix

Table 6-1. Results for criteria for assessing gelation via cloud test. All temperatures are in °C. Y:yes it is cloudy; N:no it's clear. ? represents a state in between the two. The range of temperatures over which the transition occurred is shown in bold.

Oven temperature (°C)	E50	E15	E5	E4M	F450	F4M	F50
39.5	N	N	N		N	N	N
40	N	N	N	N	N	N	N
40.5							
41							
41.5	N	N	?	N	N	N	N
42							
42.5							
43							
43.5	N	N	N	N	?	N	?
44							
44.5							
45							
45.5	?	?	?	N	?	N	N
46							
46.5							
47							
47.5	?	Y	Y	?	Y	N	N
48							
48.5							
49							
49.5	Y	Y	Y	N	Y	Y	Y
50							
50.5	Y		Y	Y	Y	Y	?
51							
51.5	Y	?	Y	Y	Y	Y	Y
52							
52.5							
53	Y	Y	Y	Y	Y	Y	?
53.5	Y	Y	Y	Y	Y	Y	Y
54							
54.5							
55							
55.5	Y	Y	Y	Y	Y	Y	Y
56							
56.5							
57	Y	Y	Y	Y	Y	Y	Y
57.5							
58							
58.5							
59	Y	Y	Y	Y	Y	Y	Y
59.5							
60							
60.5	Y	Y	Y		Y	Y	Y
Range	**43.5-49.5**	**43.5-47.5**	**43.5-47.5**	**49.5-50.5**	**41.5-47.5**	**47.5-49.5**	**47.5-49.5**
Midpoint (± variation)	46.5(±3)	45.5(±2)	45.5(±2)	50(±0.5)	44.5(±3)	48.5(±1)	48.5(±1)

Table 6-2. Effect of changing concentration, gelling time and gelling temperature on melting temperature of sample F450, as determined by the tip-test. G=gelled. M=melted. ? indicates a state in between the two.

Oven Temperature (°C)	3% at 75°C for 40 mins	4% at 75°C for 40 mins	3% at 75°C for 80 mins.	4% at 75°C for 80 mins.	3% at 95°C for 40 mins.	4% at 95°C for 40 mins.	3% at 95°C for 80 mins	4% at 95°C for 80 mins
39.3	M	M	M	M	M	M	M	M (?)
41.3	M	M	M	M	M	M	M	M?
43.1	M	M	M	?	M	M?	M	?
45.1	M	?	M	G?	M	?	M	?
47.1	M	G?	M?	G	M	G	M	G?
50.3	M?	G	M?	G	M?	?	M?	?
52.6	M?	G	G?	G	?	G	G?	G?
54.2	M?	G	?	G	M?	G?	M?	G?
56.3	G?	G	G?	G	M?	G	?	G
57.7	?	G	G?	G	M?	G	?	G
59.4	?	G	G	G	G?	G	?	G
61.6	G	G	G	G	G (?)	G	G (?)	G
Temperature of transition (°C)	55	46	53	44	58	47.5	55	47

Table 6-3. List of references used in the QDA to help attribute definition

Furniture polish, cardboard and paper (both wet and dry), lapsang souchong and an ash tray to illustrate smokiness, soil, papier maché, paint stripper, plastic, a mix of flour and water as an example of starchy, ink, newspaper (both wet and dry), wallpaper paste, a burnt rubber bung to illustrate burnt rubber, salty, a solution of quinine to illustrate bitter, a solution of methyl salicylate to illustrate medicinal, nail polish, nail polish remover, and paint stripper.

Table 6-4. Table showing all the flavour attributes generated during the first profiling session of the QDA. Rateable attributes refers to those that it was thought discriminated samples and are shown in bold.

Appearance	
Attributes generated	Bubbles, clear, yellow-tinted, hair gel-like, pale apricot, neutral, tinted, saliva-like, golden, glossy, straw-coloured, shiny, bright, viscous, **intensity of straw colour, thick**
Odour	
Attributes generated	Fusty, gluey, starchy, damp paper-like, cardboard, floral, wall-paper paste, musty, esterey, nail varnish, medicinal, woody, earthy, mouldy, damp, paper, industrial, smokey, plastic, furniture polish, paint stripper, ink, chemical, **musty, chemical**
Taste/Flavour	
Attributes generated	Smokey, plastic, rubber, bitter, burnt, furniture polish, chemical, toxic, starchy, old, floral, mouldy, tar, cardboard, damp paper, dirt, salty, woody, medicinal, flavour lab fridge, musty, earthy, uncooked packet dessert, gluey, **damp paper, chemical, floral, bitter**
Mouth-feel	
Attributes generated	Drying, smooth, sticky, hard to swallow, syrup-like, viscous, starchy, lingering, mouth-coating, glue-like, gloopy, thick, claggy, clarty, **thick, mouth-coating, sticky, cloying**
Aftertaste	
Attributes generated	Musty, rubbery, bitter, smokey, lingering, cardboard, pharmaceutical, salty, medicinal, harsh

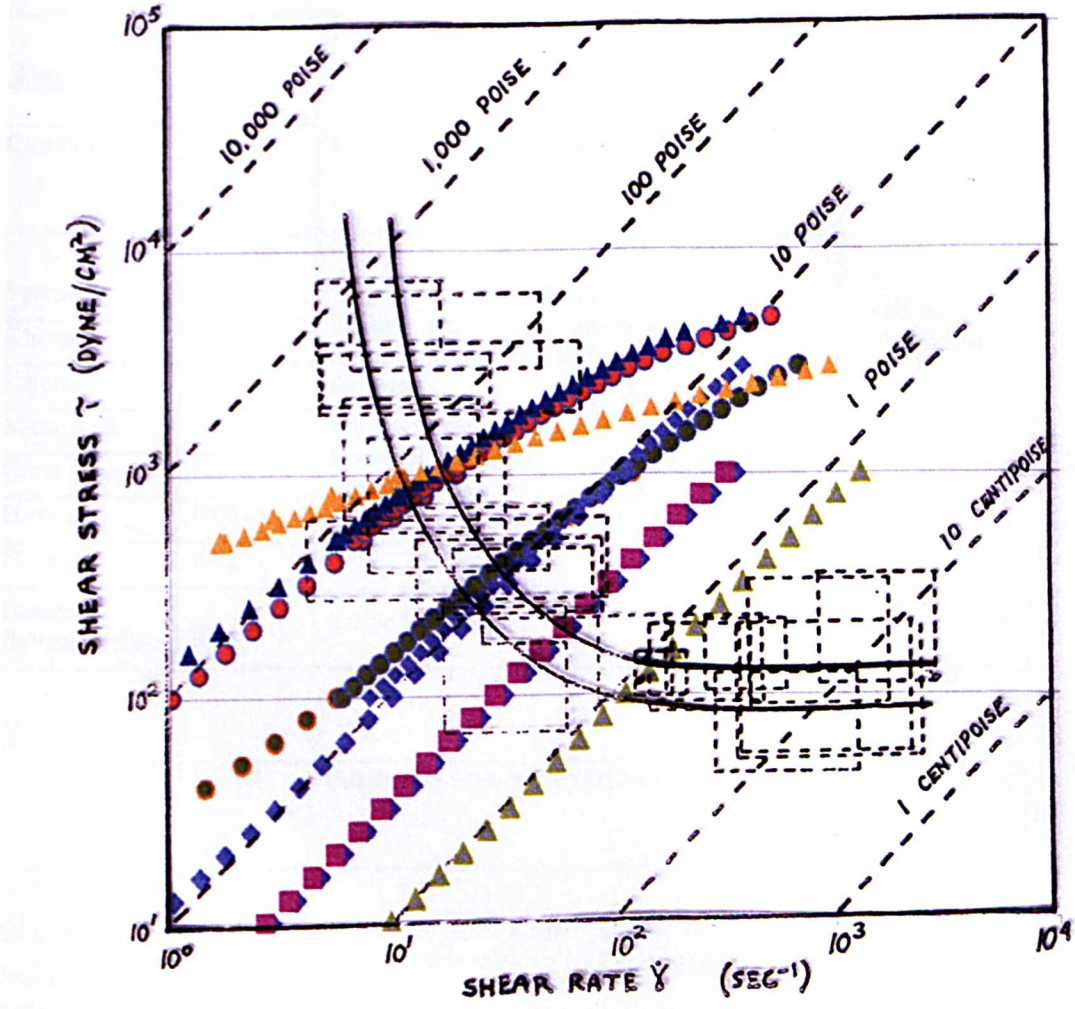


Figure 6-1. Figure showing my data superimposed on the curved space defining the limits of oral viscosity evaluation within the shear stress-shear rate plane according to Shama and Sherman (1972).

Table 6-5. Modification of concentrations used to make the five banana samples based on comments from bench testing

Sample I:

Component	Initial conc (g/L)	Comments from bench testing	Final conc (g/L)
Sucrose	10	Slightly too "green", but reducing the green hexenal and hexanal levels may result in it losing its unripe character, so levels were kept the same. Slightly bland/weak in overall flavour. However, an unripe banana does have little overall flavour, compared to the more ripe versions, so it was thought that this didn't need to change. Good astringency very reminiscent of a real unripe banana.	Same as initial concentration
Glucose	0		
Fructose	0		
Malic acid	0.4		
Citric acid	0.2		
Hexenal	0.0315		
Hexanal	0.021		
Banana flavour	0.05		

Sample II:

Component	Initial conc (g/L)	Comments from bench testing	Final conc (g/L)
Sucrose	20	Too acidic (but it may become too sweet if reduce acid levels), so acid levels not changed. Good astringency	Same as initial concentration
Glucose	1.5		
Fructose	1		
Malic acid	0.5		
Citric acid	0.2		
Hexenal	0.016		
Hexanal	0.01		
Banana flavour	0.06		

Sample III:

Component	Initial Conc (g/L)	Comments from bench testing	Final Conc (g/L)
Sucrose	50	Too sweet, so sugar levels reduced.	35.5
Glucose	5	Lacking in aromatic profile - could include very low levels of unripe and ripe aromas, but this may make it more like a mixture of an unripe and ripe flavour, rather than a mid-ripe flavour, so aroma profile kept the same	2.5
Fructose	4		2
Malic acid	0.6		0.6
Citric acid	0.2		0.2
Banana flavour	0.07	Seems much riper than B (not evenly spaced), but it was thought thag reducing sugar levels would help correct this	0.07

Sample IV:

Component	Initial conc (g/L)	Comments from bench testing	Final Conc (g/L)
Sucrose	60	Too sweet, so sugar levels reduced Too ripe, but it was thought that reducing the sugar levels would help correct this	45
Glucose	9		7.5
Fructose	9		7.5
Malic acid	0.7		0.7
Citric acid	0.2		0.2
IBA	0.025		0.025
IPA	0.025		0.025
Banana flavour	0.08		0.08

Sample V:

Component	Initial conc (g/L)	Comments from bench testing	Final conc (g/L)
Sucrose	54	Very short in terms of persistence of flavour – with a real ripe banana, flavour lingers slightly more. Possibly add a sweetener - sweetness lingers, may help whole flavour to linger. But sweeteners have undesirable side-effects such as imparting bitterness, so were thought to not be appropriate for use here.	50
Glucose	16.2		15
Fructose	13.5		12
Malic acid	0.8		0.8
Citric acid	0.2		0.2
IBA	0.05	Also a bit too sweet, so sugar levels reduced	0.05
IPA	0.05	Similar ripeness to D (spacing not equal), but evening out the sugar levels was thought would help this	0.05
Banana flavour	0.09		0.09

6.1. Flavourist's advice on how to improve the flavours for potential dish development

The flavourist Jack Knight was provided with the five banana flavours of different ripeness, prepared at concentrations outlined in Table 4-6 in the medium of bottled water (Evian). He made the following comments on how their quality could be improved.

Table 6-6. Table outlining the recommendations from the flavourist to make the banana flavours more authentic

Advice	Recommended quantities	Reasons justifying advice
Replace the aldehydes hexenal and hexanal with their equivalent alcohols - cis-3-hexenol and hexylalcohol in the unripe sample	Alcohols are a tenth of the strength of the aldehydes, so should be added at 10 times the concentration of the equivalent aldehydes	Aldehydes can have quite a fatty character
Replace isoamylacetate with either isoamylvalerate, or with a 80:20 IAA:IAB (isoamylbutyrate) mix in the ripe sample	The same quantity as IAA	To broaden the flavour profile of the ripe samples. Both isoamyl acetate and isoamyl butyrate have already been identified in banana aroma (Salmon and Martin 1996)
Add small amounts of eugenol to all banana samples at the same level	At a concentration in between their recognition and detection threshold, to prevent the banana samples from actually having the flavour characteristics elicited by the compounds	To improve their authenticity - eugenol has previously been quantified in the banana at low levels (Mayr, Mark et al. 2003).
Add small amounts of maltol to the ripe banana samples	As for eugenol	To give them a certain roundness and authenticity that isn't achieved solely from the addition of esters[1]
Add small amounts of furaneol to the ripe banana samples	As for eugenol	For the same reasons as for maltol
Add small amounts of mandarin oil extract to the ripe samples	As for eugenol	Mandarin oil is often used by flavourists when developing fruit flavours – it is a natural product and helps enhance fruit flavours.

[1] As a banana ripens, it is thought that there would be an increase in volatile sugar degradation products, as an attractant for seed distributors. Although nowadays bananas are propagated by root offsets, this was unlikely to have always been the method and probably doesn't apply to all plantains (Jack Knight, personal communication).

6.2. Flavour development when Angel Delight was used as the matrix

Milk solutions I, II and IV were prepared according to the concentrations for I-V outlined in Table 4-6, and then unflavoured Angel Delight (AD) base (Premier Foods, St Albans, UK) was added to each at a final concentration of 23% w/w, according to the manufacturer's instructions. The mixture was blended using a hand held whisk (250W, Kenwood, Havant, UK) equipped with stainless steel beaters for 30 seconds on the lowest speed setting.

The three different flavoured Angel Delights (*AD I, III and V*) were sampled, and bench testing was used to develop and improve their authenticity, as outlined in Table 6-7.

Table 6-7. Process of modification and development of flavours for the unripe, midripe, and ripe banana flavours in the matrix of angel delight. Quantities are expressed in g/L.

Unripe (*AD I*)

Compound	Flavour *I* (as in Table 4-6)	1st modification	2nd modification	3rd modification	**Final modification**
Sucrose	10				
Malic acid	0.4	0.4	1.2	1.2	**1.2**
Citric acid	0.2	0.2	0.6	0.6	**0.6**
Hexenal	0.0315	0.0315	0.0315	0.063	**0.06**
Hexanal	0.021	0.021	0.021	0.042	**0.042**
Banana flavour	0.05	0.05	0.05	0.1	**0.1**
Comments	Still quite unripe, but too sweet Quite appley?	Better without sugar, but still pretty similar Green; but not very bananry	Best But needs more banana flavour - up bananary notes Up the green aromas	Seems unripe A little too green? Too much HexE?	**Reminiscent of unripe banana**
Action to take	Remove added sugar	Try adding acid	Try doubling all aroma levels	Try reducing HexE levels	

Midripe *(AD III)*

Compound	Flavour *I* (as in Table 4-6)	1st modification	2nd modification	3rd modification	**Final modification**
Sucrose	35.5	12.8	12.8	12.8	**12.8**
Glucose	2.5	1.2	1.2	1.2	**1.2**
Fructose	2.0	1	1	1	**1**
Malic acid	0.6	0.6	0.6	0.6	**0.6**
Citric acid	0.2	0.2	0.2	0.2	**0.2**
Banana flavour	0.07	0.07	0.1	0.14	**0.11**
Comments	Green note comes through (flavour A?) Too sweet A bit synthetic? (flavour A?) Bland/not very bananary	Better with less sugar Still a bit subtle - needs a bit more banana	Still quite bland, making it more reminiscent of an unripe banana which is lacking in flavour	Not as good	**Reminiscent of mid-ripe banana**
Action to take	Reduce sugar	Try increasing flavour A	Try further increasing flavour A	Reduce flavour A slightly	

Ripe *(AD V)*

Compound	Flavour *I* (as in Table 4-6)	1st modification	2nd modification	3rd modification	4th modification	**Final modification**
Sucrose	50	60	50	50	50	**52**
Glucose	15	20	15	15	15	**16**
Fructose	12	16	12	12	12	**13**
Malic acid	0.8	0.8	0.8	0.8	0.8	**0.8**
Citric acid	0.2	0.2	0.2	0.2	0.2	**0.2**
IBA	0.05	0.05	0.07	0.07	0.07	**0.07**
IAA	0.05	0.05	0.07	0.05	0.06	**0.06**
Banana flavour	0.09	0.09	0.12	0.12	0.12	**0.12**
Comments	Bananary Slightly synthetic Could be sweeter? Still quite synthetic Needs more aroma and flavour A?	Far too sweet	Effective, ripe. Artificial at the beginning, but afterwards very like a ripened banana. Bit too strong IAA.	Not as good with less IAA	Better with this IAA level Could be a tiny bit sweeter (since this is the sweetest sample)	**Reminiscent of ripe banana**
Action to take	Try increasing sweetness	Try increasing aroma levels and flavour A	Try less IAA.	Add a bit more IAA	Add a little more sugar	

The concentrations used in the final modifications of the 3 flavours *AD I, III* and *V* (as shown in Table 6-7) were used to predict concentrations for the intermediate banana flavours *II* and *IV* in the matrix of angel delight. The concentrations outlined below (see Table 6-8), when present in the milk used to make each angel delight product, produced five samples that were perceived by bench testing to increase linearly in ripeness.

Table 6-8. Concentrations of tastants and aromas added to the milk used to prepare angel delight samples of increasing banana ripeness flavour. Quantities are expressed in g/L.

Compound	I	II	III	IV	V
Sucrose	0	0	12.8	30	52
Glucose	0	0	1.2	8	16
Fructose	0	0	1	6	13
Malic acid	1.2	1	0.6	0.7	0.8
Citric acid	0.6	0.4	0.2	0.2	0.2
IBA	0	0	0	0.04	0.07
IAA	0	0	0	0.04	0.06
Hexenal	0.06	0.04	0	0	0
Hexanal	0.042	0.02	0	0	0
Banana flavour	0.1	0.12	0.11	0.11	0.12

The prototype was also coloured to improve the presentation and the ripening experience, as shown in the photo (see Figure 6-2).

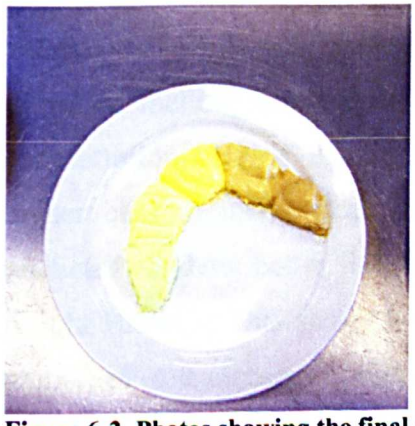


Figure 6-2. Photos showing the final coloured angel delight prototype

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