Relationship between texture of gels and flavour release.

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ABSTRACT

To provide further insight into the relationship between the structure of hydrocolloid solutions and gels and perception of taste and flavour, solutions of gelatin and locust bean gum, and gels prepared from mixtures of (a) high acyl and low acyl gellan (b) carrageenan and locust bean gum were studied. Both solutions contained sodium chloride and the gels were flavoured with ethyl butyrate.

The gels were classified from rheological measurements into 3 categories: strong/brittle, intermediate and soft/elastic. Volatile release was measured by monitoring nose space volatile concentration during consumption using Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS). In addition headspace measurements were performed with APCI-MS. The headspace concentrations did not exhibit significant differences between the gels systems but the release of ethyl butyrate in-nose was affected by the matrix, showing a higher intensity for the more brittle gels containing high levels of low acyl gellan. The release of Na^+ following a two bite compression was monitored by the use of an ion specific electrode. The more brittle gels containing high levels of low acyl gellan and high amount of κ-carrageenan exhibited significantly higher release of Na^+. Strain at break correlated inversely with salt release (r^2=-0.87) and nose space volatile concentration (r^2=-0.55).

In a later stage gelatin was added (1-5%) in the previous mixtures of HA-Gellan and LA-gellan (constant polysaccharide concentration of 0.6%). The rheological analysis of the gels yielded different behaviour of the gels. At low levels of LA-gellan the rheological data can be explained by polymer blending laws. At higher levels of LA-gellan, development of elastic behaviour from the previous brittle gels observed does not fit polymer blending law theory. Flavour release during diffusion experiment showed that at 37°C the gels containing gelatin exhibited higher salt release. Temperature sweeps have shown that a drop of G′ is observed around 27-28°C indicating that the gelatin present in the mixture is melting. However the level of the drop of G′ indicates that the continuous phase of the gel composite was the gellan system. Volatile release was measured by monitoring nose space volatile concentration during consumption using Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) but showed no significant differences between the different gels. Headspace experiments performed at different temperatures showed that the gels containing high amounts of gelatin when compared to control gels that contained 0% gelatin exhibited higher release of ethyl butyrate.

To mimic the mixing of gelatin with saliva after melting, gelatin solutions at 50°C containing salt were mixed with water. Even at high concentrations (30%) of gelatin mixing efficiency and release was very efficient. In contrast when locust bean gum solutions containing salt were mixed with distilled water it was found that both salt release and mixing efficiency decreased at polysaccharide concentrations above c*.

It is concluded that the intensity of flavour perception in hydrocolloid solutions and gels is dominated by the release of the tastant. In solutions this is favoured by good mixing behaviour between the hydrocolloid solution and saliva and in gels by a low strain at break. A gelatin replacement should not only show melt in the mouth behaviour but good mixing between the melted gelatin and saliva.

It was shown that thermal processing at 121°C induced deacetylation of HA-gellan at much lower pH than would normally be needed in a typical deacetylation process. Therefore new textures can be achieved through deacetylation through heat processing. When gels which were prepared by deacetylation by heat processing were compared with blends that had an equivalent acyl content different textures were obtained even though the Young's modulus was very similar at the same total acyl content.

The implications of this work for gelled petfood products is discussed

ACKNOWLEDGMENTS

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My family deserve a big thank you as well for their continuous love and support. During my time in Nottingham I have made numerous good friends which have all made my life enjoyable and fun but listing them all

will be difficult and bad for the environment so a big thanks to all of them (you know who you are).

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Introduction

The present work has been funded by Mars. Several of the company's major petfood brands are delivered wholly or partly as gelled products. The main objective of the work presented in this thesis was to further understand the mechanical properties of current and potential gelling systems for petfoods and to investigate the relationship between the textural properties and flavour release. The hypothesis that was driving this project was related to a textural parameter, brittleness and was: ''Do brittle gels give a better flavour release?

The first objective was to take a simplified version of the gel used in the final product and develop protocols for a robust physical description of gel texture and afterwards characterize mixed gel systems in terms of the parameters defined. The gelling systems chosen were κ-carrageenan and locust bean gum (LBG), which is a similar system as to the one currently used by the petfood industry and also mixtures of low acyl (LA) and high acyl (HA) gellan gum which offers great potential due to their wide range of textures as well as the possibility of gum production on site. In addition to this, some experiments were carried out in collaboration with another

project funded by Mars that looked into the effect of heat sterilization on HA-gellan.

After achieving various textures by altering the proportions of the components in the gelling systems used, the next step was to check whether a correlation between the texture and flavour release existed. The hypothesis that brittle gels give better flavour release drove the work, as will be explained further later on. Flavour can be broken down into aroma which is perceived by the olfactory epithelium in the nose and taste which is perceived by the taste buds on the tongue. Therefore the approach was twofold. (a) Gels were spiked with ethyl butyrate and the head space and nosespace were measured. (b) Salt was added to the system and the salt release was monitored during a two bite Texture Profile Analysis experiment (TPA).

The gel formed in a petfood product has a third potentially important component. During heat processing gelatin leaks out from the meat chunk into the gel network. Gelatin is renowned for its excellent textural and flavour properties and it could give some advantageous properties to gelled petfood, so the work was extended to include gelatin and to investigate the effect of gelatin on texture and flavour release.

Chapter 1

Literature Review

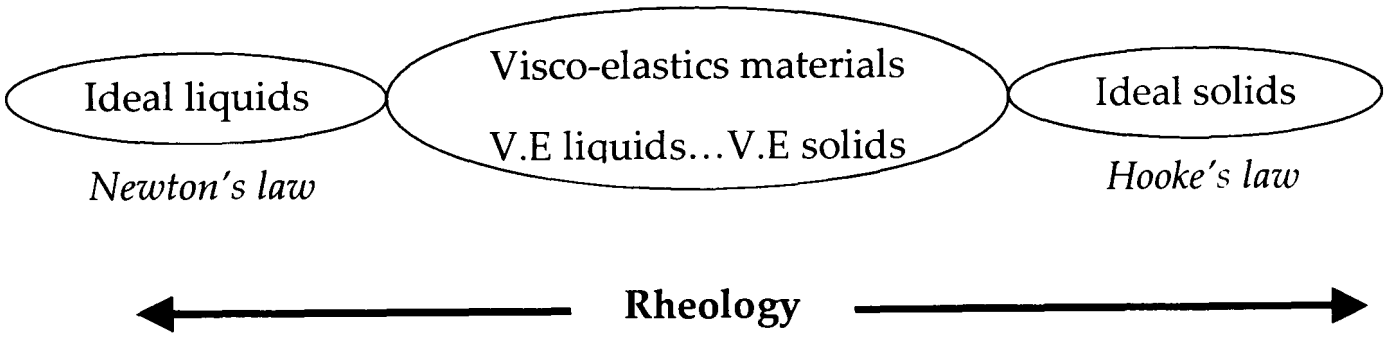
1.1 Introduction

It is essential to this study to measure the textural characteristics of different polysaccharide systems and then establish whether any correlations between the texture and flavour release do exist. In order to characterise the texture of the gels and solutions an overview of the rheology involved will precede the description of the physicochemical properties of the polysaccharides used.

1.2 Introduction to rheology

An important part of this thesis involves the rheological characterisation of gels therefore the basic concepts will be discussed here. First of all a brief overview of the rheology of solutions will be discussed, however since the majority of the work involved gelled samples the rheology of gels will be covered in more depth.

Continuum mechanics distinguish fluids mechanics and solids mechanics As it will be described below, ideal fluids are described by Newton´s Law and ideal solids by Hooke´s law. However, many materials are visco-elastic, exhibiting properties intermediate between ideal solids and ideal liquids. Rheology is defined as the science of the deformation and flow of materials when a stress is applied. Central to the subject is the relation between stress and strain.



1.2.1 Stress and strain definition

Stress is determined as the force applied per unit area. Stress is expressed by equation 1 and its unit is Pascals (Pa).

$$stress = \frac{F}{A} \qquad \text{(Equation 1)}$$

stress obtained when a force F is uniformly applied over a body with an area A.

Stress can be applied in tension/compression or shear. Films are frequently studied in extension. Solid foods are commonly studied in compression although small deformation measurements are also made in shear. Liquids

are almost always studied in shear. To avoid confusion, the symbol used is the greek lower case σ (sigma) for stress applied in tension and compression tests and Greek lower case τ (tau) for stress applied in shear conditions.

Tensile and shear stress are depicted in Figure 1

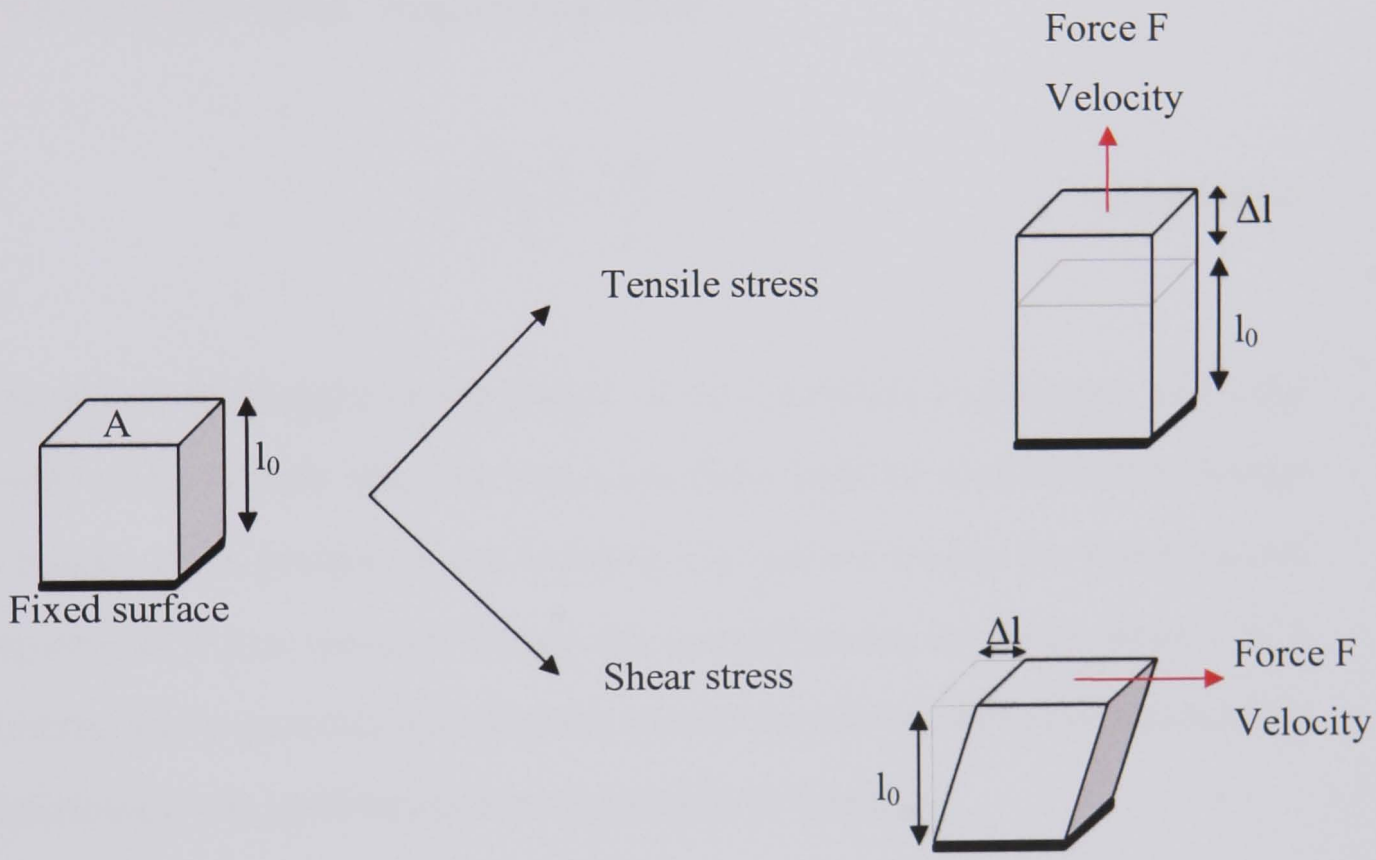


Figure 1. Tensile stress and shear stress applied to a cubic volume of material and corresponding deformation

Strain is the result of stress applied to a material and refers to the change in size or shape of the material. Strain is denoted by the Greek letter ε

(epsilon) when in tension or compression or the letter γ (gamma) when a shear stress is applied.

1.2.1.1 Strain in tension and compression

In compression or tension there are two ways to describe strain. Equation 2 defines a strain called "engineering strain".

$$\varepsilon = \frac{l_0 - l}{l_0} = \frac{\Delta l}{l_0} \qquad \text{(Equation 2)}$$

Where l_0 is the height or the length of the unstressed specimen and l the height or the length after the stress has been applied and Δl is the change in height. Δl is positive if the material has gained length (in tension) and negative if it has reduced length (in compression), however since ε is a dimensionless quantity the absolute values may be quoted and stated if the experiment was performed in compression or tension.

True strain or Hencky strain accounts for the fact that a given degree of incremental stretching or compression will have a different incremental strain as the extension of compression of the sample continues. Hencky strain is defined in Equation 3.

$$\varepsilon_T = \int d\varepsilon = \int_{l_0}^{l_f} \frac{dl}{l} = \ln \frac{l_f}{l_0} \qquad \text{(Equation 3)}$$

Hencky strain (Equation 3) is linked to engineering strain (Equation 2) by the following relationship:

$$\varepsilon_T = \ln\frac{l_f}{l_0} = \ln\frac{l_0 + \Delta l}{l_0} = \ln(1+\varepsilon) \qquad \text{(Equation 4)}$$

Finally it must be noted that the true strain and engineering are virtually indistinguishable at small deformations.

1.2.1.2 Strain in shear

A similar expression of the strain in shear can be established:

$$\gamma = \frac{\Delta l}{l_0} \qquad \text{(Equation 5)}$$

where Δl is the deformation and l_0 the distance between top and bottom surfaces as depicted in Figure 2.

1.2.1.3 Strain rate

The strain rate is the change in strain over the change in time and is denoted as $\dot{\gamma}$ and $\dot{\varepsilon}$. It is expressed in units of reciprocal seconds (s^{-1}).

In shear $$\dot{\gamma} = \frac{\partial \gamma}{\partial t} = \frac{v}{l_0}$$ (Equation 6)

In tension $$\dot{\varepsilon} = \frac{\partial \varepsilon}{\partial t} = \frac{v}{l_0}$$ (Equation 7)

where l_0 the original length for tensile tests and v the speed of deformation as depicted in Figure 2. Therefore, the strain rate is not only the rate of deformation with time but also the velocity gradient established in a fluid as a result of an applied stress.

If a shearing force is applied over an area A of the surface of a fluid in contact with a flat stationary plane then the upper layer of the fluid will move with a velocity v, and then it will drag the layer immediately below with a slightly slower velocity and so on through the layers and a velocity gradient is set up within the fluid (Lewis, 1996). If this velocity gradient does not change with position then the shear rate in the sample will be constant.

1.2.2 Strain-stress relationships for ideal materials

Strain-stress relationships exist for shear and elongational stress, for both ideal solids and ideal liquids (Macosko, 1994).

The relation between force and deformation for ideal solids is described by Hooke's law: the force is proportional to the deformation or

In shear : $\tau=G\gamma$ (Equation 8)

In tension : $\sigma=E\varepsilon$ (Equation 9)

where τ (resp. σ) is the stress in shear (resp. tensile) tests and γ (resp. ε) the strain in shear (resp. tensile). The constant of proportionality for shear test is G, called the elastic modulus and E for tensile tests and called the Young's modulus.

Young's and elastic modulus, both determining the elasticity of a material in tensile and shear tests respectively are related by the equation 10.

$$E=2\,G\,(1+\mu) \qquad \text{(Equation 10)}$$

where μ is the Poisson's ratio. For incompressible and isotropic materials, Poisson ratio equals 0.5 and thus the tensile modulus is three time that measured in shear (Equation 11).

$$E=3\,G \qquad \text{(Equation 11)}$$

For the liquids, the simplest equation is Newton's law of viscosity, indicating that the stress is proportional to the strain rate (in shear):

$$\sigma = \eta\dot{\gamma} \qquad \text{(Equation 12)}$$

where η, the constant of proportionality is the viscosity.

As stated earlier viscosity is almost always measured in shear, though there is an increasing interest in measuring extensional viscosities. The recent advances in the development of filament stretching extensional rheometers has made possible the acquisition of data regarding the extensional viscosity (Tirtaatmdja and Shridar, 1993; Spiegelberg et al., 1996a,b). The prevalence of extensional flow in most processing methods, coupled with the inability to obtain the extensional properties from shear rheometry experiments, emphasizes the need to perform extensional rheometry. A relationship between shear viscosity and extensional viscosity similar to Equation 10 can be found, but further discussion on this does not fall within the scope of the current document.

The law governing viscous liquids and elastic solids will be described separately below. However, it is important to mention that the material behaviour observed is extremely dependant on the time scale of observation. For example, mountains and rocks do behave like fluids if the time frame is million of years, and liquid water behaves as a solid at a time scale of millisecond.

1.3 Viscous behaviour and polymers rheology

Viscosity can be defined as the internal friction between the molecules of a material, i.e. its resistance to flow (Lewis, 1996). As will be discussed in Chapter 4 viscosity of liquids is a very important parameter that can actually be used to predict the behaviour of solutions in mouth and how that can impact on flavour release.

Viscosity is also a measure of the rate of flow. For example water which has very low viscosity will flow easily out of a glass, whereas honey will flow but with more difficulty and some other fluids will not flow in a reasonable time scale. For ideal viscous liquids, the viscosity is expressed in Newton's law as the coefficient of proportionality between the shear stress and the stress rate (see Equation 12) and is in Pa.s in the SI Units. It is also expressed in centipoises (cP, 1cP=1mPa.s).

In this section a more focused description on the factors that affect the viscosity of hydrocolloid solutions will be discussed.

1.3.1 Viscosity definitions

When hydrocolloids are dissolved in a solvent (water for example) the viscosity of the solution (η) is always higher than that of the solvent (η_s). The ratio of solution viscosity to solvent viscosity is called the relative viscosity (η_{rel}, see Equation 13).

$$\eta_{rel} = \frac{\eta}{\eta_s} \qquad \text{(Equation 13)}$$

Another associated term, specific viscosity η_{sp} describes the fractional increase in viscosity upon addition of polysaccharide:

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} = n_{rel} - 1 \qquad \text{(Equation 14)}$$

For very dilute solutions the increase in η_{sp} and η_{rel} with increasing concentration can be described by the Huggins and the Kramer equations:

Huggins equation $\eta_{sp} = [\eta]c + K'[\eta]^2 c^2$ (Equation 15)

Kramer equation $\ln(\eta_{rel}) = [\eta]c + (K' - 0.5)[\eta]^2 c^2$ (Equation 16)

where K′ is the Huggins coefficient.

The parameter [η] is called intrinsic viscosity. It is often obtained by a double extrapolation to the zero concentration of Huggin's and Kramer relationships (Equation 17, Morris et al., 1981).

$$[\eta] = \lim_{c \to 0}\left(\frac{n_{sp}}{c}\right) = \lim_{c \to 0}\left(\frac{n_{rel}}{c}\right) \qquad \text{(Equation 17)}$$

The unit of intrinsic viscosity is volume per unit weight, cm^3/g. The intrinsic viscosity is not a true viscosity measure but a measure of the hydrodynamic volume occupied by the isolated polymer chains in a given solvent divided by the molecular weight. It depends primarily on the molecular structure (linear vs branched, rigid vs flexible) and molecular weight of polysaccharides as well as on the solvent quality (Launay et al., 1986). However it does not depend on polymer concentration like specific viscosity or apparent viscosity (Cui, 2005).

1.3.2 Viscosity dependences

1.3.2.1 Shear rate dependence of viscosity

According to Newton's law for an ideal viscous liquid, the applied stress is proportional to the rate of shear strain but is independent of the strain which means that the viscosity defined by Equation 12 is independent of shear rate or shear stress. Many biopolymer solutions do not obey this condition and are called non-Newtonian fluids where the viscosity is shear-rate dependent. There are three classes of non-Newtonian fluids: shear-thinning, shear thickening and plastic (see Figure 2).

For biopolymers solutions, the viscosity of the solution generally decreases with increasing shear rate, particularly at higher concentrations and the solution is then termed shear-thinning. The situation where the solution viscosity increases with increasing shear rate is termed as shear thickening and is much rarer for food thickeners (Lapasin and Pricl, 1999). For a plastic fluid, a minimum shear stress is required for flow to begin which is known as yield stress. As soon as the yield stress is exceeded, the liquid usually shows shear thinning behaviour (Cui, 2005).

As the viscosity is dependant on the shear rate, the limit value η_0 of the viscosity when the shear rate tends to zero is often extrapolated and named zero-shear viscosity (Lapasin and Pricl, 1995).

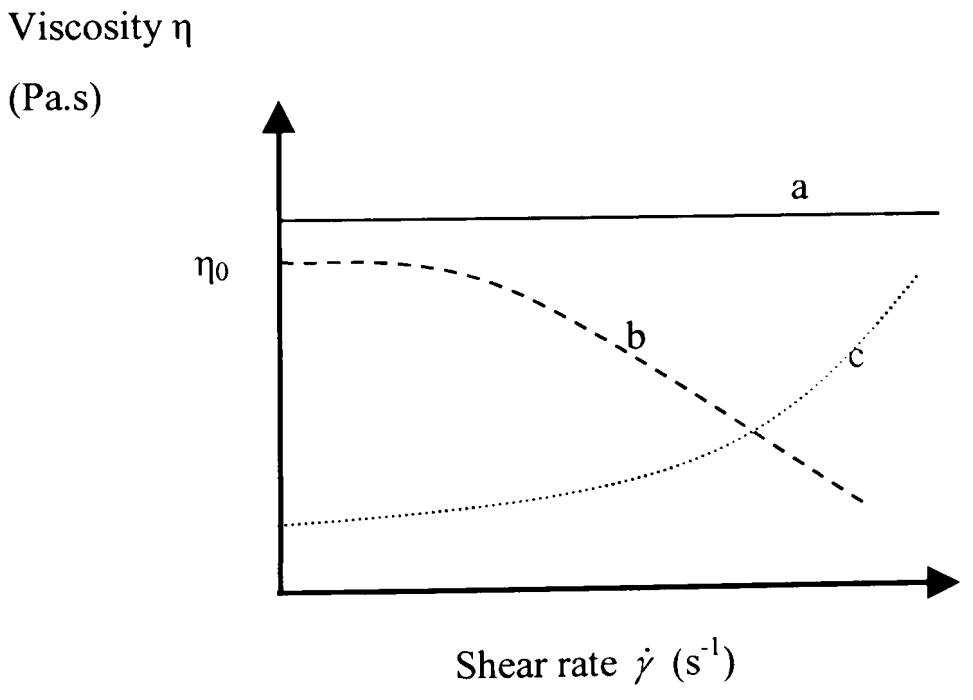


Figure 2. Flow curves (viscosity as a function of shear rate) for (a) Newtonian, (b) shear-thinning, (c) shear thickening.

The degree of shear thinning of a polysaccharide solution depends on the intrinsic molecular characteristics including conformation, molecular weight and charge for anionic polysaccharides. Extrinsic factors such as concentration, temperature and pH may also influence flow properties. Stiff polysaccharide chains are more pseudoplastic. The degree of pseudoplasticity increases with the polysaccharide concentration and molecular weight (Porter and Johnson, 1963). The origin of the concentration dependence of the shear thinning behaviour will be studied later.

1.3.2.2 Time dependence of viscosity

For some non-Newtonian liquids flow viscosity may depend on the length of flow time and flow history. In general two types of flow can be distinguished thixotropic and rheopectic (Lewis 2002). In a thixotropic fluid when a constant shear rate is applied, the viscosity decreases with time until it reaches a stationary state. In a rheopectic fluid the viscosity gradually increases with time. Rheopectic behaviour is not commonly observed in polysaccharide systems used in food applications (whipping cream could be an example), thixotropic behaviour is often observed in disperse systems where the dispersed phase is made up of molecular aggregates. The application of shearing conditions results in a gradual reduction of aggregate dimensions, leading to a progressive decrease in viscosity with time. Thixotropy is a reversible phenomenon. The viscosity returns to its original value after cessation of shear with a delayed period rather than instantaneously. Thixotropy is usually associated with shear

thinning behaviour. To detect thixotropy viscosity measurements are first made at increasing shear rate followed by decreasing shear rate. Because at a given shear rate the sample will be sheared for a longer time in the decreasing shear rate part of the experiment its viscosity will be lower. In many polysaccharide solutions thixotropy is a result of strong interchain association through hydrogen bonding (Cui, 2005).

1.3.2.3 Temperature and pressure dependence of viscosity

Viscosity is temperature and pressure dependent. For all liquids, viscosity decreases with increasing temperature and decreasing pressure (Macosko, 1994). Therefore it is very important to control the experimental temperature and also to state the temperature when making and reporting measurements (Whorlow, 1992, Bourne 2002).

Looking into the effect of temperature on LBG solutions as studied by Kok et al (1999). the viscosity was governed by three parameters:

- Reversible decrease in viscosity with increasing temperature due to increasing macromolecular motion
- Increase in viscosity due increased solubilisation
- Loss of viscosity due to a decrease in molecular weight as a result of thermal degradation.

1.3.2.4 Concentration

The viscosity increases with increasing concentration of the hydrocolloid. In a double logarithmic plot of zero shear rate viscosity against the concentration, a specific critical concentration c* can be identified, above which there is a significant increase in gradient (Figure 3). The slope, prior to c*, when n_0 is plotted against concentration on a double-logarithmic graph has been reported to be around 1.3 (Morris et al., 1981). As the concentration increases a critical point is reached (c*) and the slope increases abruptly to a value typically around 3.3. See Figure 3.

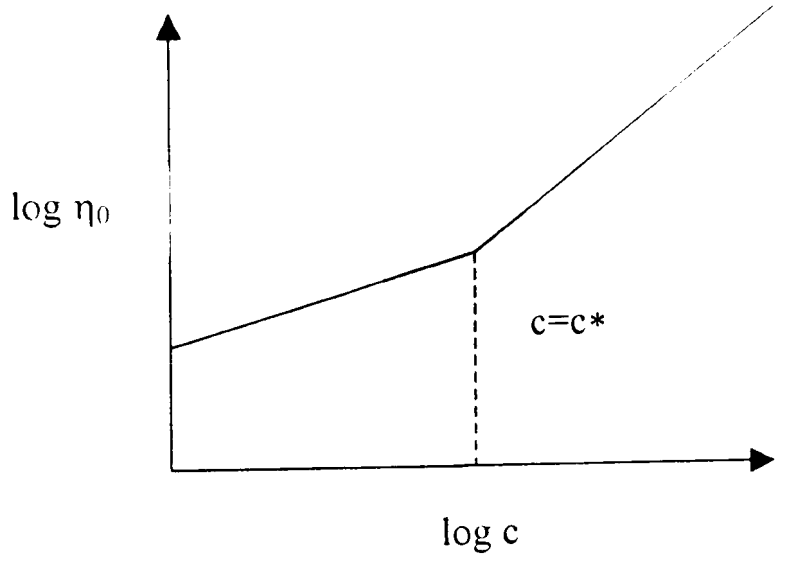


Figure 3. The concentration dependence of zero shear viscosity (η_0) for a typical "random coil' hydrocolloid (Morris et al., 1981)

The c* transition will be discussed further in section 1.3.3.1. The concentration relative to c* has been investigated regarding its effect on flavour release and suppression. Baines and Morris (1987) investigated sweetness and strawberry flavour perception in solutions thickened with

guar gum. They concluded that the key determinant of flavour suppression in such systems was the thickener concentration relative to its coil-overlap concentration. This will be discussed further in Chapter 4.

1.3.3 Entanglement of polymer solutions

Some polysaccharides molecules, such as xanthan, are much stiffer and their behaviour in solution is modelled by rigid rods. However, the majority of polysaccharides behave like random coils.

Polysaccharides of sufficiently high molecular weight and concentration can form non specific physical entanglements, creating a dynamic network due to Brownian motion. The viscosity of an entangled polysaccharide network is proportional to the extent of entanglements (expressed by the average number of entanglement points per molecule), which in turn is proportional to the chain length or molecular weight. Therefore for a given polysaccharide, the viscosity of a semi-dilute solution also increases with molecular weight (Cui, 2005). The dependence of the viscosity upon shear (shear thinning behaviour) and concentration of polymer can thus be explained at the molecular level, considering polymer chains as random coils.

1.3.3.1 C*

The schematic representation of the polymer chains in solutions for concentrations below and above c* is depicted in Figure 4. For concentrations below c*, the solution is defined as in the "dilute regime".

At the critical concentration, the chains of the polymer are beginning to overlap in solution. Above the critical overlap concentration, the solution is defined as in the "semi dilute regime". The total hydrodynamic volume of the individual chains exceeds the volume of the solution. The freedom of molecular movement is dramatically reduced and consequently this induces a sharp increase in solution viscosity as mentioned previously.

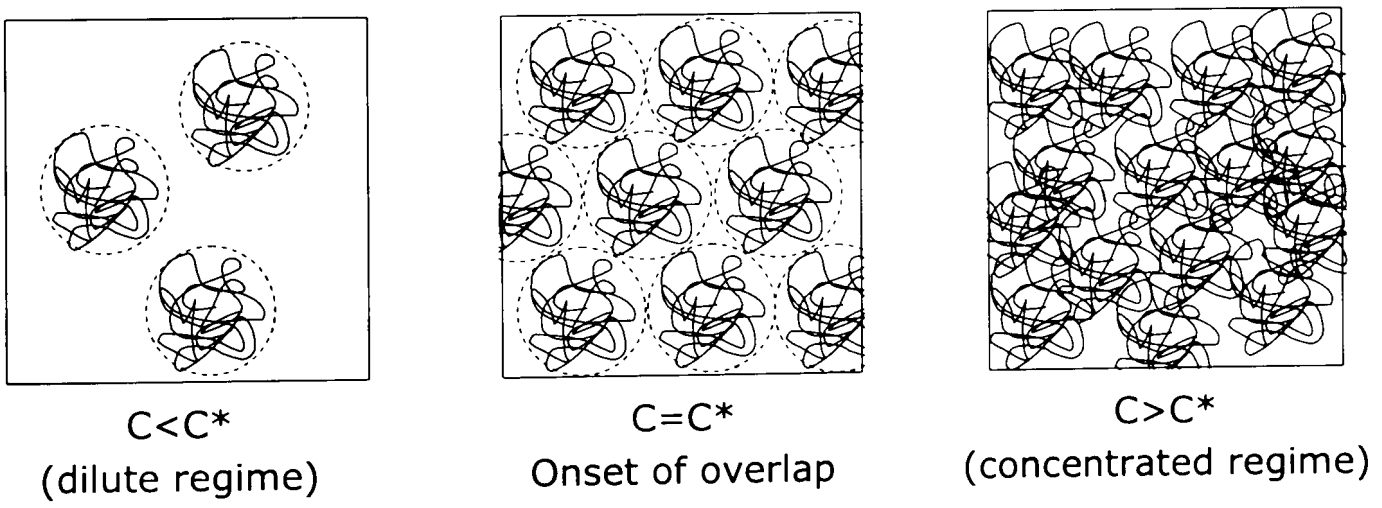


Figure 4. Polyssacharide behaviour at concentration <, = and > C* (Lapasin and Pricl, 1999)

1.3.3.2 Alignment with flow of polymer chains

For some polysaccharides especially the random coil type, or highly branched globular type, dilute solutions under shear show essentially Newtonian behaviour therefore their viscosity is independent of shear rate. However as mentioned previously polysaccharides solutions often exhibit shear thinning behaviour. This is particularly seen at concentrations above c*. The shear thinning behaviour of a semi-dilute solution is caused by the decreasing number of chain entanglements between the chains as the shear rate increases. At high shear rates, the newly formed entanglements cannot compensate for those being disentangled, thus viscosity decreases (Cui, 2005). In dilute solutions below c* a decrease in viscosity can be observed through alignment of linear molecules. For random coils this can be a consequence of a change in shape as illustrated in Figure 5.

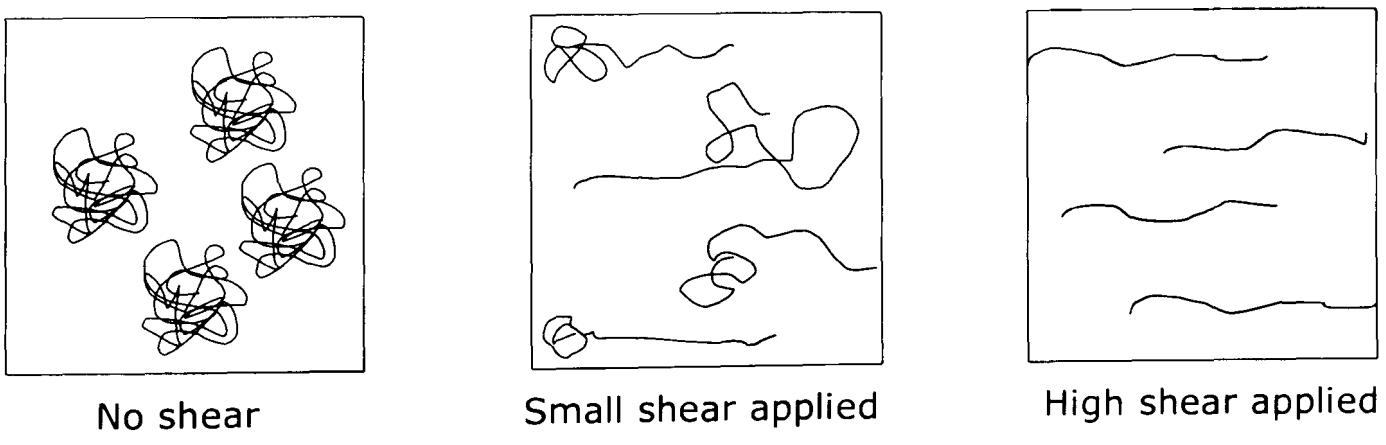


Figure 5. The shear dependence of the conformation in a dilute solution of a random coil polymer.

1.4 Visco-elastic properties and solids rheology

Like liquids, solids are also deformed by the application of forces. In this section the rheology of gels will be discussed. Understanding the way that gels break up is very important due to the correlations that may arise with consumer acceptance of a particular product, correlation between particular textures and improved mouthfeel and flavour release.

In the mastication process the forces that a food is subjected to are very complex. During that process the food is broken down, volatiles that are present are released and retronasaly perceived by the olfactory epithelium, tastant molecules are released, mixed with saliva and perceived by the taste buds on the tongue, various mechanical receptors are responding to the texture of the material and all of this information is converted to electric pulses (nerves) that are then processed by the CPU (brain) in order for us to decide whether we like a product or not. It is as complex as it sounds and it does involve many different disciplines to investigate all of this, however this study is focused on how the sample might break up in the mouth and how this will influence the flavour release in addition to the role of other textural/rheological parameters. Texture will be the key parameter determining to what extent the mechanical receptors will be stimulated, texture as we will demonstrate later will also drive the tastant release (consequently the tastant release will affect the taste perception) and finally texture may also have a role in the aroma release (not as strong as with taste). Texture therefore plays a fundamental role in driving perception of flavour.

Therefore it is very important to characterize gels in order to distinguish between various textures that are achievable and determining which ones are preferred for various products. All gels show viscoelastic behaviour and a complete rheological description requires the measurement of a number of parameters over time (Mitchell, 1976), but before looking into the viscoelasticity of gels a short review of the different rheological methods available will be given.

1.4.1 Viscoelastic behaviour of gels

The term viscoelastic for a material implies that it possesses both elastic properties of an ideal solid as well as some of the flow properties of an ideal liquid. Gels like all polymer systems show viscoelastic behaviour. This type of behaviour can be characterized by static experiments like the creep compliance experiment where a constant stress is applied and the strain is followed as a function of time (Mitchell, 1980). The results are expressed in terms of the creep compliance (J(t)) as shown in equation 18.

$$J(t) = \frac{strain(t)}{stress} \qquad \text{(Equation 18)}$$

An alternative static experiment which has been utilised in this study is stress relaxation experiment. In this type of experiment a constant strain is applied and the stress required to maintain this strain is measured over time. For an ideal elastic body, for example rubber, the stress will be

independent of time. The results are expressed in terms of the stress relaxation modulus G(t) as shown in equation 19 (Mitchell, 1980). Data obtained from stress relaxation can be subjected to further analysis as will be discussed in Chapter 2.

$$G(t) = \frac{stress(t)}{strain} \qquad \text{(Equation 19)}$$

1.4.2 Oscillatory methods

Viscoelastic materials can also be characterized by dynamic experiments, where a sinusoidally oscillating stress or strain is applied to the material (Figure 6).

G*, the complex shear modulus, expressed in Pascals (Equation 20) and is the ratio of the stress amplitude (σ_o) to the strain amplitude (γ_o).

$$G^* = \frac{\sigma_o}{\gamma_o} \qquad \text{(Equation 20)}$$

G*, the complex result of dynamic experiments can be broken down into 2 components, the real and the imaginary part. The real part of G* is G′, the storage modulus (in Pascals). It corresponds to the elastic (solid) behaviour of the material: G′ is the ratio of the stress component in phase with the strain/strain. The imaginary part of G* is G″, the viscous modulus or loss modulus and correspond to the viscous part of the behaviour of the

material (Mezger, 2006). G'' is thus the ratio of the stress component that is 90°out of phase with the strain/strain (Mitchell, 1980). The ratio of these two moduli is the loss tangent tanδ as defined in equation 21.

$$\tan\delta = \frac{G''}{G'} \qquad \text{(Equation 21)}$$

For a perfectly elastic material (Hookean solids) G'' will be zero and G' will equal the shear modulus G*. In contrast, for a perfectly viscous fluid, G' will be zero. Viscoelastic materials have both G' and G'' different from zero. For "liquid like" viscoelastic products, G''>G' whereas for "gel like" viscoelastic products, G'>G''.

It follows that for perfectly elastic material, that is also incompressible and hence Poisson's ratio is 0.5 then Equation 11 becomes Equation 22. For many materials, particularly soft polymers, volume changes are very small and it can be considered that Poisson's ratio equals 0.5 (Whorlow, 1992). Equation 22 gives a theoretical relationship between data from oscillatory shear tests and compression tests

$$E=3G' \qquad \text{(Equation 22)}$$

where E is the Young's modulus and G' the storage modulus as obtained from oscillatory shear tests.

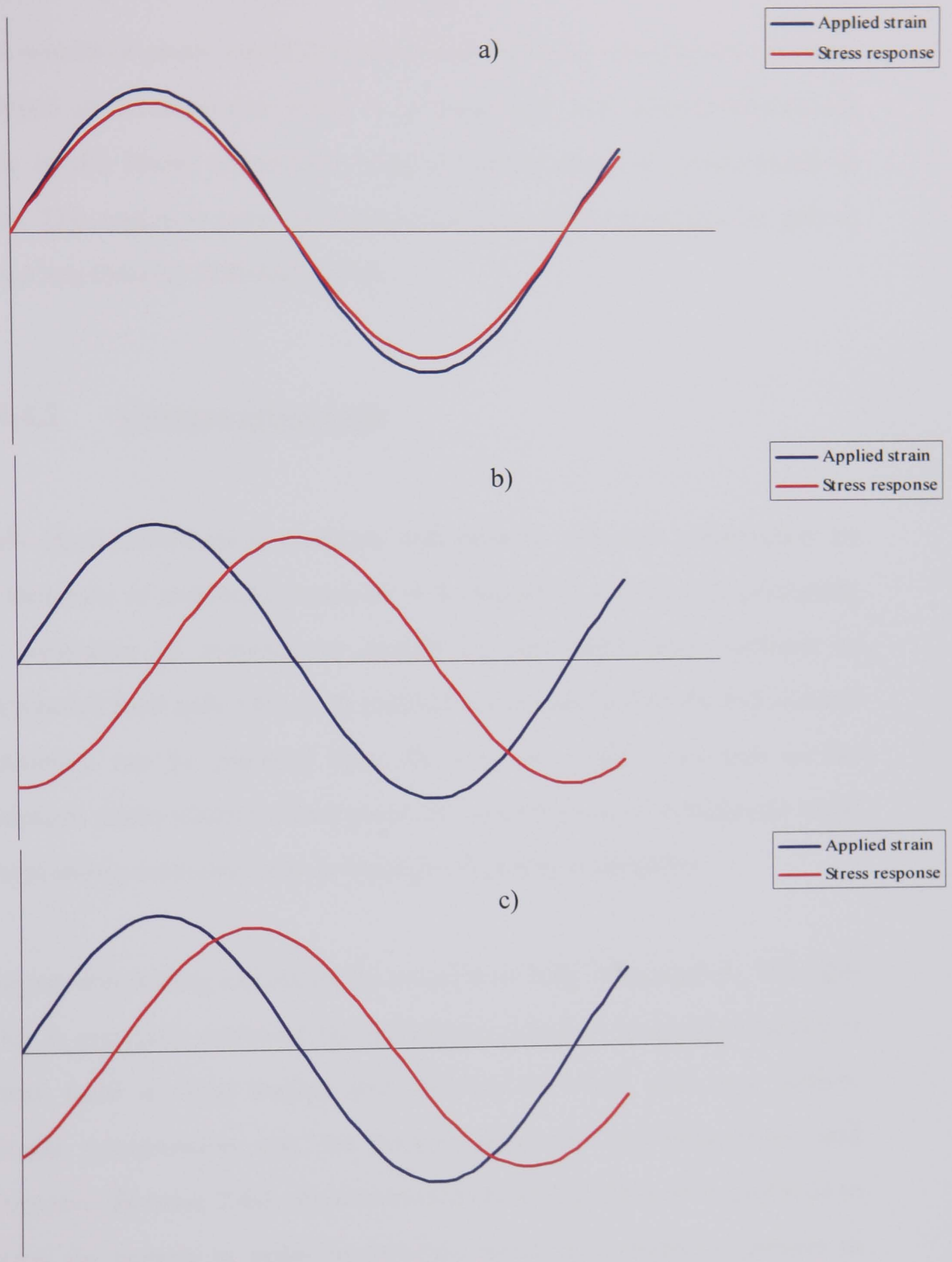


Figure 6. Differences between elastic, liquid and visocelastic materials under small amplitude oscillatory testing. a) Elastic solid, b) Newtonian liquid, 90°C phase difference, c) Viscoelastic material

If the results of creep, stress relaxation and dynamic experiments are to be analysed in fundamental terms it is important that measurements are made in the linear viscoelastic regions where stress is proportional to strain. This region is only encountered at low strains typically for gels at strains less than 0.1 (Mitchell, 1976).

1.4.3 Compression tests

These small deformation methods will provide valuable information on the structure of materials; however it is important in terms of predicting the behaviour of material in mouth to use destructive methods to characterize food gels. One such method is a compression test and various parameters can be assessed like: the force to rupture, as well as the maximum strain (deformation) prior to rupturing and fundamental small deformation parameter such as Young's Modulus of elasticity.

Compression testing can either be uniaxial or bulk compression. The later which is generally achieved by applying a pressure hydrodynamically is seldom used in food testing and will not be dealt with any further. Uniaxial compression can be broken down to non-destructive and destructive (Bourne, 2002). As mentioned above it is often of importance to destroy the sample in order to simulate better the conditions present in mouth.

The parameters that are often obtained from a compression test are:

- Force to rupture in Newtons (N) and this will be used in the present document.
- The strain at rupture (engineering strain)
- Young's modulus

1.4.4 Measurements of deformation under stress

The various methodologies for testing food can be grouped into A) Fundamental methods, B) Empirical methods and C) Imitative Methods.

1.4.4.1 Fundamental methods

These methods are designed to measure well defined physical properties of a sample that is tested and to relate this property to textural characteristics assessed by sensory panels (Lewis, 1996). Such physical properties are stress-strain relationships, viscoelastic behaviour, viscous behaviour (liquid foods). In all these cases the rheological behaviour of the food can be described mathematically and related to sensory characteristics.

Bourne (2002) states that fundamental methods are slow to perform and may not correlate as well with sensory evaluation as do empirical tests. This is based on data acquired in the 70's regarding sensory data of red

delicious apples. However current data as it will be demonstrated in Chapter 5 regarding gels, show that fundamental parameters can be correlated with flavour release.

Fundamentals measurements of gels frequently involve the oscillation experiment shown schematically in Figure 7.The application of small harmonic deformations allows the viscous and elastic moduli (G″ and G′) of the material to be determined. Two typical geometries can be used in rotational rheometry: parallel plate and cone plate (Figure 7). Where the cone or the plate is rotated the torque required to rotate the moving element or exerted on the stationary plate is measured.

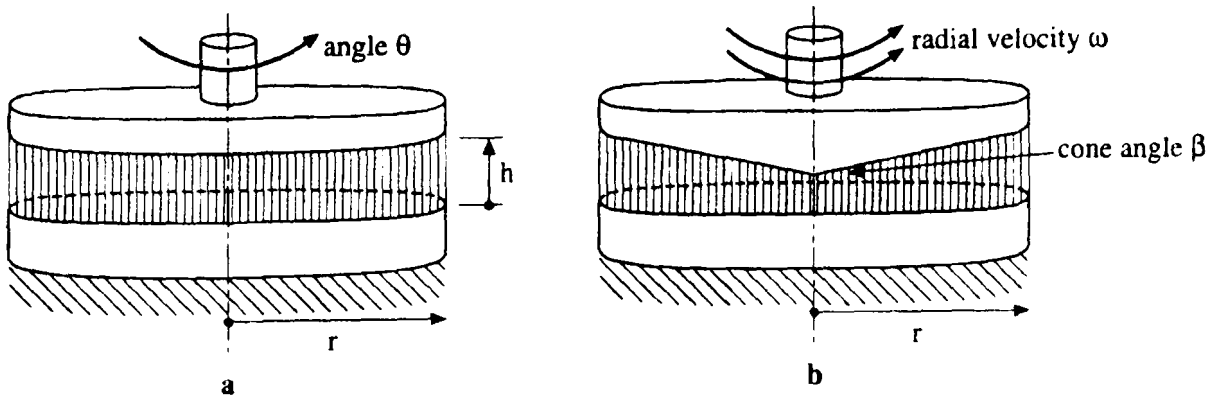


Figure 7. a) Parallel plate and b) cone/plate geometry. In cone/plate the cone is assumed to touch the centre of the bottom plate. In reality the tip is truncated and the corresponding gap is set (Kavanagh, 1998).

The primary advantage of a cone and plate is that the shear rate is uniform within the fluid sample, thus the shear stress at a given shear rate can be obtained from a single measurement (Venerus, 2007). The shear rate is

dependent on the angular velocity and the angle of the cone as can be seen in equation 23 (Macosko, 1994)

$$\dot{\gamma} = \frac{\omega}{\beta} \qquad \text{(Equation 23)}$$

1.4.4.2 Empirical methods

These tests measure parameters that are not well defined and cannot easily be expressed in fundamental terms, but from practical experience are found to be related to textural quality (Bourne 2002). The results acquired are usually characteristic of the sample in question, under the specified experimental conditions, therefore to ensure that the results reported are reproducible the specified conditions should be reported along with the data (Lewis, 1996).

1.4.4.3 Imitative methods

The tests that try to imitate the conditions that a food sample is subjected in practice are included in this category (Bourne 2002). An example of an imitative test is the texture profile analysis (TPA). This has a sensory and a physical dimension. The physical test is able to measure various textural parameters simultaneously (Figure 8).

Since this technique was initially proposed by Friedman et al. in 1963 the methodology and instrumentation available have changed considerably allowing for many ways of interpreting the data. In TPA the plunger of the texturometer (in this study the instrument used was Texture Analyser Plus from Stable Micro Systems) compresses the sample twice, in an attempt to understand the sample behaviour in conditions that simulate in-mouth conditions. The two compressions are called the first bite and second bite respectively.

Figure 8 shows a typical TPA response as well as the parameters derived from each part of the curve.

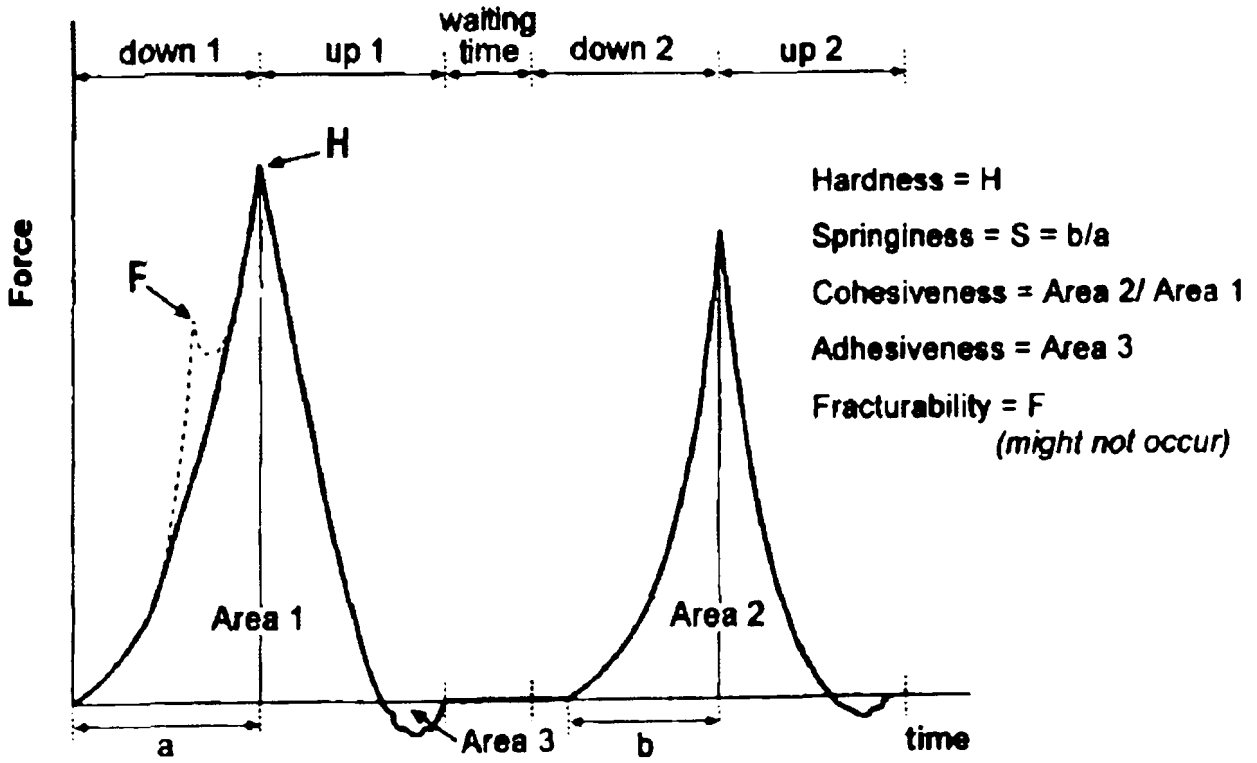


Figure 8. Texture Profile Analysis (TPA) (Fiszman, 1998)

Fiszman et al. (1998) have applied TPA to cylindrical samples of gelatin, κ-carrageenan/locust bean gum and gellan gum gels and have concluded that gelatin gels recovered almost all of their initial height (high springiness) whereas the κ-carrageenan/locust bean and gellan gels were not able to recover all of the lost height. It should however be appreciated that the results will depend on a number of factors most importantly the extent to which the sample is compressed.

1.5 Introduction to polysaccharides used in this study

At this point the main hydrocolloids used in this study will be discussed. In particular mixed κ-carrageenan and locust bean gum, which is currently used by the pet food industry and mixtures of low acyl(LA) and high acyl(HA) gellan gels. Their chemical and physical properties will be outlined as well as their gelation mechanism.

1.5.1 κ-carrageenan

Carrageenans take their name from the town of Carragheen on the west coast of Ireland, where sun-dried and bleach red seaweed (also known as Irish moss) has been used for centuries, mainly as a fertiliser, but it has also been used in gelled milk deserts. They are extracted from the red seaweeds

with a dilute alkaline solution and the potassium salt of a carrageenan is normally produced (Glicksman, 1969).

The manufacturing process for extracting carrageenan is presented in Figure 9. During the alkaline extraction, the alkali can be chosen in order to determine the particular salt type of carrageenan produced which will then have different consequences for the properties of the resulting extract (Imeson, 2005). In the diagram an alternative process is also shown which involves the ability of kappa carrageenan to form gels with potassium salts. The precipitated gel synerises and water is further removed under pressure. The precipitated carrageenan may be frozen and thawed to assist in the dewatering step.

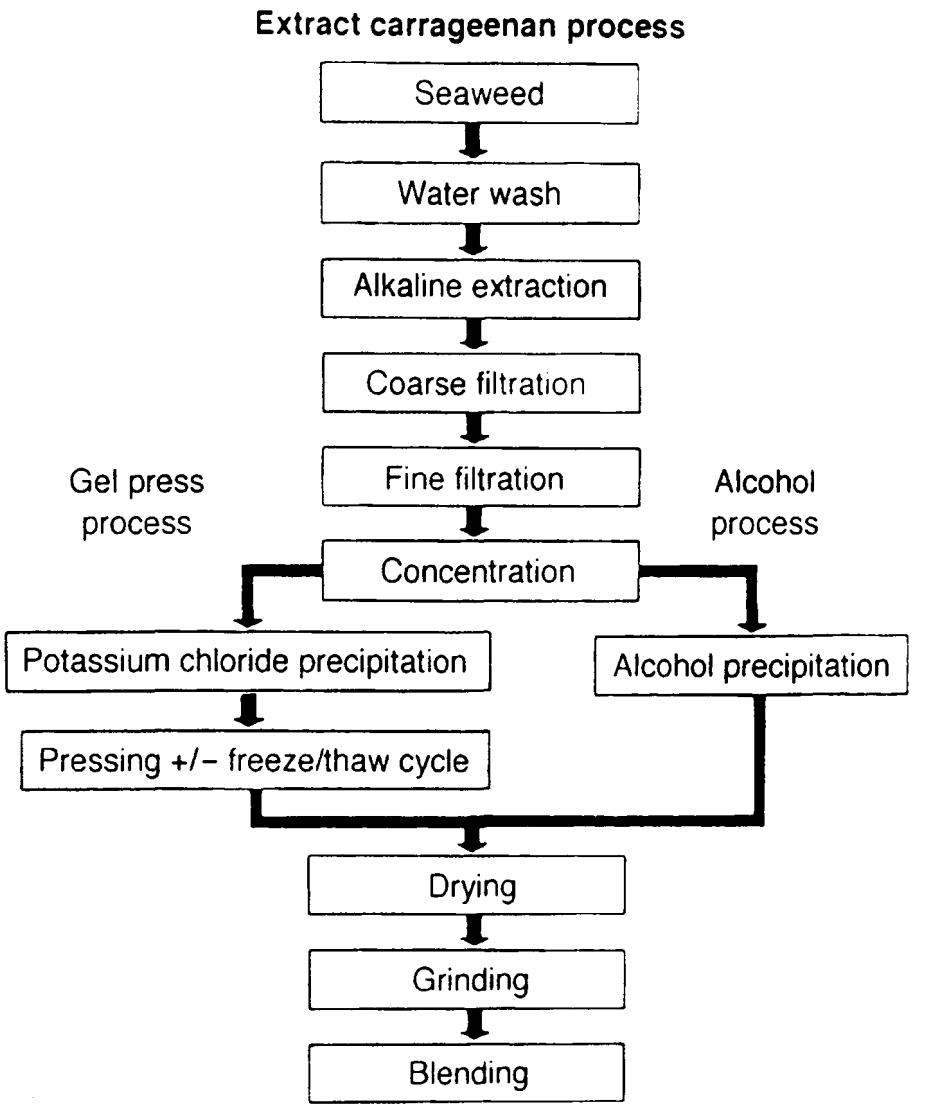


Figure 9. Manufacturing process for carrageenan (Imeson 2005)

Carrageenan is a high molecular weight linear polysaccharide comprising repeating galactose units and 3,6-anhydrogalactose (3,6 AG), both sulfated and non-sulfated, joined by alternating α-(1,3) and β-(1,4) glycosidic linkages. Carrageenan is not a single polysaccharide but a group of sulphated galactans (Therkelsen, 1985). There are three main structures in the carrageenan family that can be seen in Figure 10, kappa (κ), iota (ι) and lambda (λ) and each structure possesses different properties (Whistler and BeMiller, 1992).

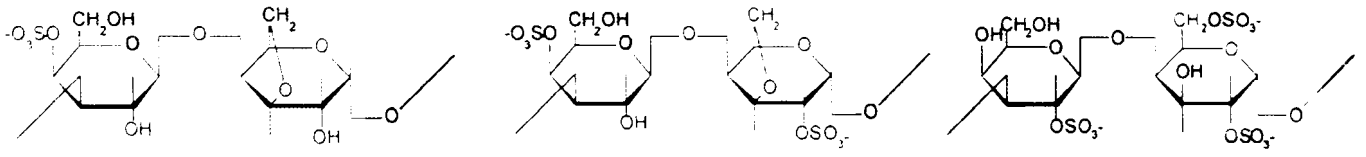


Figure 10. Ideal repeating disaccharide units for kappa, iota and lambda carrageenan (Vincekovic 2005)

The sulphate groups strongly affect the properties of different type of carrageenans in aqueous solutions. Kappa carrageenan possesses one single sulphate group, iota- two sulphate groups and finally lambda three sulphate groups per disaccharide unit (Vincekovic, 2005).

The gelation mechanism as well as the properties of κ-carrageenan will be described now, however it must be pointed out that the sulphate present on the galactose backbone makes interactions with cations possible, particularly with potassium.

1.5.1.1 Gelation of κ-carrageenan

A solution of κ-carrageenan will form a stiff, brittle and thermoreversible gel upon cooling. Ordered assembly of κ-carrageenan into closely packed junctions requires suppression of intermolecular electrostatic repulsion by positive counterions (Figure 11).

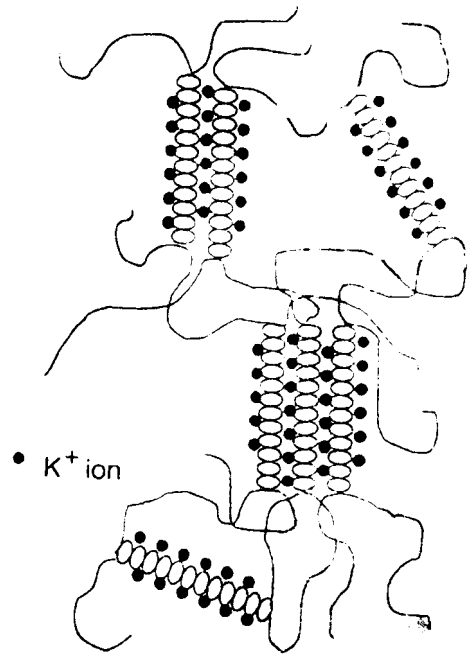


Figure 11 Gelation of kappa -carrageenan with cations (Imeson 2005)

Large group I cations (K^+, Rb^+ and Cs^+) are the most effective in promoting conformational ordering and gel formation (Rochas, 1980). The mechanism of gelation involves coil-helix transition of the κ-carrageenan molecules and it has been described by Morris et al. (1980). The gels formed from kappa carrageenan are the strongest among carrageenan gels and also exhibit syneresis due to the extension of junction zones within the structure (Whistler and BeMiller, 1992).

Gels from iota carrageenan are softer and more resilient, do not synerise and have good freeze-thaw stability. Carrageenann in the sol state assumes a random coil conformation. Upon cooling, lower temperatures induce double helices to be formed as a result of both the alternating α-(1,3) and β-(1,4) glycosidic linkages and the presence of the 3,6 anhydride link within

the second galactose sugar ring. The helices are then stabilised via an aggregation step which takes place almost instantaneously (Goodall et al., 1980). Sequences devoid of the anhydride link function as a helix breakers. Subsequently the aggregation of double helices forms a cross-linking domain and leads the infinite network structure enough to complete gelation. Counter ions are found to play a pivotal role in gelation , due to the electrical charge that the sulphate groups are giving to the carrageenan repeat units. Morris et al suggested the modified gelation model by inducing the counter ion mediated between double helices (Morris, Rees & Robinson, 1980). However Grasdalen and Smidsrod (1981) have reported that k-carrageenan may form helices without gelling in the presence of certain iodine salts. Viebke et al (1994) have also demonstrated that it is possible to produce a gel isothermally from a helical solution by modifying the helix-helix interaction by salt. The other most known carrageenan is lambda carrageenan and differs from iota and kappa in that anhydride residues are almost absent and therefore lambda carrageenan is incapable of double helix formation, and therefore does not gel.

The gelation temperature depends on the carrageenan type and the concentration of counterions, but it shows relatively little sensitivity to carrageenan concentration. The strength of the gels though is highly dependent upon the carrageenan concentration as well as the type and concentration of the cation used. If the cation concentration exceeds a certain limit (~ 0.2M) the gel will become weaker (Therkelsen, 1985).

There are many applications for the carrageenans. It can be used as a gelling agent in dairy deserts, pet food, as stabilizers in ice cream and similar products, for preparation of evaporated milk, infant formulas, for whipped cream, for improving the texture and quality of low-fat meat products (Whistler and BeMiller, 1992).

1.5.2 Gellan-gum

Gellan is an extracellular polysaccharide (Figure 12) that is produced by the bacteria *Sphingomonas paucimobilis,* previously known as *Pseudomonas elodea* (Sutherland, 1994). Gellan gum has a molecular structure based on a tetrasaccharide repeating unit composed of (1-3)-β-D-glucose, (1-4)-β-D-glucuronic acid, (1-4)-β-D-glucose, and (1-4)-α-L-rhamnose as the backbone with acyl substituents of L-glycerate and acetate at C-2 and C-6 (ca. 50%) positions of the (1-3)-linked D-glucose, respectively (Kuo, Mort, & Dell, 1986).

The acyl groups inhibit inter-helical associations in the presence of potassium, which minimizes electrostatic repulsion. This may be due to a steric effect, leading to decreased elasticity of the gelled system and to reduced thermal hysteresis. However, in the absence of potassium, the acyl groups rather promote inter-helical associations (Noda et al, 2008). This may be due to lowered charge density of the gellan gum molecules, leading to increased elasticity of the gelled system without enhancing thermal hysteresis. The acyl groups, particularly glycerate, promote the sol-gel transition upon lowering temperature by stabilizing the double

helix in the presence or absence of potassium. AFM images also showed that the macroscopic network structures developed through the elongation and branching of non-associated fibers or strands, in which end-to-end type inter-helical associations predominate over side-by-side type ones, supporting the fibrous model as the gelation mechanism (Funami 2008). Furthermore the O-acyl substituents of high-acyl gellan inhibit the close packing of the helices into crystalline domains. Highest crystallinity and formation of more rigid and brittle gels is observed when the O-acyl groups are absent (Kang and Pettitt, 1993). Therefore by varying the degree of acylation, gellan gels can be produced with a variety of textures.

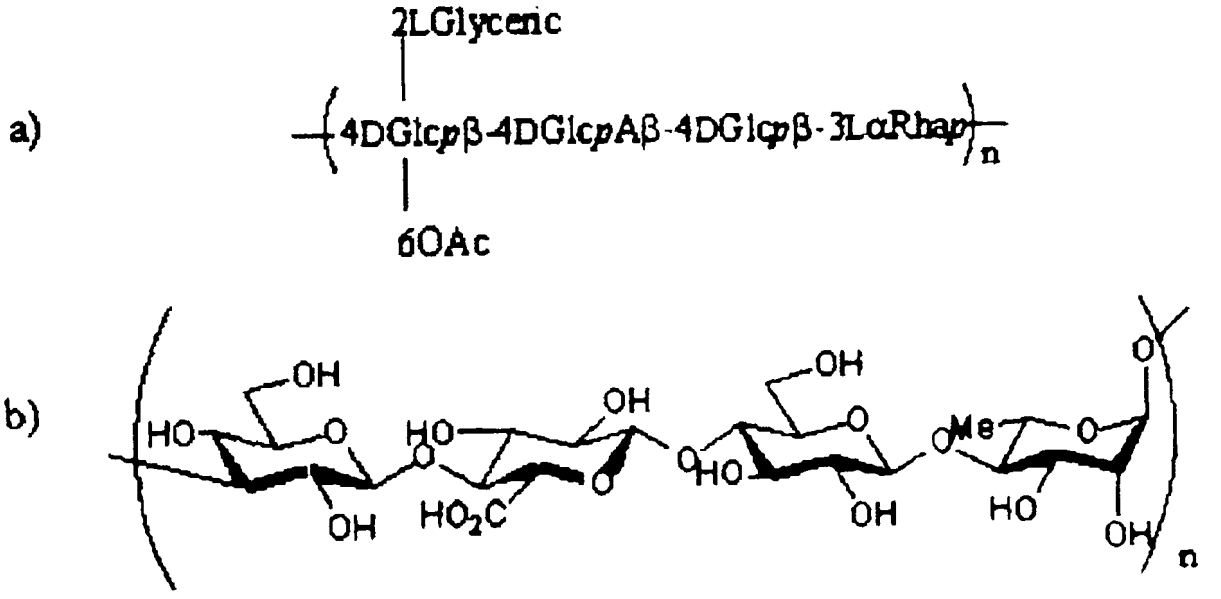


Figure 12. Chemical structure of a) native gellan and b) deacylated gellan (Rinaudo & Milas, 2000)

The possible applications of gellan gum are very diverse and range from confectionery products like jellies, fillings and marshmallow, to jams, fabricated foods (vegetables, meat substitutes), water based gels, pet foods,

icings and frostings, dairy products, as well as applications outside the food industry like microcapsules or for photographic films (Sanderson 1990).

1.5.2.1 Gelation and rheology of gellan

According to Morris (1991) the mechanism of gelation is based on helix formation upon cooling which is considered to promote end-to-end association, via double helix formation into fibrils. These fibrils can afterwards thicken into fibres by means of a chain end linking to the middle of a separate chain. Gel promoting cations promote inter-fibril or intra-fibre crystallization and a permanent network is formed. The gelation mechanism is shown in Figure 13.

There is wide range of cations that are able to induce gelation of deacetylated gellan. Divalent metal ions promote aggregation by site-binding between pairs of carboxyl groups on neighbouring helices (similar to the egg-box structure). Monovalent metal ions bind to the surface of individual helices, thus lowering their charge-density and reducing the electrostatic barrier to aggregation (Morris et al, 1996).

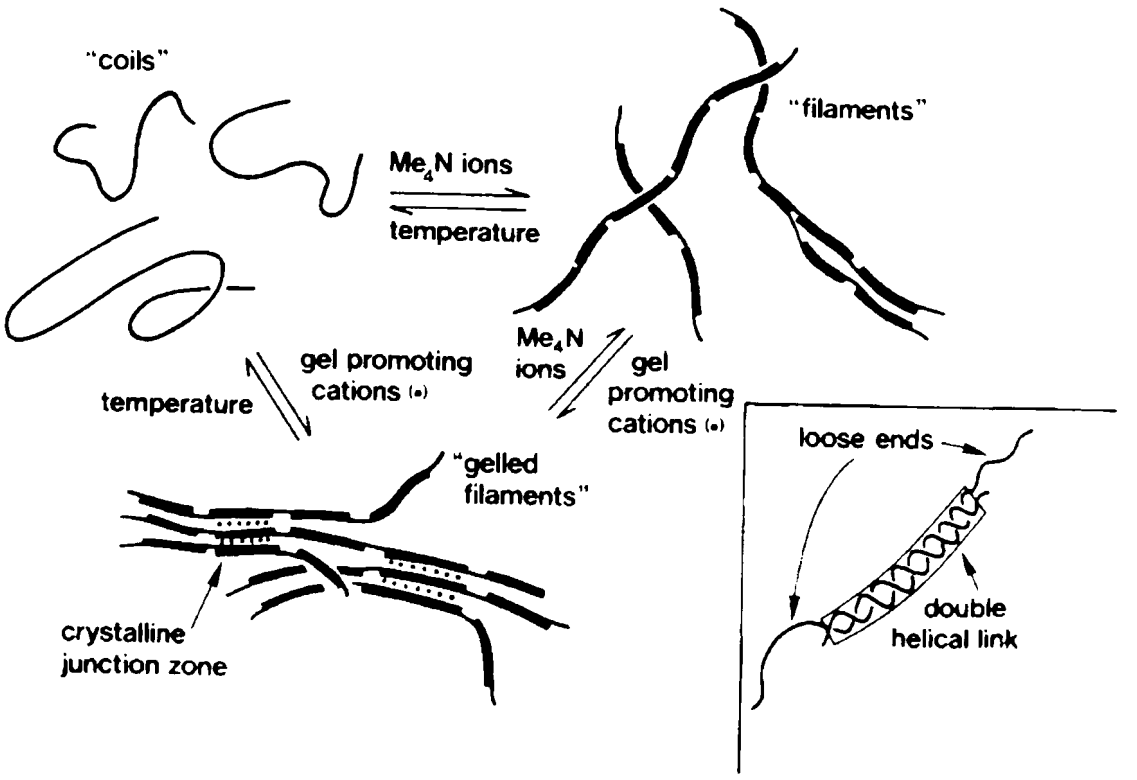


Figure 13. Model for the gelation of gellan gum. The effect of gel-promoting cations and temperature are shown. At the right bottom corner the end-to-end association of the polymers by double helix formation is shown. (Morris VJ, 1991)

Early work on the characterisation of low-acyl gellan gels by Nussinovitch, (1990) has concluded that the strength and stiffness of the gels smoothly increased with the gum concentration. Also the gels solidity as measured by the asymptotic modulus in stress relaxation and compliance in creep also increased with gum concentration. Mixed gellan gels are more deformable and exhibit similar compressive strength compared to low acyl gellan gels at similar polymer concentrations (Mao et al., 1999). Mixtures of high acyl and low acyl show two separate conformational transitions at temperatures characteristic of the individual components which indicate that there is no formation of double helices involving strands of both types (Morris, 1996). The effect of adding high acyl gellan to low acyl can be

compared with the effect of adding LBG to κ-carrageenan gels where the addition of LBG also makes the gels more deformable.

1.5.3 Gelatin

Gelatin is one of the most used hydrocolloids in the food industry. Although it is a protein many of its characteristics are equal or better to polysaccharide gums, which has earned gelatin the 'distinguished' title of 'Honorary polysaccharide' (Harding, personal communication). Gelatin can form gels over a wide range of concentrations and therefore can be used in a large range of products in a number of different industries, including pharmaceutical, photographic, cosmetic industries and in most direct relevance to this project, in foods. In the food industry, gelatin's primary role is a gelling agent, however it is used as an emulsifying agent, thickener, foaming agent, film forming. Among many food products that contain gelatin are jellies, mousse, marshmallows, fruit gums etc.

One of the most important characteristics of gelatin is its unique ability to form thermo-reversible gels. The melting temperature of gelatin gels is usually around 28°C, below mouth temperature, making gelatin gels far superior in texture and flavour release when compared to gels produced by other hydrocolloids. In addition to that gelatin has another very important characteristic which enhances the flavour release, its ability to mix well with saliva. This will be demonstrated latter on in this study.

Gelatin is produced by degradation and solubilization of the collagen that exists in bone and skin tissue. The collagen monomer exists as a triple helix, which is 300nm long and 1.5nm in diameter and has a molecular weight of about 300,000. On mild heating (40°C) the helix unfolds to yield a mixture of α chains, β chains which consist of two covalently bound α chains and γ units consisting of three α chains (Ledward 2000). Collagen contains an unusual amino acid, hydroxyproline, found in almost no other protein. The primary structure of collagen, and of the gelatin derived from it, is based on a repeating sequence, which can be written as Gly-X-Y; proline occurs predominantly in position X and hydroxyproline in position Y; other amino acids can occur in either position. In native collagen, each molecule has three covalently linked peptide chains wound together into a triple helix. The triple-helix structure depends on the occurrence of glycine at every third position in the peptide chain. Glycine is the smallest amino acid; its sidechain is a single hydrogen atom, which is located in the core of the helix, in a position that could not accommodate a larger sidechain, therefore glycine plays a predominant role in gelatin's ability to form a triple helix (Harrington and Morris, 2009)

An important observation regarding collagen in animals is the fact that with increasing age of the animal the collagen tends to become stronger (high tensile strength). This accounts for the more tender meat from younger animals and the reason behind this is the number of intramolecular crosslinks between collagen molecules increases with time and also the linkages change from divalent to trivalent. Trivalent linkages

can link three rather than two collagen molecules and these stable crosslink require quite severe processing in order to yield soluble gelatins and this accounts for why commercial gelatins are so heterogenous in size (Ledward, 2000).

There are mainly two different processes to obtain commercial gelatin and this classifies gelatin into Type A and Type B gelatin. Type A gelatins are obtained from skins and bones of different origin through an acid process, which yields gelatin with an isoelectric point of 6-9.6. Type B gelatins are obtained through an alkaline process of cattle skins and bones. Their isoelectric point is around 4.5-5.5 (Ledward, 2000).

1.5.3.1 Gelation of gelatin

When gelatin is kept at temperatures above 40°C it behaves as random coil and when cooled thermoreversible gels are formed, at concentrations above 1%, depending on the quality of the gelatin and the pH. During the gelling process, the chains undergo a conformational disorder-order transition and partly regenerate the collagen triple-helix structure. The network is formed by associating helices in junction zones and stabilized by hydrogen bonds.

When the gel is matured at high temperature only a few collagen-like junctions zones will form and weak gels will be formed. With further cooling additional parts of each chain will become ordered, either by the

formation of new junction zones or the growth of existing ones. At lower temperatures the gel strength increases faster with time (Ledward, 2000).

1.5.3.2 Bloom value

The key parameter that determines the functional properties and the price of commercial gelatin is the Bloom value. In order to measure the Bloom value, a 112g sample of 6.666% w/w gelatin gel is prepared in a standardized container and conditioned following a highly standardized time and temperature protocol. After ageing the sample is brought to 10°C and an instrument measures the force need to push a plunger of 12.5mm in diameter 4mm into the gelatin. The force is produced by dropping shot into a cup in controlled manner until the plunger reaches the required, 4mm depth. The actual Bloom value is the weight required expressed in grams (Bloom, 1925). The Bloom value as well as the viscosity of gelatin, are dependent on the average molecular mass of the gelatin molecules.

1.6 Behaviour of Biopolymer Mixtures

In this section the behaviour of biopolymer mixtures will be discussed. It is of great interest because both synergistic effects and segregative phase separation can be observed. Both these affects will be discussed in terms of the behaviour of biopolymers and biopolymer gels.

1.6.1 Multicomponent gels

Perhaps the most useful property of polysaccharides is their ability at relatively low concentrations (less than 1%) to form firm gels. In ordered to develop a solid-like structure the polymer chains must come together into a three dimensional network where the pores are filled with water. In natural polysaccharides the linkages are not covalent, instead the network is formed by association of chain segments into long conformationally ordered junction zones. The junction zones are held together by hydrogen bonding, hydrophobic associations (Van der Waals attractions) or ionic cross bridges (Whistler and BeMiller, 1992).

A binary gel is the simplest form of a multi-component gel. Various structures arise when two polysaccharides are mixed and gelled. A big part of this study will involve two component gels and some of the work will involve three component gels, so very briefly here the gelation mechanism when more than one gelling agent is used will be describe prior to discussing the gelation mechanism of the individual gelling agents used in this study.

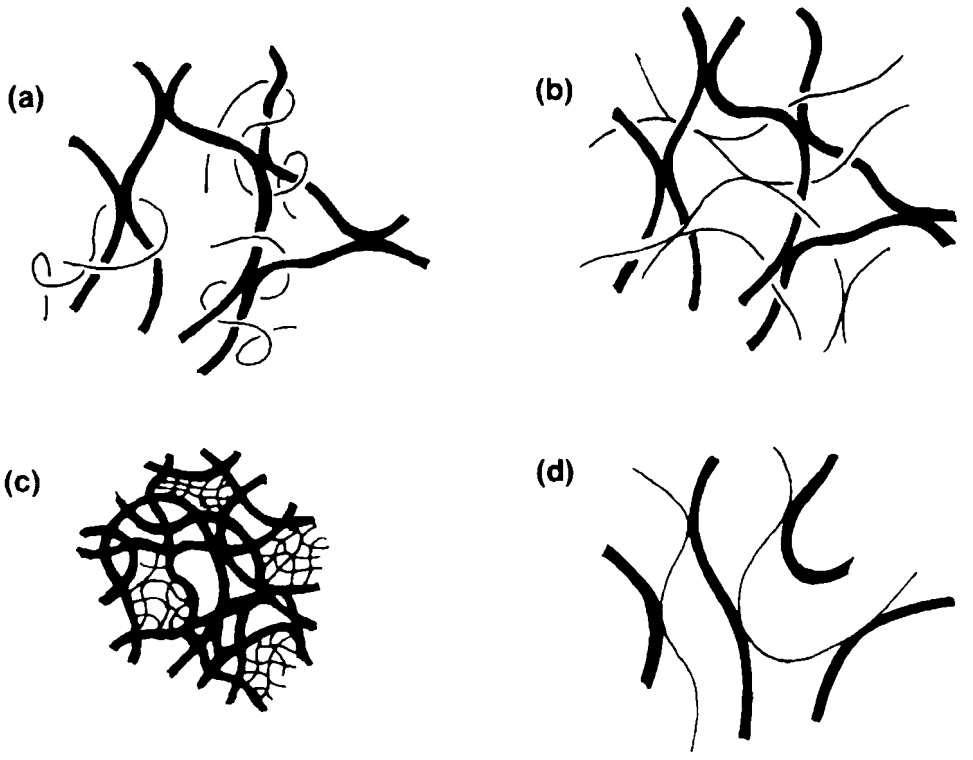


Figure 14. Binary polymer gel networks: a) Network formed from only one polysaccharide; b) Interpenetrating network formed by independent gelation of the individual polysaccharides; c) phase-seperated network formed by demixing and subsequent gelation of the two polysaccharides; d) coupled network formed by intermolecular binding between the two polysaccharides (Morris, 1991).

In Figure 14 (Morris, 1991) the schematic models for the different types of possible binary gels are shown. In Figure 14a only one of the two polysaccharides will form the network. On the contrary in Figure 14b both of the polysaccharides will form independent networks. In this case there must be some demixing prior to gelation, then a phase-separated network will be formed (Figure 14c) and finally if there is binding of one polysaccharide to the other there will be the formation of a coupled network (Figure 14d). The food allowed polysaccharide that participates most extensively in coupled networks is locust bean gum.

1.6.2 Locust bean gum (LBG)

Locust bean gum is the refined endosperm of the seed of the carob tree. Locust bean gum (LBG) is a plant galactomannan containing (1-4)-β-D-mannopyranosyl backbone with attachment of (1-6)-α-D-galactose single units (Dea and Morrison, 1978). There are about 3.5 (2.8-4.9) mannose residues for every galactose residue. LBG dissolves in hot water and forms a viscous solution with rheology typical of that of macromolecular solutions (Doublier and Launay, 1981). It has been shown that the structure of locust bean gum consists of a high proportion of substituted couplets, a lesser amount of substituted triplets and a non-random distribution of the galactose units that yields a relatively high proportion of un-substituted blocks that were termed "smooth" regions (McCleary, 1985). LBG would not gel on its own however when mixed carrageenan or xanthan it will form an elastic gel. This will be discussed further shortly afterwards.

LBG has been utilised industrially for a long time. It has been used in papermaking and its derivatives have been in use in the textile industry and petroleum industry. However locust bean gum has a major role in the food industry. Its unique swelling and water binding properties granted its use in dairy and frozen dessert products. LBG provides ice-cream products with heat shock resistance, smooth meltdown as well as the desirable texture. Further uses of LBG in the food industry include soft cheese products, meat products and petfoods, glassed and canned products, bakery products, dietary products and many others. Particularly in canned petfood, locust bean gum is an excellent stabilizer due to its temperature

dependent hydration properties which provide good retort stability (Maier et al., 1993). However the behaviour of LBG during thermal processing is dependent on the quality of the polysaccharide. Kok, et al. (1996) have compared a higher quality crude locust bean gun with a lower quality crude locust bean gum and have concluded that the functionalities of the samples were greatly influenced by the non-galactomannan fraction. Furthermore in a later study it was established that the thermal stability of the galactomannan fraction differs between the refined and crude samples (Kok et al., 1999). Finally Kok (2007) has demonstrated that indeed there is a difference on the ratio of mannose/galactose ratio between crude LBG and refined LBG of 3.1 to 3.9 respectively.

1.6.3 Synergy between κ-carrageenan and LBG

Synergy is the phenomenon in which two forces/agents act together and produce an effect greater than when you simply add the separate effects of the forces/agents. The binary system of κ-carrageenan and LBG exhibit a form of synergy and has been extensively studied after the observation by Dea and Morrison (1975) that at a concentration below the gelation threshold for κ –carrageenan alone gelation was observed on the addition of LBG. However it must be taken into consideration that the total polysaccharide concentration was increased in that gelling system by the addition of LBG.

Initially it was believed that a coupled network was formed (Figure 15) with unsubstituted mannan backbone binding to the carrageenan helix to

form a number of junction zones (Dea and al., 1972), however an alternative view was reported by Cairns and al. (1986) who found no evidence of intermolecular interaction. The authors suggested that mixtures of κ-carrageenan and LBG consist of chains of the LBG entrapped within the network formed by κ-carrageenan.

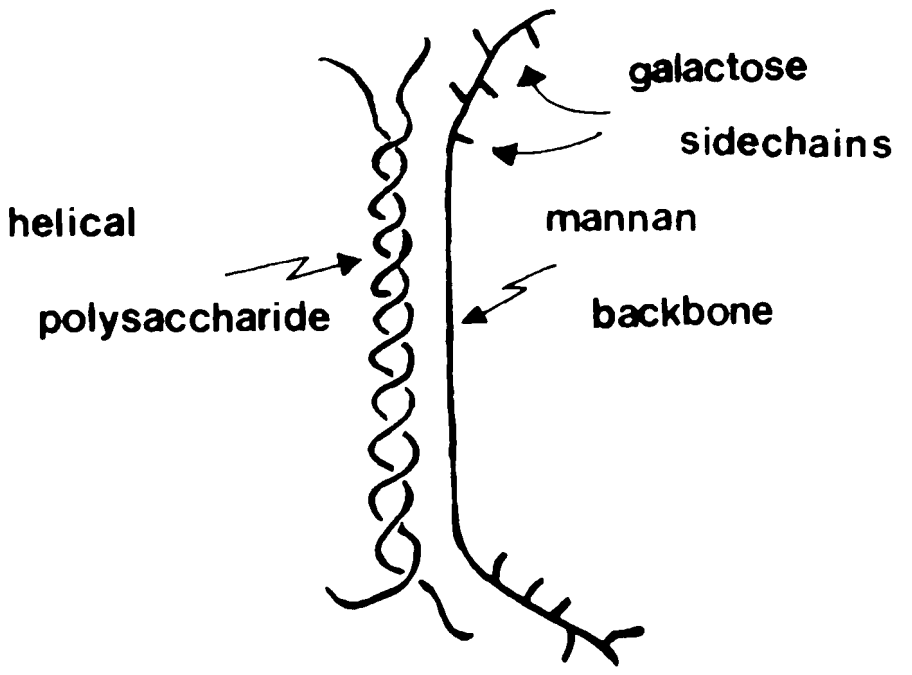


Figure 15. Original model proposed by Dea et al. (1972) to explain gelation in synergistic polysaccharide gels

Turquois et al. (1995) by small angle x-ray scattering have concluded that there is significant decrease of κ-carrageenan aggregation due to the presence of LBG. They suggested a mechanism of absorption of mannan chains on the surface of κ-carrageenan bundles that results in a more regular distribution of κ-carrageenan molecules in the medium which in

effect modifies to a large extent the rheological properties of the system by modifying the microstructure of the system.

1.6.4 Phase behaviour of gels systems

Many food systems and in particular wet petfood include as major components mixtures of biopolymers in solution in water. There is a direct link with the morphology of these mixtures with their rheological properties and therefore a direct linkage with the texture of the product. Therefore the phase behaviour of such mixtures is very interesting to investigate (Donald et al., 1995).

Phase separation in biopolymer mixtures is more often observed than miscibility among polymers which in fact is the exception (Albertsson, 1986). If the interaction between different polymer segments is repulsive then phase separation will occur above certain polymer concentrations and there will be two separate phases each one rich in one of the polymers. If on the other hand the forces between the two polymers are attractive then the polymers will collect in the same phase and there will be another phase with lower polymer concentrations. In order to be fully miscible the two polymers must be very similar in their properties (Albertsson, 1995).

Mixtures of two different biopolymers have been the subject of extensive research during the last decades and as a result some general principles have been established. The interactions between two polymeric constituents can be described as associative or segregative, depending on

whether they are enthalpically more favourable (associative) or less favourable (segregative) than interactions between individual polymers of the same type (Picullel, Bergfeldt and Nilsson, 1995). Associative interactions normally occur by electrostatic attraction, for example negatively charged polysaccharide molecules and protein molecules that are positively charged. The resulting complexes may be soluble or insoluble and that depends on the ionic environment and the relative and absolute concentrations of the two polymers. The more usual outcome is the formation of insoluble complexes. Segregative interactions which is also known as ''thermodynamic incompatibility'' is more common and at sufficiently high concentrations of the two polymers two phases can be formed, each rich in one of the biopolymers, and typically one phase dispersed within another. This can lead to bulk phase separation, via coalescence of the dispersed phase and due to either creaming or sedimentation, as a result of density differences between the two phases. At lower concentrations the polymers may co-exist without bulk phase resolution occurring. Phase separation can play an important role in enhancing gel strength of gelling polymers by confining the polymers to a fraction of the total volume, effectively increasing the concentration in the respective phase volumes. Restriction of molecules to only part of the total volume causes a large reduction in entropy, and segregation will occur only if the loss of entropy is outweighed by the enthalpic advantage of individual molecules being surrounded by others of the same type (Harrington and Morris, 2008).

In order to understand the phase behaviour of a two phase systems a phase diagram is used, which is unique for the system in question given the fact that the conditions are set (pH, temperature and salt concentration). A typical phase diagram for a two phase aqueous system is presented in Figure 16. The binodal curve divides the diagram into the bottom region where, for those component concentrations, the two components will be present in as one phase and the top region where the system will exist in two phases. The tie line connects two nodes on the bimodal, which represent the final concentration of phase components in the top and bottom phases. When moving along the tie line coordinates, the systems denoted will have different total compositions and volume ratios, but the same final concentration of phase components in the top and bottom phases (Kaul, 2000).

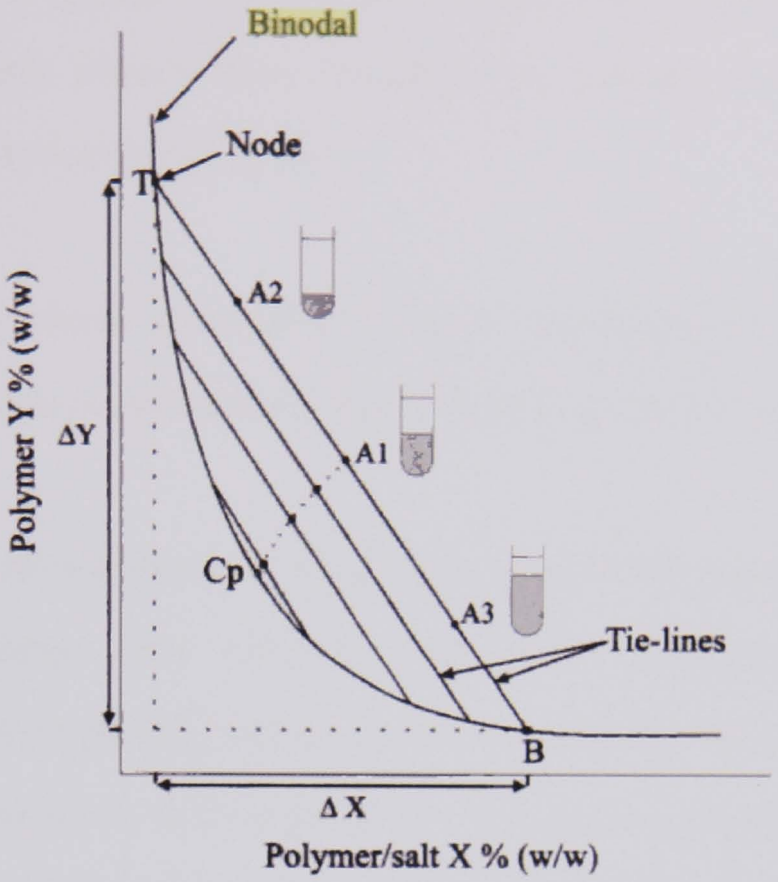


Figure 16. Illustration of a phase diagram. Bottom phase polymer /salt X(%w/w) and top phase polymer Y (%w/w). On the tie line TB A1, A2 and A3 have equal total concentrations of polymer Y + X however the phase volumes are different . The phase composition is though identical on the same tie line (Kaul, 2000)

Piculell et al. (1995), classify the phase behaviour observed for a mixture of polymers in solvent into three types:

- Segregative phase behaviour which is the most common form and where two phases are formed and each phase is enriched in one of the polymers.

- Associative phase separation, where two oppositely charged biopolymers attract thus creating two phases, one of which is enriched in both biopolymers.

- Borderline phase separation is a very rare situation in which one of the polymers is distributed equally between the two phases.

When dealing with solutions, if there is not a quenching process involved, like rapid freezing, then demixing ultimately produces bulk phase resolution. However gelling systems have a mandatory quenching process which usually prevents such separation. The gelling process may totally prevent a bulk phase separation or the phase separation may not be obvious on a macroscopic scale but evident microscopically (Clark, 1995).

In Figure 17 the possible behaviour when mixing two gelling agents X and Y are presented. In Figure 17A, there is a one phase system where both polymers are participating in the formation of the matrix (for example xanthan and LBG). In Figure 17B, polymer Y is forming the continuous phase and polymer X exist as spherical inclusions within the matrix. An example of such a system is agarose and gelatin. When more gelatin is added to the system phase inversion is observed (Figure 17 C), where the polymer that constituted the continuous phase is now observed in inclusions and polymer in the inclusions is now forming the continuous phase.

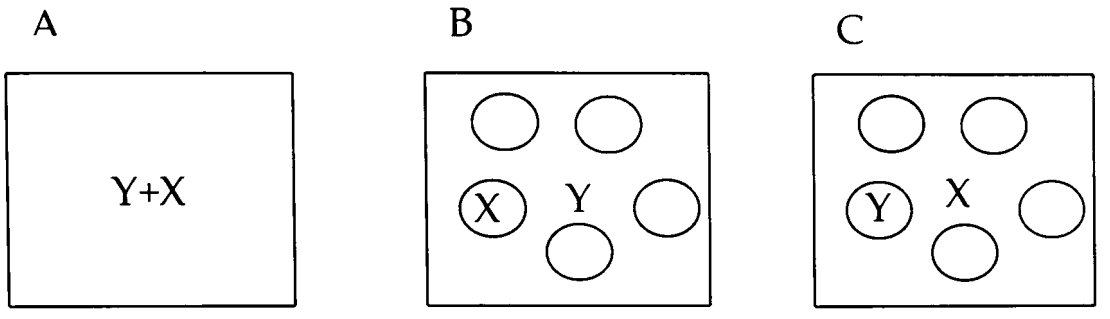


Figure 17. Different possible phase behaviours. A) Both biopolymers participate in the formation of the network (i.e xanthan and LBG), B) one of the polymer Y is forming the continuous phase and the second biopolymer exist as inclusions (agar and gelatin), C) phase inversion, polymer X constitutes the continuous phase and polymer Y exists as inclusions (i.e high amounts of gelatin and agar)

1.7 Objectives

In summary the present work will be based on two distinct mixed gel systems, κ-carrageenan & LBG and LA-&HA-gellan, which show some similarity in behaviour but also, as will be demonstrated in the following chapters, show distinct differences. Furthermore gelatin and its influence will also be taken into consideration which will make the gelling system more complex. The main ideas behind characterisation of texture have been described and during the next chapter the methods applied in this study will be described, focusing more on characterisation of mixed gel systems.

Chapter 2

Materials and Methods

2.1 Materials

Bovine gelatin type B (225) and locust bean gum (LBG) for the preparation of the solutions were supplied by Sigma-Aldrich. Mixed high acyl /low acyl gellan gels and mixed κ-carrageenan locust bean gum (LBG) gels were prepared for instrumental analysis. The κ-carrageenan was supplied by Sigma-Aldrich and low acyl gellan gum and high acyl gellan gum was supplied by CP-Kelco. Potassium chloride, potassium phosphate monobasic and potassium phosphate dibasic for the preparation of the buffer solution were supplied by Sigma-Aldrich. Ethyl butyrate and sodium chloride were also supplied by Sigma Aldrich.

2.2 Methods

In this section the general methods used will be described. In the following chapters if a deviation from the method occurred this will be described in detail. There has been a small part of this study that involved preparation

of solutions and this will first be described followed by the preparation and characterisation of gels.

2.2.1 Solutions

2.2.1.1 Preparation

Distilled water was heated to 90°C and then the gelatin or locust bean gum was added and the solution was mixed for 2min at 16000rev/min (Ultra Turax T-25 basic mixer). Concentration ranges investigated were gelatin (1-30%) and locust bean gum (0.1 to 1.0%). For the mixing experiments the samples that were photographed, were prepared in water and for the subsequent salt release experiments in 0.2M NaCl. Visual observations did not show any effect of the salt on the mixing behaviour of the solutions.

2.2.1.2 Visual observation

5ml of each of the LBG and gelatin solutions, which contained a small amount of red food colouring, were carefully added to the bottom of a 50ml glass beaker containing 20ml of distilled water with a plastic syringe (Ferry et al.; 2006a). The temperature of the water and the added solutions were at 40°C. The solutions were rapidly stirred by hand with a teaspoon for 2-3s in a circular motion and photographed afterwards. The

concentration of Na^+ close to the top surface was measured with a specific ion electrode as described by Ferry et al., (2006b).

2.2.2 Gels

2.2.2.1 Preparation of buffer

The buffer solution contained the following salts per 1L (minimum purity 99%, supplied by Sigma-Aldrich, Steinheim, Germany):

KH_2PO_4 9.1g

K_2HPO_4 6.1g

KCl 10g

In order to prepare the buffer, the salts were dissolved in slightly less distilled water than the final required buffer volume, using a Fischer magnetic stirrer. The pH was adjusted to a final value of 6.5 by the addition of small volume of HCl or NaOH (minimum purity 99%, supplied by Sigma-Aldrich, Steinheim, Germany). The solution was transferred to an appropriately sized volumetric flask and made up to the correct volume using distilled water (1L).

2.2.2.2 Gel preparation

The total concentration of polysaccharides used in the mixed gels was 0.6%. The concentration was chosen firstly to be comparable to the polysaccharide concentrations used in the petfood industry and secondly to give free standing cylinders of gels for the subsequent compression tests. All of the gels were prepared in a buffer solution and the pH was controlled at 6.5. 1L of buffer solution was heated at 90°C and afterwards 6.0g of the hydrocolloid mixture was added and intense mixing followed for 2 min (Ultra Turax T-25 basic mixer at 16000revs/min) which was then followed by stirring at 90°C for 10min. For the subsequent compression testing the solution was then poured into cylindrical Perspex moulds in order to have cylindrical samples of 20mm height and 20mm diameter. The gel samples were refrigerated at 4°C for 24h and the samples were left to equilibrate at 20°C for one hour prior to analysis.

2.2.2.3 Rheological characterisation

Gels were characterised by compression followed by stress relaxation and oscillatory testing.

2.2.2.3.1 Compression testing

Compression measurements were made using a TA-TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), fitted with a 40mm diameter Perspex probe at a constant speed of 2mm/s (more details on the parameters are give on Table 1) until compression to an engineering strain of 0.75 ($\Delta L/L_o$ the initial gel height is Lo and ΔL is the height at the end of the compression). Four replicates of each gel were analysed. Samples that had clear defects or a high level of air bubbles were not used.

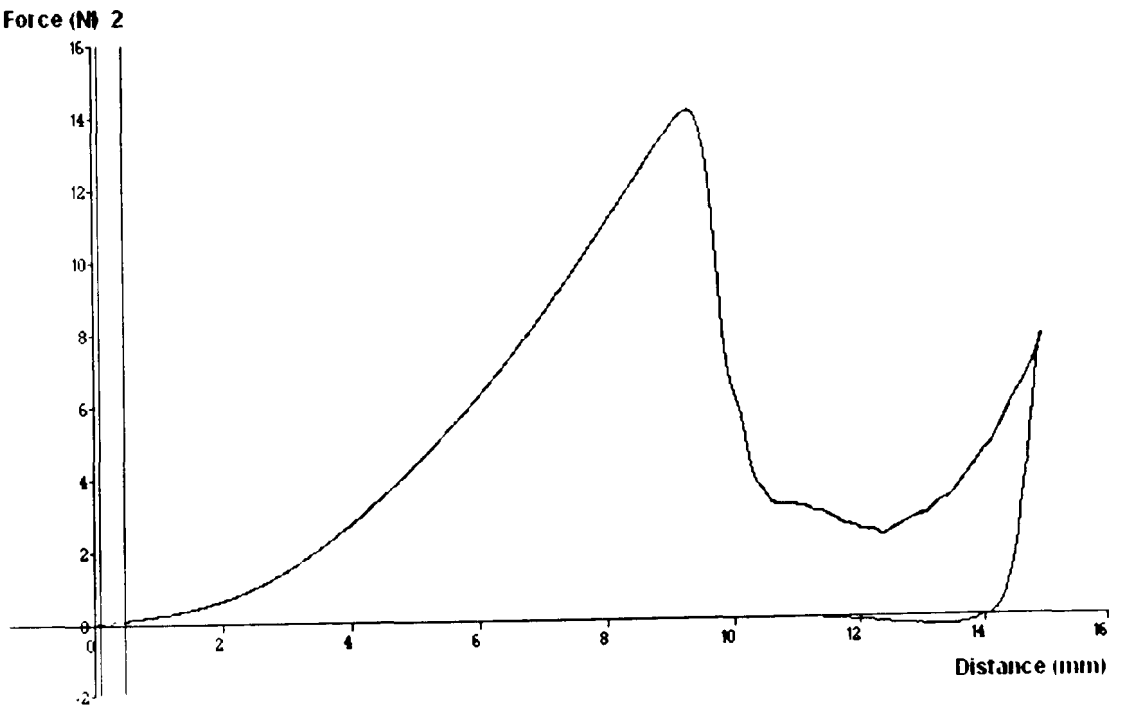


Figure 18. Force deformation curve as acquired by the TA-TX2 software. At the initial part of the curve the slope was taken to calculate the Young's Modulus (E).

Young's modulus (E) was calculated from the initial slope (Figure 18) of the force deformation response (dF/dL) using Equation 24.

$$E=\frac{dF}{dL}\times\frac{L_0}{A_0} \qquad \text{(Equation 24)}$$

where E: Young's modulus; A_0 initial gel cross sectional area and L_0 the initial gel height

Table 1. Texture analysis parameters for compression tests

Parameter	**Compression test**
Probe	Circular perpex plunger (40mm diameter)
Load Cell/kg	30
Pre-test speed(mm/s)	5
Test speed(mm/s)	2
Post-test speed(mm/s)	10
Distance (mm)	15
Trigger type	Auto (force)
Trigger force (g)	5

2.2.2.3.1.1 Determination of the initial slope

To calculate the Young's modulus from equation 24, the initial slope of the force/deformation curve is needed. However the initial slope is difficult to measure. The effect of the distance range (start-end point) chosen for the slope is shown in Figure 19.

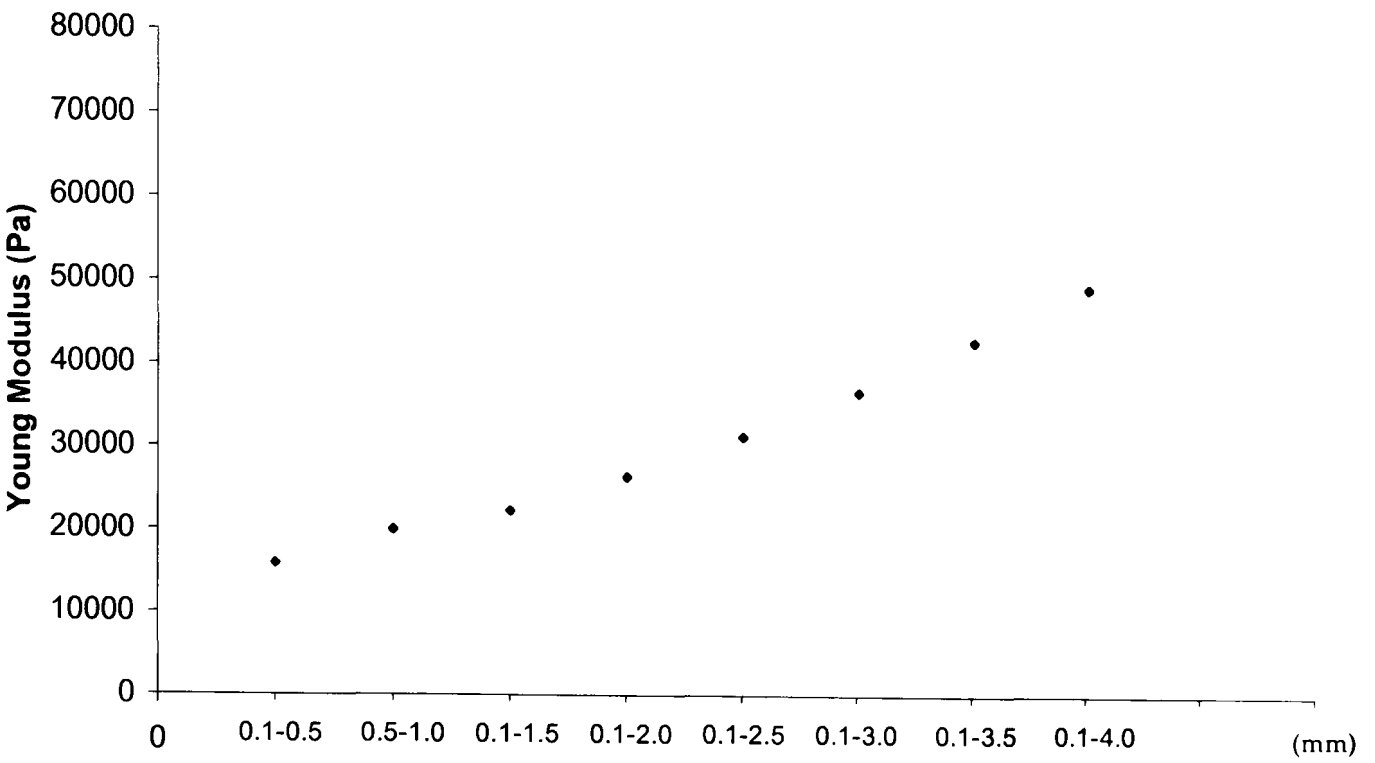


Figure 19. Investigation on the effect of the range of the "initial slope" used on the value of Young's Modulus. On the X axis are plotted different lengths of initial slope taken to establish its effect on Young's Modulus.

The investigation in the effect of the slope (Figure 19) yielded interesting results. As the range over which the slope was calculated increased in length, the values calculated for Young's Modulus also increased reaching values that are extremely high for the materials in question. Ranges starting from zero length were not considered in order to account for slight imperfections of the gel surface not visible by the naked eye.

The values calculated were compared with the storage modulus from oscillatory experiments to see if they fulfilled the relationship 3G′=E. In Figure 20 can be observed that the bigger the length of the initial slope then bigger the deviation from the 3G′=E relationship (G′ was measured at a frequency of 1Hz). Therefore for the rest of the study when E is being

referred to, that has been calculated from the gradient of the initial slope from 0.1-0.5mm which was found to give best agreement with the oscillatory data. Another interesting observation from this graph is the fact that the agreement with 3G'=E is poorer for the lower modulus gels. This will be further discussed in Chapter 3 when the results for the characterisation of the gels are presented.

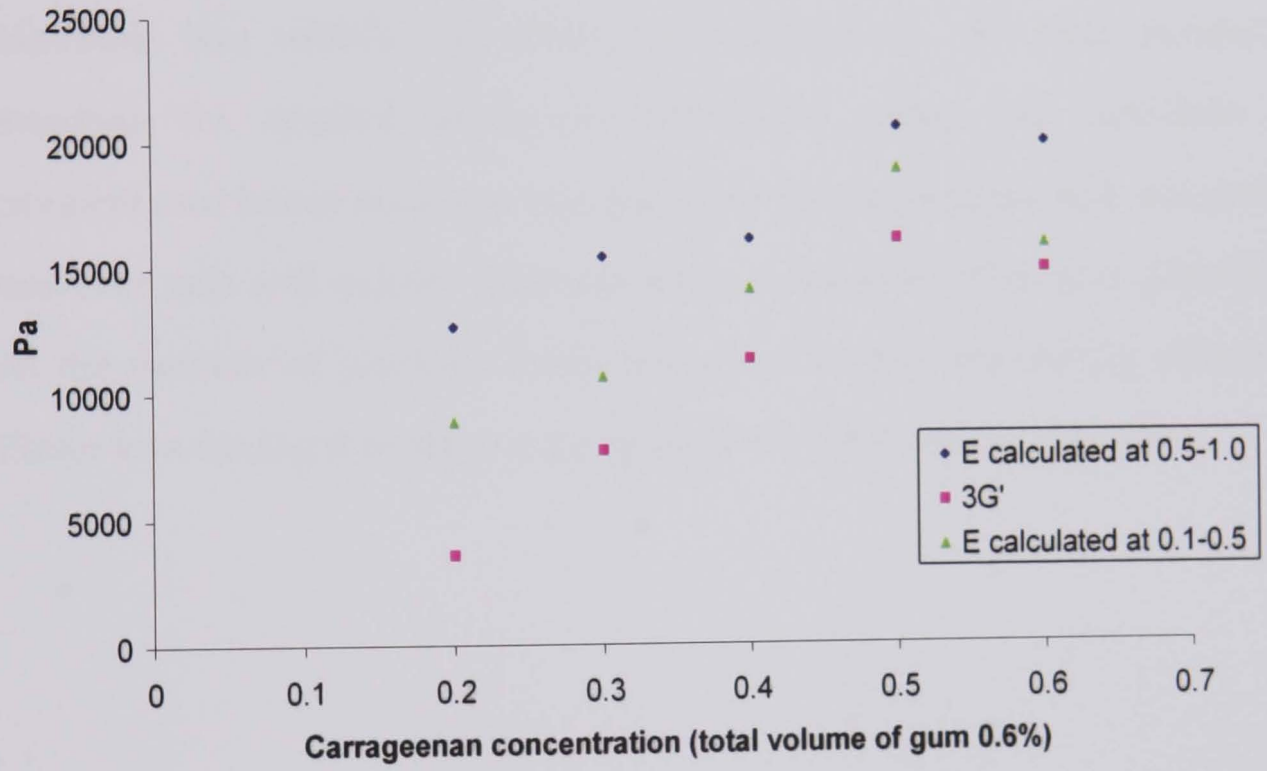


Figure 20. Comparison of Young's Modulus values when different initial slopes are used for calculating. On the X axis the concentration of carrageenan is plotted and the rest of the concentration up to 0.6% is made up from LBG

2.2.2.3.2 Stress relaxation

As mentioned in Chapter 1, in this type of experiment a constant strain is applied and the force required to maintain the applied strain is measured over time. In this case the gel was compressed to a strain of 0.2 (Δl/Lo) at a constant speed of 2mm/s and the decay in force with time followed for 300s (more details on the experimental parameters are given on Table 2). In Figure 21 some different stress relaxation behaviours can be observed. Materials like rubber will show no reduction in the force needed to maintain the applied stress (in vulcanised rubber the crosslinks are covalent and hence none are lost during stress application and relaxation), however gels will exhibit different stress relaxation behaviour depending on the number of junction zones being lost or moving during relaxation Water loss during that stage may account for some stress relaxation.

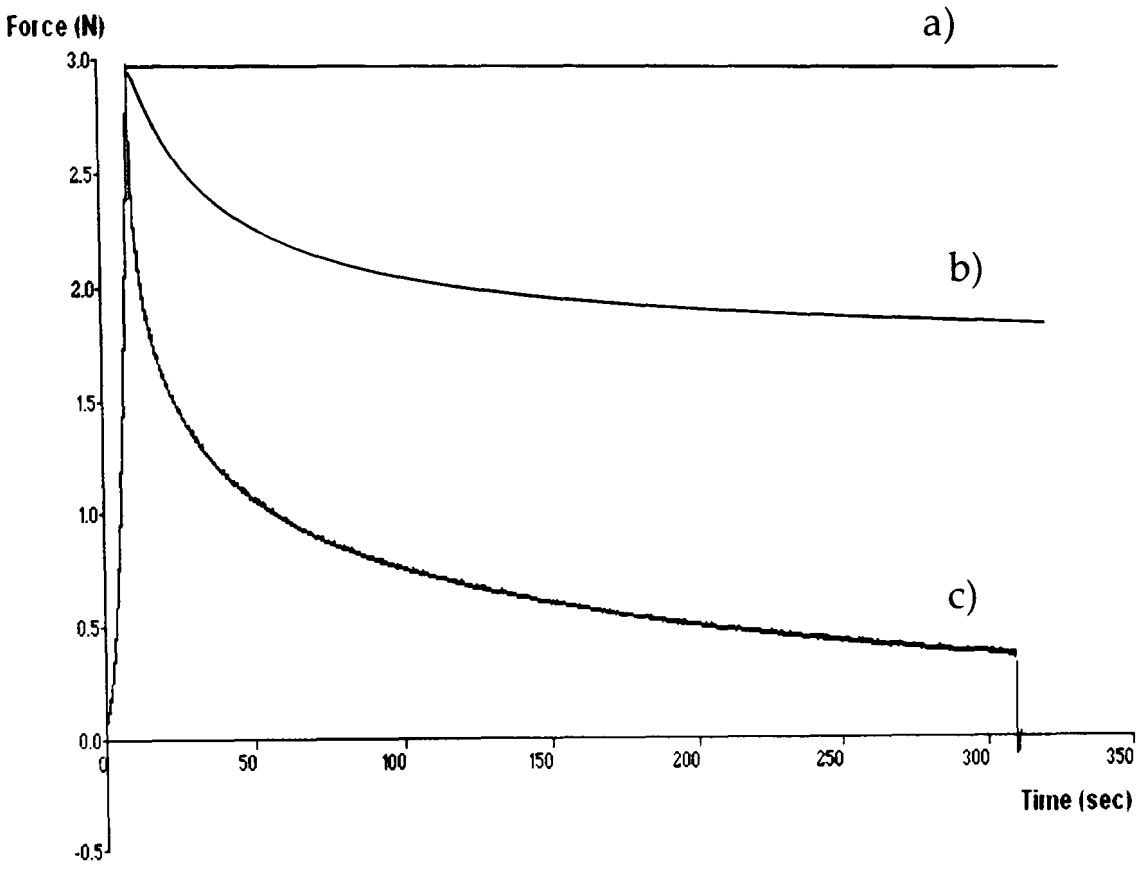


Figure 21. Stress relaxation curve. a) Elastic body(rubber), b) Elastic gel, c) Brittle gel

The stress relaxation curves can be normalized and linearised as described by Peleg (1979) using the relationship given in Equation 25:

$$\frac{F(0).t}{F(0)-F(t)} = k_1 + k_2.t \qquad \text{(Equation 25)}$$

where Fo is the maximum force and F(t) is the force at time t.

The derivation of Fo (Equation 25) is well described by Peleg (1979) and further investigated by Peleg and Normand (1983). In this section the basic principles of the method will be described and also how the parameters attained by the normalization of the data can be used to characterize different gel textures. By applying Equation 25 to the stress relaxation

curve straight lines can be obtained (Figure 22) from which the slope will be identified as k_2 and the point of interception of the Y axis will be k_1. The slope of the straight lines (k_2) cannot be less than 1. From a rheological perspective, the slope can be considered as an index of how 'solid' the compacted specimen is in short time scale (Moreyra and Peleg, 1980). A value of k_2 of 1 will indicate 'liquid' properties of the sample, meaning that the stress will finally reach a value of zero. In contrast any value greater than 1 will indicate that there will be some stresses that will remain unrelaxed. The larger the magnitude of the slope, therefore the higher the k_2, the more solid the sample will be with an ideal elastic body being expressed by $k_2 \dashrightarrow \infty$.

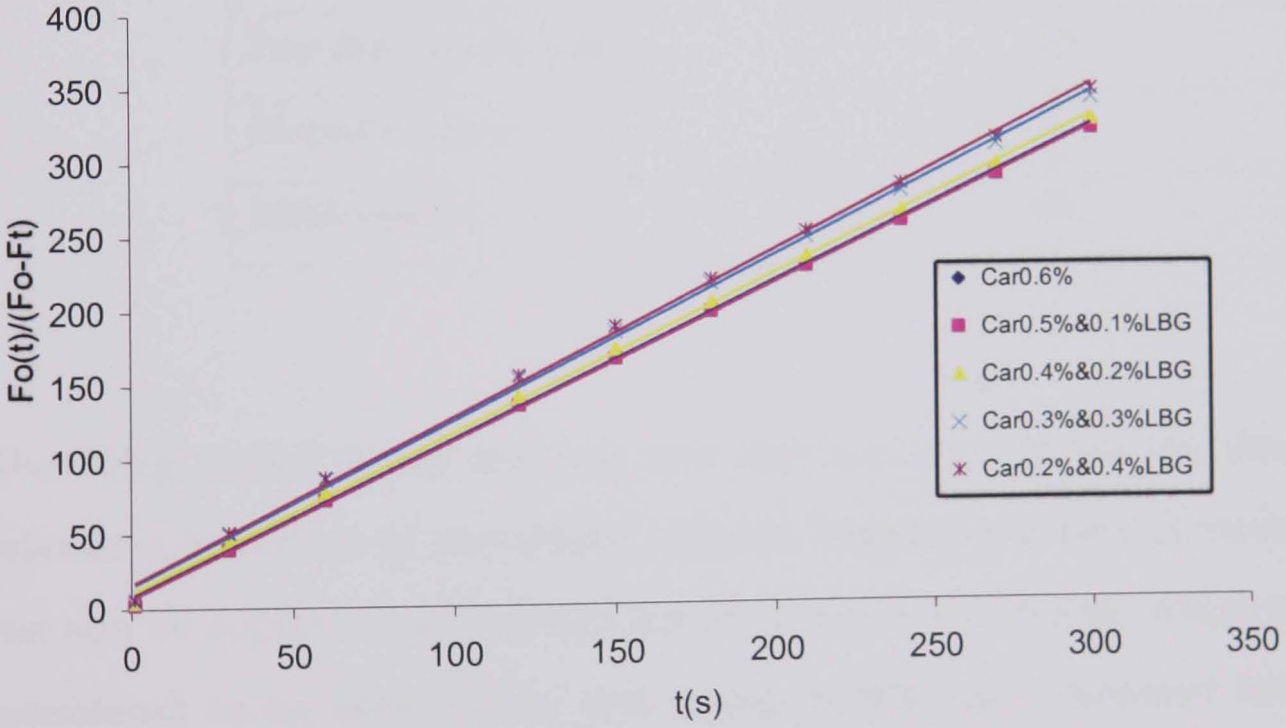


Figure 22. Straight lines attained after normalizing and linearizing the stress relaxation curves according to Equation 25

The k_1 represents the rate at which the stress initially relaxes. The higher the value of k_1 the slower the rate that stress relaxes and similarly to k_2 an

ideal elastic body will have $k_1 \rightarrow \infty$. In terms of the relaxation curve itself a lower k_1 will express steeper descent of the relaxation curve towards the residual value (Peleg, 1979).

Table 2. Texture analysis parameters for stress relaxation tests

Parameter	**Stress relaxation test**
Probe	Circular perpex plunger (40mm diameter)
Load Cell/kg	30
Pre-test speed(mm/s)	3
Test speed(mm/s)	2
Post-test speed(mm/s)	10
Distance (mm)	5
Hold time (s)	300

The Peleg model is not the only one that can characterize the stress relaxation behaviour of viscoelastic material. Massless mechanical models can also be applied. These models are composed from springs, which are considered to be ideal solids, and dashpots that are considered ideal liquids. The springs will account for the elastic properties of the material and the dashpots for the viscous properties. The ways that the springs and dash pots are combined gives rise to different mechanical models (DelNobile, 2007). In particular the most common models used are the

Kelvin-Voigt model and the Maxwell model (Figure 23). These can be combined various ways.

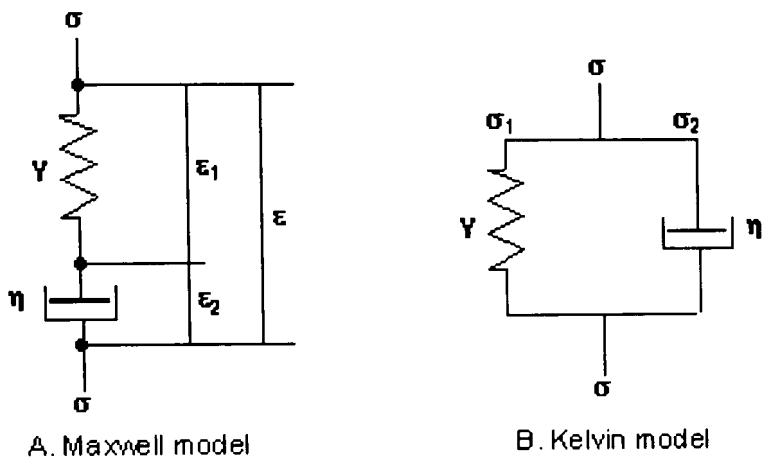


Figure 23. Elements of A) Maxwell model and B) Kelvin-Voigt model

The Kelvin-Voigt model is composed of a dashpot and spring in parallel. It is the basis of development of models to describe creep behaviour, although the creep behaviour of biological materials is better described by the Burger model which is composed of Kelvin-Voigt and Maxwell elements in parallel. Mitchell and Blanshard (1976), have demonstrated that it is possible for a model consisting of one Maxwell element in series with 2 Kelvin-Voigt model to describe the viscoelastic behaviour of alginate gels.

The Maxwell model consists of a Hookean spring and a Newtonian dash pot in series and is suitable for understanding stress relaxation data however it does not predict an equilibrium stress. In order to describe the stress relaxation of food the general Maxwell model is used where several elements are connected in parallel with a spring (Steffe, 1996).

A comparison of three popular stress relaxation models was performed by Hassa et al. (2005). They used the generalized Maxwell , Nussinovitch and Peleg model for describing the viscoelastic properties of eight date cultivars and their Khalal (balah) and rutab stages of maturity and they found that all models were valid for quantifying the relaxation behaviour of the products.

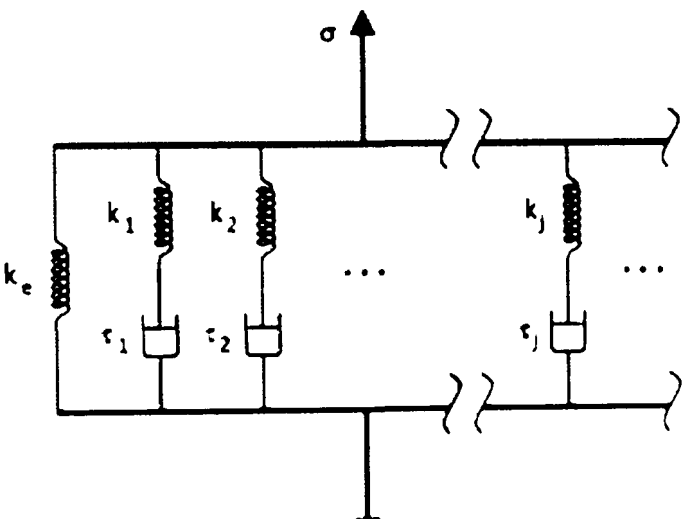


Figure 24. The Generalised Maxwell model takes into account that relaxation does not occur at a single time, but at a distribution of times. Due to molecular segments of different lengths with shorter ones contributing less than longer ones, there is a varying time distribution and this is exhibited by having as many spring-dashpot elements necessary to account for this distribution. A single spring element is included to account for an equilibrium stress.

2.2.2.3.2.1 Degree of Compression

One important factor that may influence the values attained for k_1 and k_2 is the degree of compression (Figure 25). This has already been investigated by Winwood et al. (1985) and they concluded that the gels appear to be more viscoelastic (lower k_2 values) at higher degrees of compression. The cause for this could be internal failure occurring in the gels at the higher degrees of compression, which has been discussed by Peleg et al, (1979).

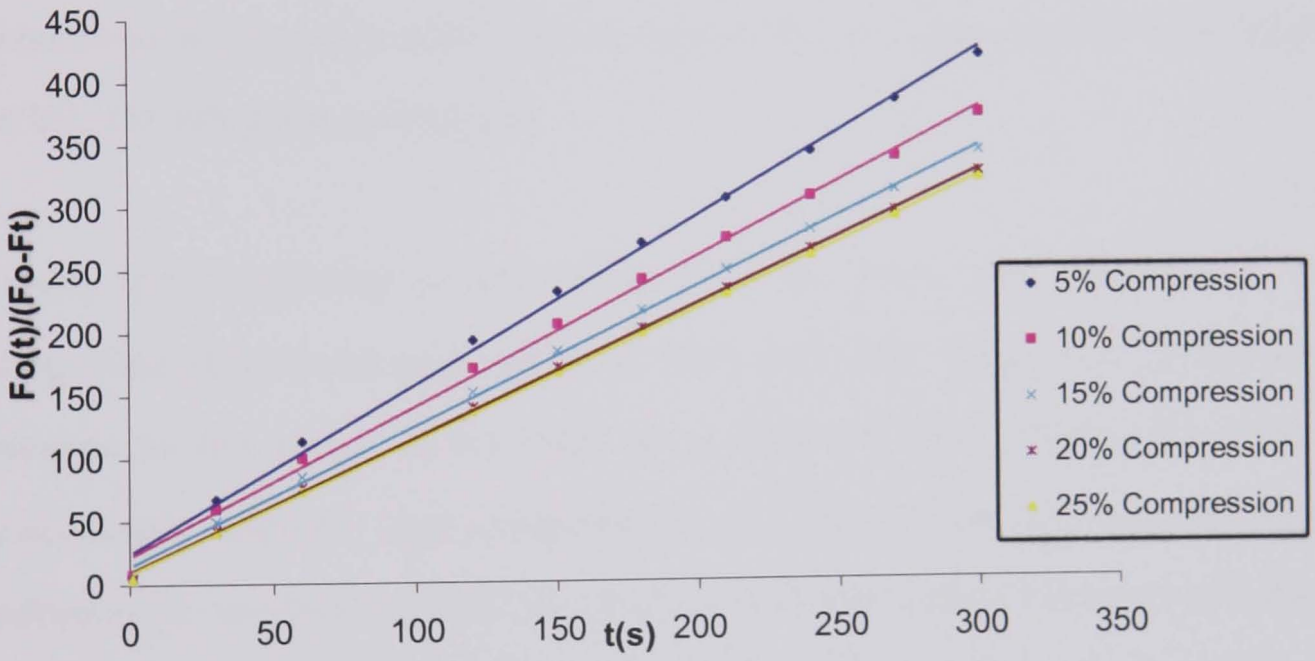


Figure 25. Effect of degree of compression of κ-carrageenan gels on the relationhsip used to obtain the paramaters k_1 and k_2

Figure 25 demonstrates how an increasing degree of compression will alter the slope as well as the point of intercept for the same gels. Therefore at higher degree of compression the gels will appear more viscoelastic. However, if the same degree of compression is applied to a series of

different gels then firstly the results will distinguish between the gels and secondly the ranking order will be the same regardless of the degree of compression.

2.2.2.3.3 Dynamic rheology

Dynamic oscillatory experiments were performed using a controlled stress rheometer (Bohlin Rheometer CVO) using a concentric cylinder ribbed geometry (C14 geometry). The ribbed geometry assures that the slippage effect will be reduced to a minimum. The hot samples prepared as described earlier were added to the rheometer and the samples were aged at 4°C for 24h prior to analysis.

Firstly an amplitude sweep from 0.1-10% strain was performed to determine the viscoelastic region at 25°C and 1Hz. Afterwards frequency sweeps in the range of 0.1-10Hz at a strain of 0.1% (within the linear viscoelastic region) and temperature of 25°C were carried out. The parameters obtained were G′, G″ and tan δ. Temperature sweeps were also carried out with at 1°C per minute from 25°C -90°C at a frequency of 1Hz.

2.2.2.4 Flavour release analysis

2.2.2.4.1 Nose-space analysis

The breath volatile composition was monitored using a platform II Quadrupole mass spectrometer (Micromass, Altrincham, UK) fitted with a modified atmospheric pressure chemical ionisation source. The details of the interface are described in a European Patent application and in a US patent (Linforth and Taylor, 1998, 1999). A plastic tube was fitted in one of the nostrils of the panellists. As the panellists consumed the gel samples (2.0g +/- 0.1g), a small proportion of their breath was drawn into the API source (Linforth and Taylor, 1998) at a flow rate of 35ml/min through a heated (60°C) deactivated fused silica transfer line where it was ionised by a 4 kV corona discharge to yield characteristic ions. The mass spectrometer was used in positive ion mode, with a cone voltage of 18 V, and set to monitor m/z117 and m/z59, which are the ions yielded by ethyl butyrate and acetone respectively (acetone is present in breath and is monitored to check when the panellist swallowed and if they were breathing normally, Hodgson et al., 2003). The panellists were given the sample on a spoon and instructed to breath in, place the sample in their mouth, chew the sample 10 times, swallow, and breathe out and continue to breathe normally for 1 min. Three replicates of each gel were consumed. From the chromatogram produced by the mass spectrometer the maximum volatile intensity (Imax) for each sample was determined and the cumulative area under the curve (CAUC) was determined by integration.

2.2.2.4.2 Headspace analysis

Gels were prepared as for the in-nose analysis and 50ml of gel was placed in 100ml bottles and sealed with headspace cups. The gels were aged at 4°C for 24h prior to analysis. Air was drawn from the samples headspace into the API source at a flow rate of 2.5ml/min as describe in section 2.3.3. Four replicates per sample were analysed.

2.2.2.4.3 Na^+ release

The gel cylinders were placed in a cylindrical container of 62mm diameter and then subjected to a Texture Profile Analysis (TPA) two bite experiment to a strain of 0.75 and a probe speed of 2mm/s and immediately afterwards 50ml of distilled water (20°C) was poured into the container and the release of Na^+ was monitored with an ion specific electrode for Na^+ (Microelectrodes, Inc) connected to a Jenway pH meter. Special care was taken to place the electrodes on the side of the container close to the surface of the water and not in contact with the ruptured gel. The value was recorded every 2 seconds for 5 min. The experiment was carried out at ambient temperature (~20°C).

2.2.2.5 Statistical analysis and experimental design

A general factorial design was used for the design of the experiment. Design Expert 6.0.2 was used to provide the design of the experiment and the randomization of the samples. The data were subjected to Analysis of Variance (ANOVA) to determine if significant differences existed in terms of the rheological parameters, aroma release parameters (Imax, CAUC) and Na^+ release. Where a significant effect was found a Tukey test was used to identify which samples were significantly different to the others. Pearson correlations coefficients were calculated between the mean instrumental measures of Texture Profile Analysis and Na^+ Release. All the statistical analysis was performed with SPSS 13.0 for windows.

Chapter 3

Texture of gels and flavour release

3.1 Introduction

This chapter will be broken down into two major parts. The first one will involve looking at the rheological characterisation of the two mixed systems; κ-carrageenan&LBG and mixtures of LA-&HA-gellan. The results obtained for the parameters that have been described in the previous two chapters will be discussed followed by a comparison of the two systems. Afterwards a brief description of flavour will guide the reader towards the relationship that exists between texture and flavour release.

3.1.1 Rheological characterisation

3.1.1.1 Introduction

Both of the mixed systems used in the present study have been investigated quite extensively in terms of their rheological parameters. It was the aim to obtain a multidimensional characterisation of the gel systems that will then be correlated with odour and tastant release.

3.1.1.2 κ-carrageenan and LBG

Looking firstly at the κ-carrageenan and LBG system it was generally accepted that if part of the κ -carrageenan is replaced with LBG, the gel strength as indexed by the G′ and Young's modulus (E) increases with increasing LBG concentration and then decreases, creating a peak which denotes the so-called 'synergistic' effect. However the ratio at which the synergistic G′ peak occurs has been found to vary from 8% (Arnaud et al., 1989) to 70% (Stading and Hermansson, 1993) probably due to the different total polymer and KCl concentrations used.

Fernandez et al. (1991) studied various samples of LBG and reported the optimum κ-carrageenan /LBG ratio to be 4:1 in all types of LBG samples and KCl concentrations. The synergy also varies, for example the Young's modulus for the optimum mixture was found to be 1.3-2 times higher than that of carrageenan alone (Turquois et al., 1995; Arnaud et al., 1989).

However more recently Chen et al. (2001) and Dunstan et al. (2001) found that the introduction of LBG showed no synergistic effect in terms of gel rigidity (E and G′) at any KCl concentration when the total polymer concentration was kept constant, however there was clear evidence for synergy from measurements of failure properties.

In Chapter 1 it has been discussed that the Young's modulus and G′ should satisfy the relationship E=3G′ (Equation 22), however G′ values are often reported to be significantly lower than expected from this

relationship. The inconsistent results could be attributed to various reasons. A possible explanation could be the syneresis of the κ-carrageenan gel, from which the released water creates slip when conducting dynamic measurements. A much higher G′ has been reported when a specially designed perforated concentric cylinder geometry was used to avoid slippage effects (Richardson and Goycoolea, 1994). Winwood et al. (1985) have reported G′ values at 33,000 Pa from a 1.2% κ-carrageenan gel which was glued between two parallel plates. Dunstan et al. (2001) have reported a significantly higher G′ in the range of 10,000-30,000 Pa for a 1% κ-carrageenan gel, which was glued between two parallel plates in a similar way to the Winwood study. Another possible explanation for the significantly lower values of G′ could be the fact that E reported is higher than the true value due to the initial slope used to calculate it (see Chapters 1 and 2).

3.1.1.3 High and low acyl mixed gellan gels

High acyl gellan gels have a lower modulus than low acyl gels because the bulky acetyl and glyceryl groups prevent close association between the gellan polymer chains in double-helix formation and hinder compact packing of the cross-linked double helix. The completely deacetylated product forms very high modulus brittle gels (Baird et al., 1992). Characterisation of LA-gellan by uniaxial compression and stress relaxation has been carried out by Nussinovitch et al. (1990).

Sanderson et al. (1988) reported intermediate textural properties between those of high and low acyl gellan gels when combining low acyl gellan with high acyl gellan to form mixed gels. Mixed gels are much more deformable but exhibited similar compressive strength when compared to low acyl gellan gels (Mao et al., 2000).

3.1.2 Flavour release

The perception of flavour is considered as a combination (in the brain) of two senses, the sense of smell and the sense of taste. Therefore flavour can be broken down into two major components, the volatile compounds that are sensed by the olfactory epithelium (aroma) and the non-volatile compounds that are sensed by the taste buds on the tongue (taste). As a food is being consumed there are various factors that may influence the release of the volatile components as well as the tastant. These factors include structure break up on mastication and mixing with saliva. The work described in this chapter addresses the role of these factors for hydrocolloid gels. In a following chapter solutions will be considered.

Many studies have been carried out to investigate the effect of texture of hydrocolloid gels on flavour release with the outcomes not always being consistent. A significant number of the studies involved the use of atmospheric pressure chemical ionisation – mass spectrometry (APCI-MS). This allows the volatile release in the air expired by people while consuming a food to be followed. A nosepiece is inserted in one of the nostrils which samples air from the nose and ionises the volatile

compounds that are present by atmospheric pressure chemical ionisation. The ions that are formed are then detected in a quadropole mass spectrometer (Taylor et al., 2000).

By using APCI-MS Baek et al. (1999) reported that softer gelatin gels released larger concentrations of a volatile compound than harder gelatin gels. Boland et al. (2006) showed that gelatin gels showed higher static headspace concentrations of strawberry flavour, but decreased flavour release compared to pectin gels and when the rigidity of the gels increased, the air/gel partition coefficients decreased and the maximum in nose concentration increased. Boland et al. (2004) investigated gelatin, starch and pectin gels and they observed that flavour release was significantly affected by the texture of the gels: the gel that exhibited the highest Young's modulus of elasticity, gelatin gel, showed the lowest flavour release. Starch and pectin also showed differences in flavour release. Moreover Guinard and Marty (1995) found that firm gelatin and carrageenan gels released flavour with a lower Imax than soft or medium gels and their study also concluded that both texture and gelling agent affect flavour release. The above mentioned studies focused on the measurement of aroma release. Morris (1994) related perceived sweetness and flavour taste release to gel rheology and found a strong negative correlation between the magnitude of the strain and break and perception. Morris (1994) proposed as a likely release mechanism of taste in mouth to exposure of fresh surfaces by fracture of the gel in chewing. Bayarri et al. (2001) investigated the diffusion of sucrose and aspartame in κ-carrageenan and gellan gum gels and found that the hydrocolloid used

had an effect on the diffusion of the tastants. This was higher in κ-carrageenan gels and also diffusion was higher in low modulus compared with high modulus gels. It would be of value to predict how changes in the gel composition are likely to impact upon the flavour release (Cook et al., 2005).

The aim of the present study was to further investigate the hypothesis that the dominant flavour release perception is the release of the tastant from the hydrocolloid matrix. For gels this will be dominated by the brittleness of the gel and for solutions by the efficiency of mixing. Of particular interest is gellan gum which by combining low acyl and high acyl variants can give a very wide variety of textures.

3.2 Results and Discussion

3.2.1 Rheological characterisation

Gels were prepared as described in section 2.2.2.2 . To allow detection of Na^+ 11.54g of NaCl were added to 1 litre of buffer solution. The two bite experiment followed by detection of released NaCl was carried out as described in section 2.2.2.4.3. Figure 23 displays the two bite response for gels of different texture.

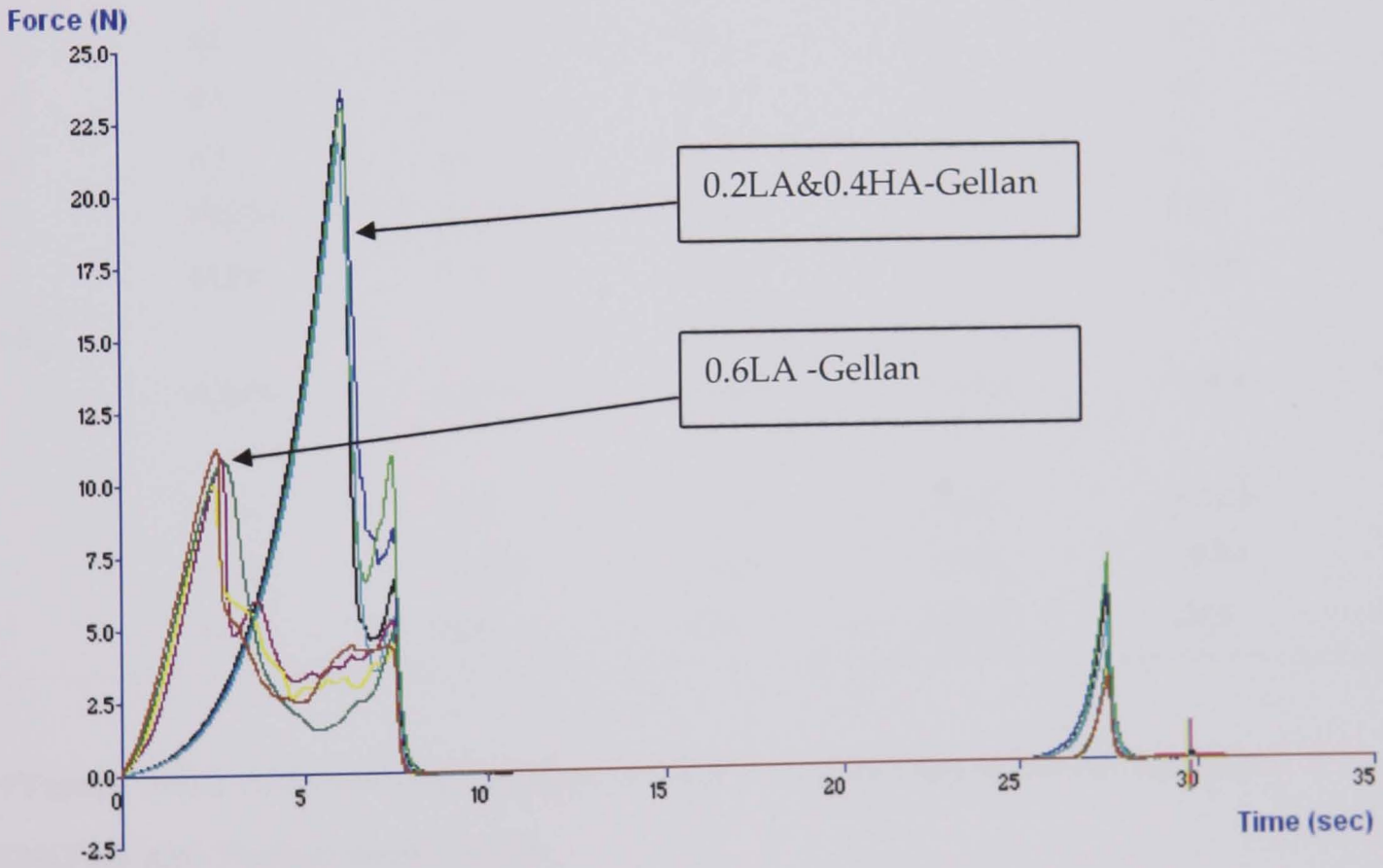


Figure 26. TPA spectra of the gels that exhibited the two extreme textures 0.6%LA-gellan and 0.2%LA-&0.4%HA-gellan

Table 3 and Table 4 show the composition and some rheological properties of the mixed gels studied. The gellan system appears to have a more diverse set of texture than the κ-carrageenan&LBG system. This will be discussed further later on.

Table 3. Composition of the gellan gels per 1000g. Rheological parameters

	gellan gel				
LA-Gellan(g)	6.00	5.00	4.00	3.00	2.00
HA-gellan(g)	0.00	1.00	2.00	3.00	4.00
Water(g)	968.20	968.20	968.20	968.20	968.20
KCl(g)	10	10	10	10	10
$KHPO_4$(g)	9.1	9.1	9.1	9.1	9.1
K_2HPO_4(g)	6.1	6.1	6.1	6.1	6.1
E[N/m2]	72378^{a}	29023^{b}	18525^{c}	11791^{d}	7533^{e}
Force at rupture[N]	12.39^{a}	8.75^{a}	11.91^{a}	12.32^{a}	20.39^{b}
Strain at rupture	0.2553^{a}	0.2790^{a}	0.3931^{b}	0.4703^{c}	0.5571^{d}
k_1	2.31^{a}	7.38^{a}	21.10^{b}	38.59^{c}	59.271^{d}
k_2	1.044^{a}	1.056^{ab}	1.106^{b}	1.283^{c}	1.771^{d}
G'(Pa)	24500	8453	7381	4330	3015

$^{a-e}$Values with different superscripts within a row are significantly different, ANOVA and Tuckey tests, $P<0.01$

Table 4. Composition of κ-carrageenan&LBG gels per 1000g. Rheological parameters

	κ-carrageenan and LBG gels				
κ-car	6.00	5.00	4.00	3.00	2.00
LBG	0.00	1.00	2.00	3.00	4.00
Water(g)	968.20	968.20	968.20	968.20	968.20
KCl(g)	10	10	10	10	10
$KHPO_4$(g)	9.1	9.1	9.1	9.1	9.1
K_2HPO_4(g)	6.1	6.1	6.1	6.1	6.1
E[N/m2]	16629^{a}	15543^{a}	12928^{b}	9916^{c}	8941^{c}
Force at rupture[N]	9.79^{a}	13.68^{bc}	15.21^{c}	18.18^{d}	13.01^{b}
Strain at rupture(mm)	0.4008^{a}	0.449^{a}	0.5164^{b}	0.5578^{b}	0.5433^{b}
k_1	9.18^{a}	8.00^{a}	11.04^{b}	15.43^{c}	15.18^{d}
k_2	1.051^{a}	1.037^{ab}	1.063^{b}	1.106^{c}	1.113^{d}
G′(Pa)	4990	5400	3800	2590	1200

[a-e]Values with different superscripts within a row are significantly different, ANOVA and Tukey tests, P<0.01

Very brittle gels were produced from 0.6%LA-gellan (high values of E and G′ and low strains at rupture, low k_1 and k_2) whereas 0.2%LA-&0.4%HA-gellan had much lower values of E and G′, high strains at rupture and higher k_1 and k_2. Mixed κ–carrageenan&LBG gels showed a narrower set of different textures ranging from brittle (0.6% κ -carrageenan, high E and G′, low strain at rupture) to elastic gel (0.2%κ-carrageenan&0.4%LBG, lower E and G′ high strain at rupture). For example E (Figure 27) divides the gels into five different groups (subsets from the Tukey test) which differ significantly whereas for the κ-carrageenan&LBG it separates the

gels into three groups. The same sub-divisions are also observed for the strain at rupture.

Also from Table 4 it can be observed that no synergistic peak is essentially observed for E and G′ (a slightly higher value is observed for 0.5%κ-carrageenan&0.1%LBG for G′) which is in agreement with observations from Chen et al. (2001) and Dunstan et al. (2001) that no synergistic peak is observed for E and G′. Furthermore the force at rupture and the strain at rupture are exhibiting peaks at 1:1 κ-carrageenan to LBG ratio.

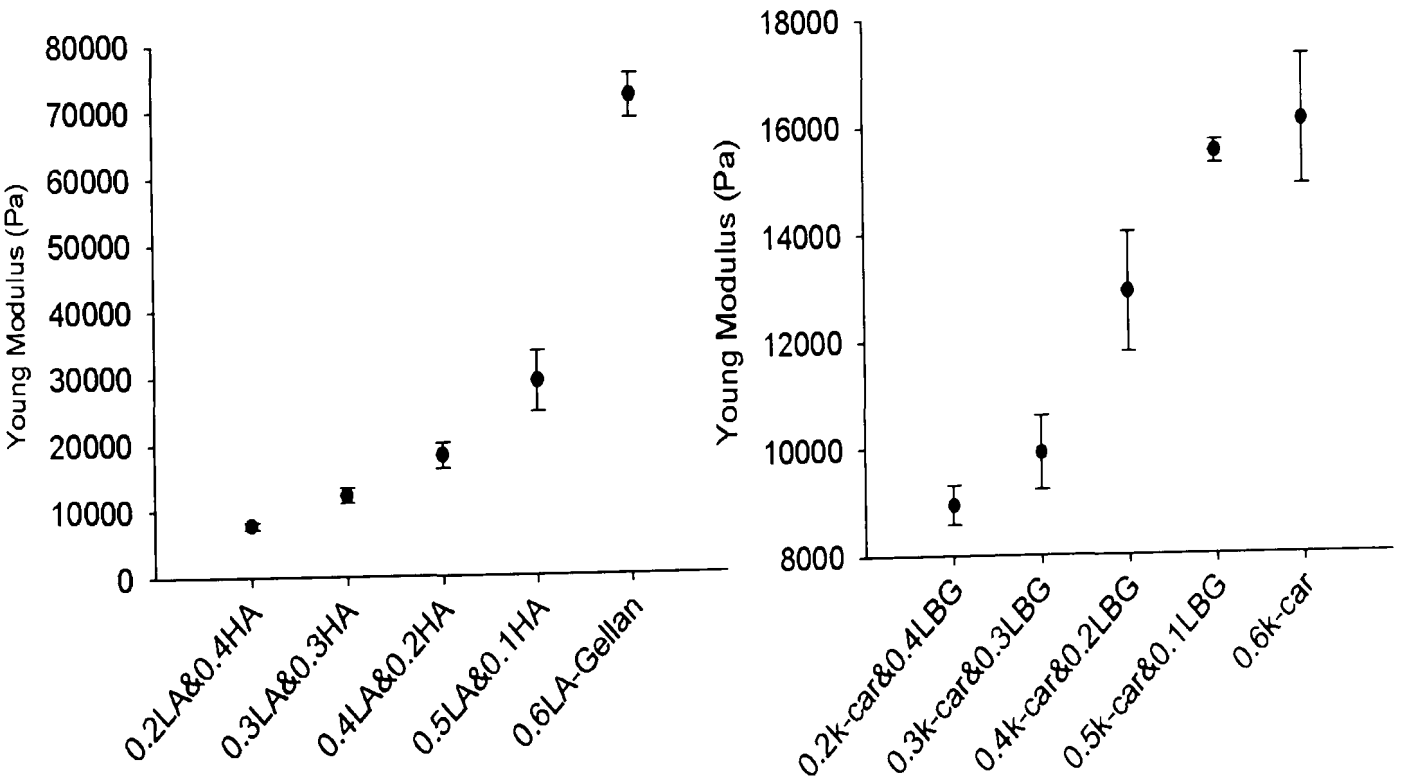


Figure 27. Young's Modulus for κ -carrageenan&LBG and LA-&HA-gellan gels

Finally although a good correlation between the compression data and data obtained from oscillation experiments was observed as reported in

Chapter 2 the relationship E = 3G′ does not hold for the gels with lowest moduli for both systems. The reason for this is not clear. Stress relaxation experiments carried out on these gels showed a substantial decay in the modulus with time i.e. the gels do not behave as perfect elastic solids. This would suggest that the modulus measured from the initial slope of the compression test would be dependent on the rate of compression. Thus the compression test could/would address different frequencies/times within the viscoelastic spectra to the oscillatory measurement. Alternative interpretations are that for the weaker gels the initial slope is less well defined and thus more prone to error and there is departure from perfect cylindrical geometry for these weaker gels.

3.2.2 Flavour Release

The headspace analysis yielded no significant differences between the different gels.

Figure 28 which displays aroma release, via in nose APCI-MS for the two carrageenan&LBG gels with extreme textures and three of the mixed gellan gels suggests that aroma release as a result of chewing in the mouth is greater for the more brittle gels. However statistically significant differences in Imax were only seen within the gellan system where the 0.6%LA-gellan and 0.4%LA-gellan gels were significantly different from the 0.2%LA-gellan both for Imax and CAUC (Cumulative Area Under the Curve). The whole range of gel mixtures was not analysed via APCI-MS,

mainly due to the poor palatability of samples which did not allow the whole range to be fed to the panellists.

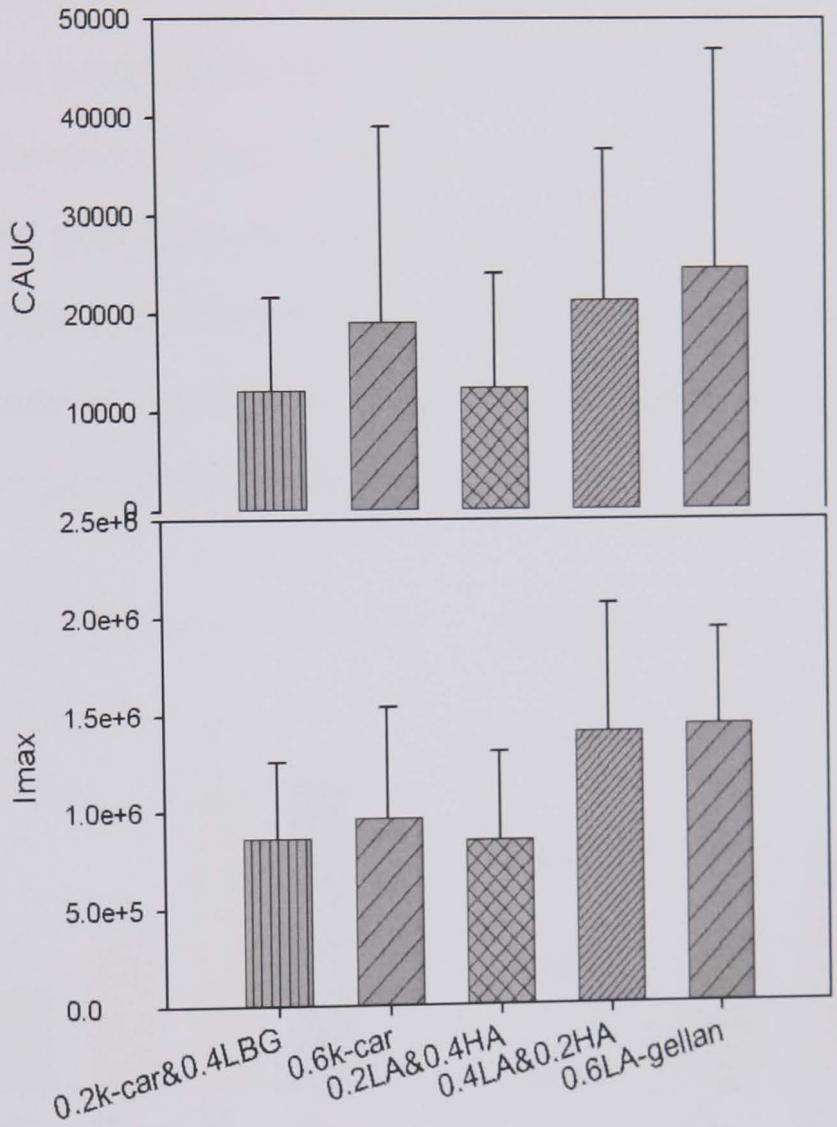


Figure 28. Comparison of Imax and CAUC for κ-carrageenan and gellan gels

The TPA spectra of the gels that had the two extreme textures 0.6%LA-gellan and 0.2%LA-&0.4%HA-gellan are shown in Figure 26 where the different behaviour of the gels can be clearly observed. Figure 29 displays the Na^+ concentration measured 20s after addition of water following TPA compression for the eight different gels). Although both Na^+ release and

Imax correlate negatively with the strain at rupture as illustrated in Figure 30 and Figure 31 the negative correlation was stronger for the salt release (r^2=-0.87) than for Imax (r^2=-0.55). Na^+ also correlated negatively with the peak force from the first and second bite measured from the TPA experiment and positively with E (Table 3). The apparent lack of a strong relationship between Imax during consumption and gel texture is consistent with previous work (Baek et al., 1999, Weel et al., 2002, and Boelrijk et al. (quoted in Renard et al., 2006). There is evidence that the rate of aroma release as opposed to Imax does correlate with gel texture and indeed sensory perception (Baek et al., 1999).

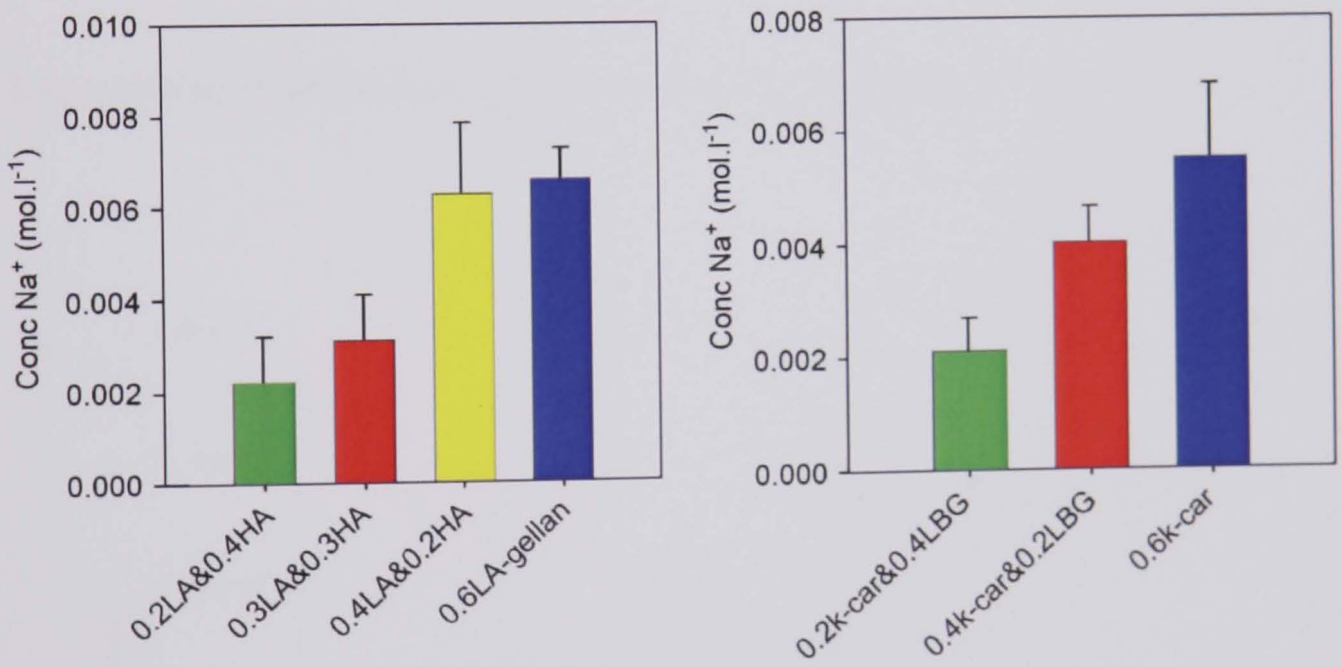


Figure 29. Na+ concentration measured 20 s after addition of water after TPA.

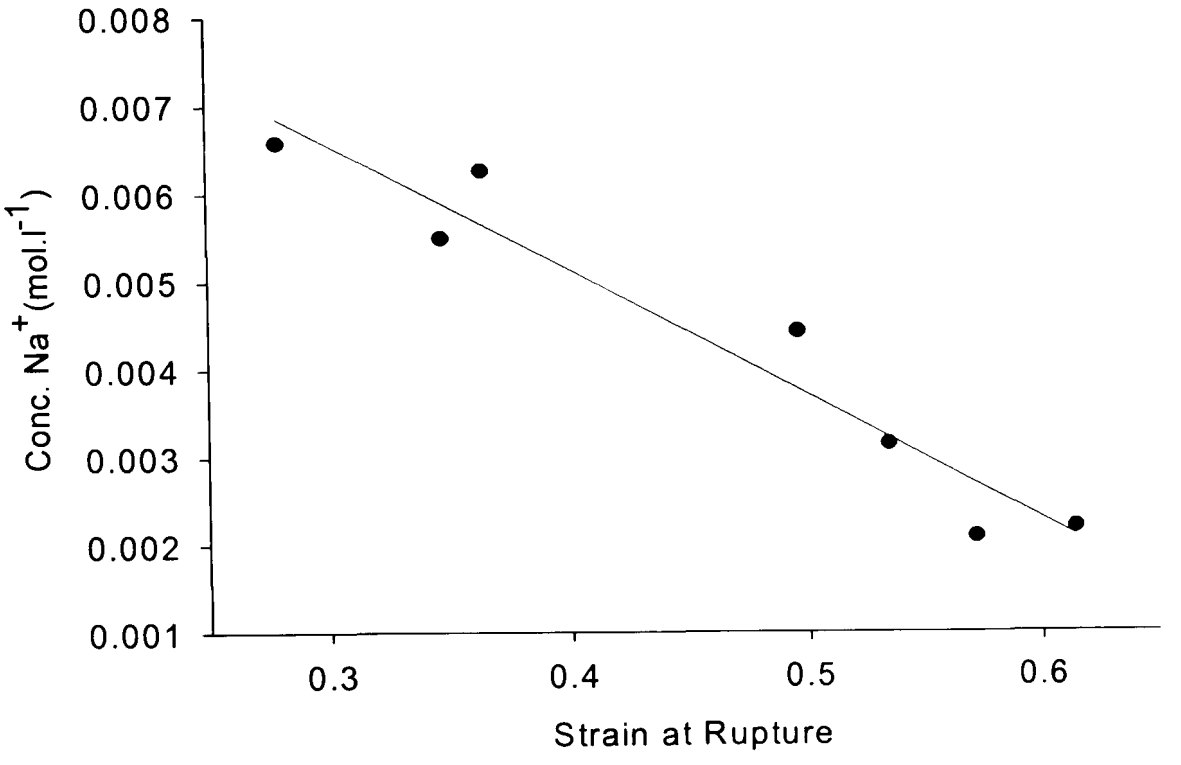


Figure 30. Correlation between Na+ release and strain at rupture

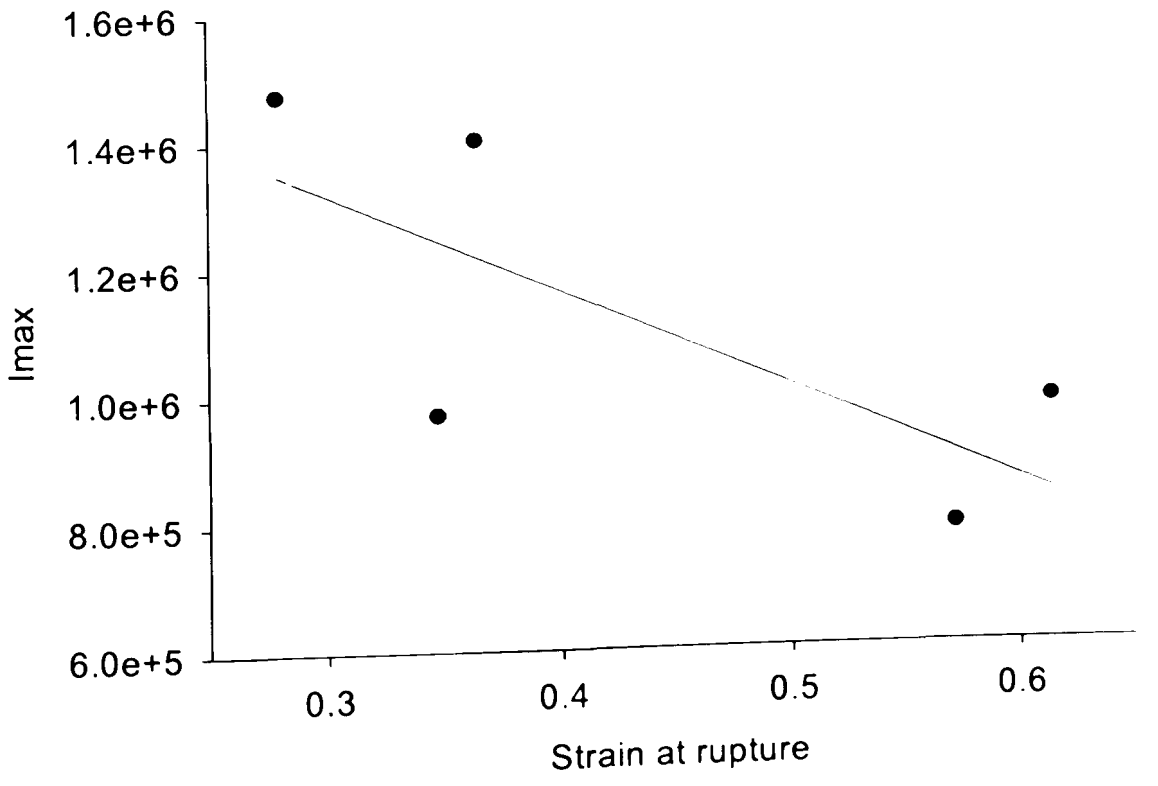


Figure 31. Correlation between Imax and strain at rupture

Table 5. Correlations of Na^+ release with rheological parameters.

		Strain	Force1st	Force2nd	E
Release	Pearson				
of	Correlation	-0.8695**	-0.7527**	-0.6468**	0.5968**
Na^{++}	Sig.(2-tailed)	1.91E-09	3.81E-06	0.0002	0.000801
	N	28	28	28	28

**** Correlation is significant at the 0.01 level**

As mentioned in the introduction to this chapter, in a study that is not often referred to, Morris (1994) reported a clear inverse relationship between strain at break and the sensory perception of flavour from gels (see Figure 32) and suggested that this was a consequence of the greater exposure of surface on chewing for the more brittle gel. The relationship was similar for perception of both sweetness and flavour sensory attributes.

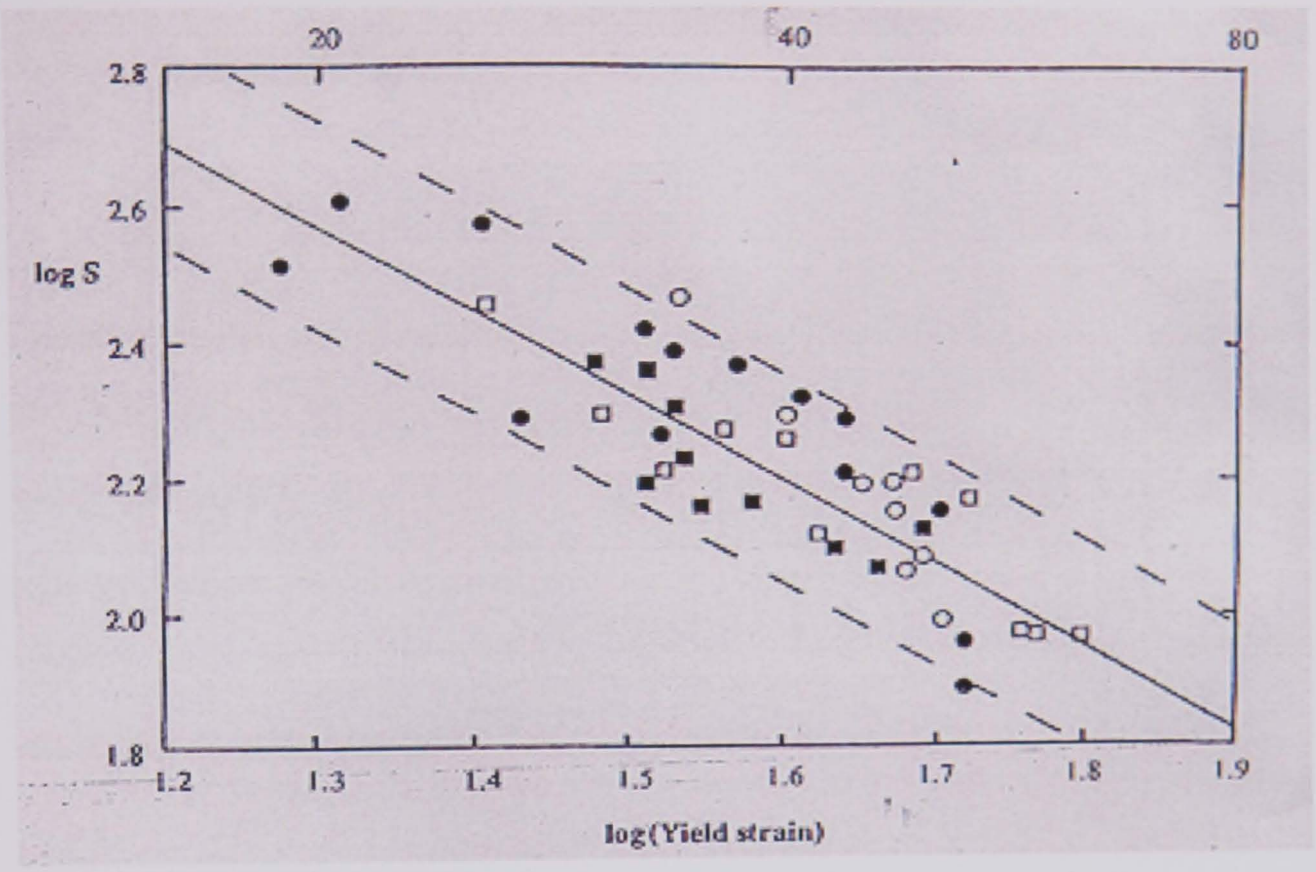


Figure 32. Variation of perceived sweetness and flavour intensity with yield strain for gels of gellan gum, kappa carrageenan, alginate calcium and xanthan/LBG (Morris, 1994)

Our work strongly suggests that the release of the tastant rather than the more frequently reported aroma release/binding is the dominating factor in this relationship and in the magnitude of perceived flavour intensity from gels. The effect of texture on the overall magnitude of flavour perception is a consequence of the well recognised sensory bimodal interaction between taste and aroma e.g. Hort and Hollowood (2004). The more extensively studied aroma release/binding must influence balance of flavour and there is evidence to suggest that it influences the rate of perception.

3.3 Conclusions

A multidimensional characterisation of gels has been achieved. A wide range of textures was produced for both systems, however it is apparent that there is a much wider range of textures achievable for mixtures of LA- and HA-gellan compared to κ-carrageenan and LBG, for the same concentration of polysaccharide. It is important to note that gels of LA-gellan are significantly more brittle than gels that contain equivalent amounts of κ-carrageenan.

For gels the decrease in the magnitude of flavour perception is a consequence of a reduction in the release of the tastant to the receptors in the mouth. This appears to be more important than aroma release. Brittle gels (low strain at rupture) show enhanced release of tastant and volatiles as a result of the increased surface area following fracture during chewing. Gelatin gels although not brittle have always been considered to give good flavour perception and mouthfeel if consumed under conditions where they melt in the mouth and this will be investigated in the following chapter.

Chapter 4

Solutions and flavour release

4.1 Introduction

This relatively small chapter will deviate slightly from dealing with gels and will briefly examine solutions. However the solutions that are going to be considered are directly related with the gels used in the petfood industry and that were also under investigation in this study. The composition of the gel matrix in petfood consists of the added polysaccharide and gelatin that leaks from the meat chunks (Jones 2004). Therefore the solutions that will be examined are LBG solutions (a primary constituent of the κ-carrageenan gels) and gelatin solutions.

For solutions it has frequently been reported that intensity of flavour perception decreases with increased viscosity. Baines and Morris (1989) reported that flavour suppression started to occur at concentrations above the coil overlap (c*) concentration for a range of hydrocolloids (Figure 33) and suggested restricted mixing as a possible interpretation. However studies by Hollowood et al. (2002) and Cook et al. (2003) have shown that the aroma release at concentrations above c* remains unaffected.

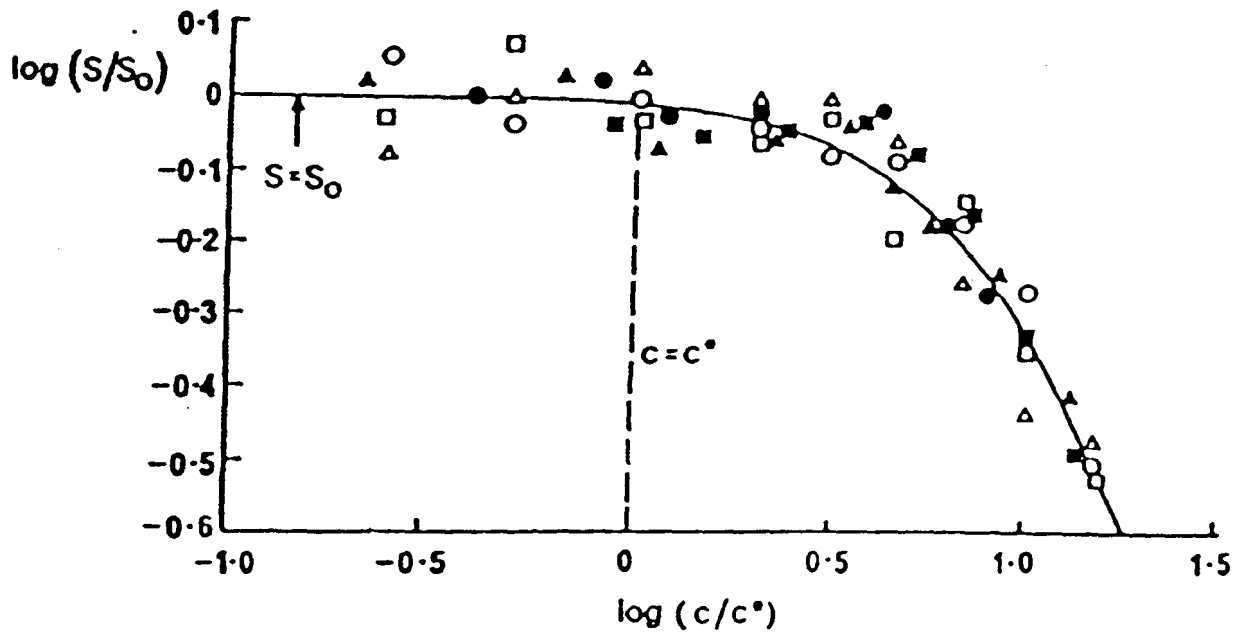


Figure 33. Variation in perceived flavour S (magnitude estimation) scaled to So (no hydrocolloid present) with hydrocolloid concentration scaled to c* (Baines and Morris, 1989)

Ferry et al. (2006a) have shown that solutions thickened by starches that exist in solution as swollen granules show little flavour inhibition at high viscosities in contrast to other polysaccharide thickeners and suggested that this was because such starches mixed very efficiently with saliva resulting in rapid release of tastant.

The objective of this work was to determine if melted gelatin would show the same inefficient mixing behaviour at concentrations above c^* as polysaccharides.

4.2 Materials and methods

4.2.1 Preparation

Distilled water was heated to 90°C and then the gelatin or locust bean gum was added and the solution was mixed for 2min at 16000rev/min (Ultra Turax T-25 basic mixer). Concentration ranges investigated were gelatin (1-30%) and locust bean gum (0.1 to 1.0%). For the mixing experiments the samples that were photographed were prepared in water and for the subsequent salt release experiments in 0.2M (1.17%) NaCl. Visual observations did not show any effect of the salt on the mixing behaviour of the solutions.

4.2.2 Visual observation

5ml of each of the LBG and gelatin solutions, which contained a small amount of red food colouring(1 ml), were carefully added to the bottom of a 50ml glass beaker containing 20ml of distilled water with a plastic syringe (Ferry et al. 2006a). The temperature of the water and the added solutions were at 40°C. The solutions were rapidly stirred by hand with a teaspoon for 2-3 s in a circular motion and photographed afterwards. The concentration of Na^+ close to the top surface was measured with a specific ion electrode as described by Ferry et al. (2006b).

4.3 Results and discussion

Figure 34 shows the appearance of LBG and gelatin solutions approximately 10s after mixing. For LBG there was a marked decrease in mixing efficiency between concentrations of 0.5% and 0.6%, whereas gelatin showed good mixing behaviour up to the highest concentration studied (30%). Studies of the concentration dependence of the viscosity of locust bean gum solutions (data not shown) indicated that the c* concentration where the viscosity starts to show a severe increase is around 0.5-0.6%. Gelatin has a much lower intrinsic viscosity than locust bean gum and previous studies have shown that the c* concentration where there is an inflection in a double logarithmic plot of zero shear viscosity against concentration is about 8% (Wulansari et al., 1998), well below the 30% concentration where good mixing continued to be observed in this study. It has been shown that high viscosity gelatin solutions show Newtonian behaviour (Wulansari et al., 1989). Since disruption of entanglements with shear rate is generally cited as the reasons for the extensive shear thinning of polysaccharide solutions above the c* concentration (Morris et al., 1981) it is reasonable to postulate that a lack of entanglement between neighbouring molecules is the reasons for both the Newtonian behaviour and the good mixing of gelatin solutions.

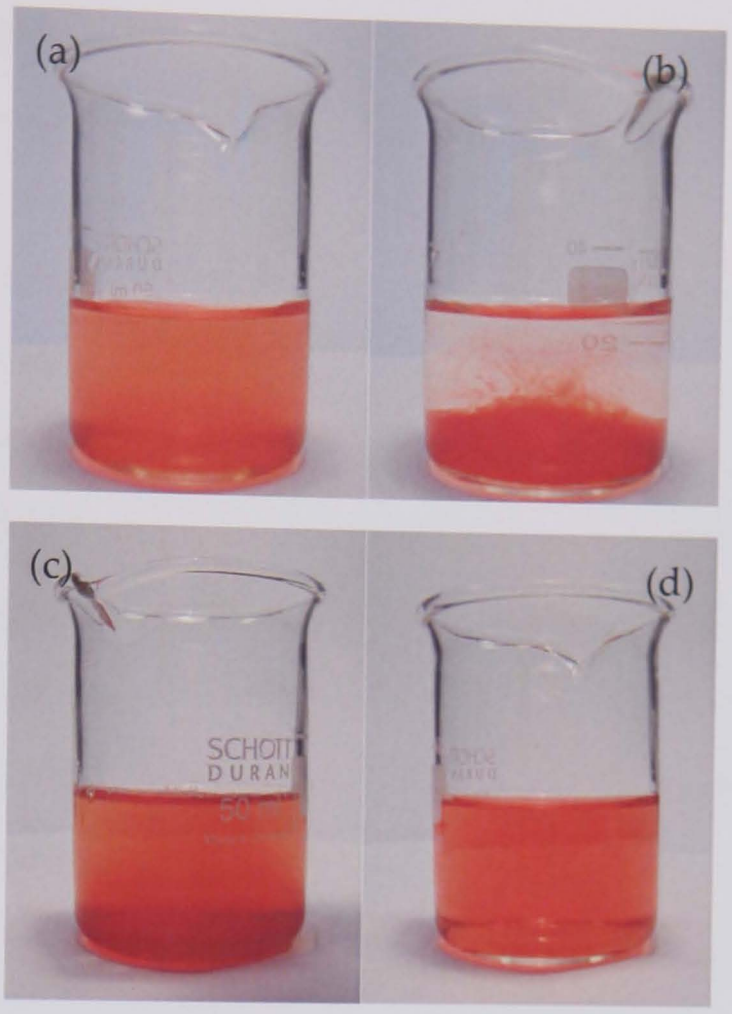


Figure 34. Appearance after mixing 5ml of viscous solutions containing a red dye with 20ml of water: (a) 0.5% LBG; (b) 0.6% LBG; (c) 10% gelatin; (d) 30% gelatin

Salt release is consistent with these visual images remaining unaffected by gelatin concentration across the whole of the range studied but showing a large decrease above concentrations of 0.5% for LBG (Figure 35). The results for locust bean gum are consistent with the hypothesis that the reduction in flavour perception above c* originally reported by Baines and Morris (1989) is as they suggested due to restricted mixing. This restriction in mixing will potentially be reducing the transport of tastants (salt in the case of our work) to the receptors on the tongue.

Sensory studies have shown that "solutions" thickened by swollen starch particles mix well, give good salt release and show good taste and flavour perception as well as a good mouthfeel at high viscosities (Ferry et al., 2006a,b). Although sensory evaluation has not been carried out for the gelatin solutions the good mixing and salt release shown in Figure 35 suggests that at high viscosities molten gelatin would give a better flavour perception and mouthfeel than hydrocolloids such as LBG. It would therefore appear that to replace gelatin by other gelling agents, a topic of substantial interest, there are two criteria that have to be met. Firstly, the gelling agent should melt at mouth temperatures but secondly the melt should be of the good mixing type.

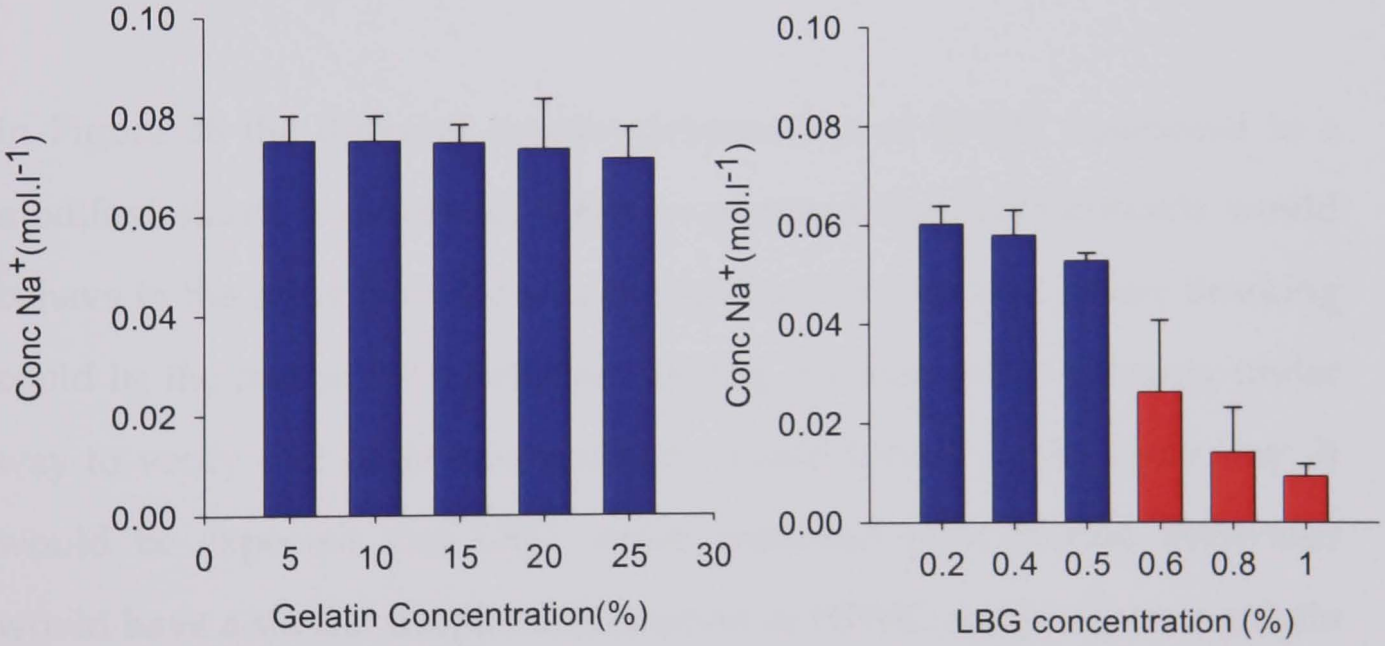


Figure 35. Na+ release for different concentrations of gelatin and LBG solutions

4.3.1 Understanding the mixing behaviour

Further work has been performed in the university of Nottingham in collaboration with Ecole des Mines by Melinda Desse (unpublished observations) by the use of rheoptics.

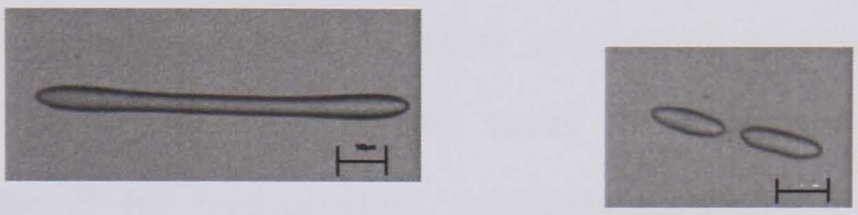


Figure 36. Droplet deformation of HPMC (left) and crosslinked waxy maize (right) at identical shear viscosity and identical stress (courtesy of Melinda Desse)

In Figure 36 the different droplet deformation of HPMC compared to a modified starch is observed. It can be assumed that hydrocolloids would behave in the same way and this elongation of the droplet before breaking could be the reason for insufficient mixing. Further work is already under way to verify that other hydrocolloids would behave in the same way. It would be expected that LBG, which exhibited poor mixing behaviour would have a similar droplet deformation as HPMC and in contrast gelatin would show a similar behaviour to cross-linked waxy maize starch. In support of this recent measurements in the department have been carried out using a CaBER (Capillary Breakup Extensional Rheometer). In this instrument a sample is placed between two plates and the upper plate moved vertically at a controlled rate until a certain gap is reached and afterwards the diameter of the filament is followed until break up.

4.4 Conclusions

As for gels, for solutions the decrease in the magnitude of flavour perception is a consequence of a reduction in the release of the tastant to the receptors in the mouth. This appears to be more important than aroma release, since Hollowood et al. (2002) and Cook et al. (2003) have demonstrated that aroma release remains unaffected at concentrations above c*. Based on the findings of this study the reduction in flavour release above the c* for hydrocolloid solutions could be attributed to restricted mixing that reduces the rate at which the tastants, e.g. salt and sugar reach the receptors at the tongue.

Gelatin and starch solutions (particularly when the granular form of the starch is retained) do not show this reduction in mixing efficiency and consequently it is predicted that it would give good flavour release at high viscosities. This failure to mix can be observed by rheoptics where it can be seen that polysaccharide droplets exhibit higher deformability, whereas starch granules break up thus enabling a more efficient mixing. No investigation of the behaviour of gelatin droplets has yet been carried out although the CaBER data suggest that these droplets will break up readily which is consistent with the ease with which they mix with water.

Tastant release will drive overall flavour perception because of a bimodal sensory interaction between taste and aroma perception. Gelatin gels although not brittle would be expected to give good flavour perception and mouthfeel if consumed under conditions where they melt in the

mouth because concentrated gelatin solutions show excellent mixing with water.

Chapter 5

Effect of gelatin addition to mixed gels

5.1 Introduction

Up to this point the work has revolved around a binary gelled system, however more often than not a combination of hydrocolloids are used in a single food product, for example desert yoghurts where guar gum, xanthan and κ-carrageenan may all be present. In gelled petfood in particular, apart from the polysaccharide that is usually added, gelatin is also present since it leaks from the meat chunks that are a major component of the product.

For this reason it is quite important to investigate the effect of gelatin on the gel matrix. A previous project funded by Mars has looked into the rheological properties of gelatin, carrageenan and LBG mixtures (Jones, 2004). Jones et al. (2004) reported that 3% of gelatin can be found in the gelling system as a result of collagen in the meat. Also the effect of autoclaving has been examined.

In the present chapter the effect of gelatin inclusion on the texture and flavour release from mixed polysaccharide gels will be reported. In most

cases the work will concentrate on the effect of 3% gelatin since it has a more direct application to the commercial product. The work will also concentrate on the gellan system since this gives more brittle gels that give better tastant release.

When gelatin is added as a third component in a polysaccharide gel system it will have an effect on the texture. The texture properties of mixtures of high and low acyl gellan gum have been investigated by Mao et al. (2000) who measured strength, deformability and firmness of mixtures of HA and LA gellan at different total polymer concentrations of 0.5, 1.0, and 1.5% and at different ratios of HA to LA gellan (25/75, 50/50 and 75/25). They concluded that the mixed gels were much more deformable but had similar strength compared to low acyl gellan gels. Furthermore the effect of calcium concentration on the textural properties was reported by Huang et al. (2003).

The gellan/gelatin system has been effectively characterized by Lee et al. (2003). In their work hardness of mixed gel decreased with increasing gelatin proportion and cohesiveness increased up to the gellan to gelatin ratio of 40-60 and then decreased. Fonkwe et al. (2003) have looked into the effect of the gelation time on texture of gellan/gelatin mixtures however the concentration of gellan was minimal (maximum addition of gelatin 0.0066g in 100ml). Papageorgiou et al. (1994) looked at steric exclusion phenomena in the system. Their results could be described on the basis of thermodynamically incompatible polymers, which gel independently in their respective phases. The system was supported by a gellan continuous

phase, however with manipulation of polymer concentration and ionic strength (addition of high amounts of salt) a phase inversion could be achieved to a system where gelatin forms the supporting matrix.

The good flavour release from gelatin gels is well known and therefore many studies have looked at flavour release (particularly aroma release) of pure gelatin gels (Baek et al., 1999; Taylor et al., 2001; Boland et al., 2004). Baek et al. (1999) reported that softer gelatin gels released larger concentrations of a volatile compound than harder gelatin gels. Boland et al. (2004) investigated gelatin, starch and pectin gels and they observed that flavour release was significantly affected by the texture of the gels: the gel that exhibited the highest Young's modulus of elasticity, gelatin gel, showed the lowest flavour release. In a similar context study, Boland et al. (2006) showed that gelatin gels showed higher static headspace concentrations of strawberry flavour, but decreased flavour release compared to pectin gels and when the rigidity of the gels increased, the air/gel partition coefficients decreased and the maximum in nose concentration increased.

However other studies are showing that aroma is not the key component driving flavour perception and texture influence is very important. Weel et al. (2002) have concluded that for a whey protein gel system it is a change in texture that determines the perception of flavour intensity and that the flavour nosespace concentration doesn't determine flavour perception. They have concluded that the mechanism is psychophysical. In Chapter 3 it was demonstrated that flavour release was mostly driven by tastant

release instead of aroma (Koliandris et al., 2007) and that tastant release was closely correlated with the texture of the gels. An inverse correlation with strain at rupture and salt release was observed. In addition to that Bayarri et al. (2007) have reported that the concentrations of aspartame that are needed to achieve a similar sweetness intensity equivalent to that of 20% sucrose were higher in soft than in hard gels. Their results imply that the harder gels are more efficient at releasing aspartame.

Mixtures of gellan gum/gelatin can be used by the industry to produce a wide range of gelled industrial and food products apart from petfood. Typical food products are fabricated vegetables, water and milk based desserts, syrups and toppings (Wolf, 1988; Shim, 1984). The flavour release from such mixtures has not been yet investigated and the question that needs to be addressed is how will gelatin affect the texture and consequently flavour release and also to what extent.

In this part of the study the aim was to characterize mixtures of high and low acyl gellan gels with the addition of gelatin at various concentrations and investigate the tastant (salt) release from the mixed systems, in particular the salt release at 37°C which would be the in-mouth temperature.

5.2 Materials and methods

5.2.1 Gel preparation

The total concentration of gellan gum used in the mixed gels was 0.6%. Gelatin was added into the mixtures at a concentration up to 5%. A pure gelatin gel 10% was used as reference gel for comparison reasons. All of the gels were prepared in a buffer solution (as mentioned in Chapter 3) and the pH was controlled at 6.5. For systems where the salt release was to be monitored the buffer strength was halved and 11.7g of NaCl were added to 1L of the gelling mixture. The buffer solution was heated at 90°C and afterwards 6.0g of the hydrocolloid mixture and the required amount of gelatin (10-50g gelatin) was added and intense mixing followed for 2min (Ultra Turax T-25 basic mixer at 16000revs/min) which was then followed by stirring at 90°C for 10min (magnetic stirrer). Where aroma release was to be measured 200mg of ethyl butyrate were added to 1L of solution at the end of this stirring period. For the subsequent compression testing the solution was then poured into cylindrical Perspex moulds in order to give cylindrical samples of 20mm height and 20mm diameter. The gel samples were refrigerated at 4°C for 24h and the samples were left to equilibrate at 20°C for one hour prior to analysis.

5.3 Results and discussion

Before discussing the rheological data an analysis on the phase behaviour of the system should take place. Unlike the gel systems discussed in Chapter 3 the current system is more complex, due to the addition of gelatin. Starting with the blend of HA- and LA- gellan Morris et al. (1996), have shown that the formation of the double helices showed no indication that strands from both type of gellan were involved. Kasapis et al. (1999) have looked into the phase behaviour of mixtures of deacylated and high acyl gellan systems and they found out that the two gellan variants are sterically incompatible and they can yield single-phase systems, or composites with disparate phase behaviour. Matsukawa and Watanabe (2007) demonstrated that when no salt was added, mixed solution of HA and LA-gellan show no phase separation.

In terms of mixtures of gellan/gelatin Kasapis (1995) reported that blends of LA-gellan with gelatin at moderate levels of salt exhibit phase exclusion phenomena with each of the polymer gelling independently in their respective phases. Furthermore the continuous phase was formed by gellan which was able to support the gelatin inclusions at temperatures above 40°C. Papageorgiou et al. (1994) have shown that gellan is capable of forming the continuous supporting phase except if the stoichiometric binding ratio of salt to polymer is so high that precipitation is promoted rather than gelation. Their work involved working with a constant gelatin concentration of 5% and varying the concentration of gellan from 0.05 to 0.5%. The conclusion was that from 0.075-0.5% of gellan, gellan was the

continuous matrix with a discontinuous gelatin filler. Therefore it would be safe to assume that in this study where the amount of gellan used was constant at 0.6% the continuous phase was gellan with inclusions of gelatin. Furthermore the data that will be shown later confirm that, and the question then would be if the LA and HA gellan are gelling in the form of interpenetrating networks or in different phases enriched in high acyl and low acyl gellan respectively (since it is proven that each type of gellan will gel on its own).

Phase separation phenomena of other complex mixtures including gelatin have also been investigated. Butler and Butler (2003) studied gelatin/maltodextrin and gelatin/dextran systems. In particular in the second system the phase separation was triggered by the conformational ordering of the gelatin molecules and could only be studied when the system has gelled. The agar/gelatin system has also been investigated, with the main difference from the gellan/gelatin system being that phase inversion was observed when 2.5-3% of gelatin was added to 1% of agar (Kelly, 1995). It should be mentioned here that phase inversion is not observed in the carrageenan/gelatin system. Phase inversion in the agar/gelatin system could possibly be explained by the lower gelling temperature of the agar. In contrast to carrageenan and gellan, agar has a gelling temperature closer to the gelling temperature of gelatin. Therefore whereas in the carrageenan/gelatin and gellan/gelatin the polysaccharide has already formed its network long before the gelatin phase begins to gel, in the agar/gelatin system both gelling agents are gelling at similar, but not

exactly the same temperature and time thus allowing for the phase inversion.

Figure 37 shows the effect of gelatin on the parameters measured by compression testing. It can be observed that increasing amounts of gelatin increase the force to rupture and the deformability of the gels as measured by the strain at break. Also an increase is observed for Young's modulus, although this is more clearly observed for gels containing high amounts of high acyl gellan. For the gels containing high amount of low acyl gellan (0.6%LA-gellan and 0.5%LA-&0.1%HA-gellan) the data do not distinguish the effect of gelatin and data acquired with dynamic rheology are in agreement with E (G' measured at a frequency of 5Hz seems to agree with E). Another interesting observation is when 1&2% gelatin is added (even up to 3%) the increase in the parameters is not apparent, implying that gels have similar textures even though the concentration of polymer present in the samples has been increased more than twice for gels containing 1% of gelatin and more than 4 times for the addition of 2% gelatin.

Figure 38 demonstrates the effect of gelatin on k_1 and k_2 parameters for the stress relaxation experiment. Regarding these parameters gelatin seems to have an effect even at a concentration of 1% which would differentiate the gels texture. Based on the stress relaxation experiment it can be concluded that addition of gelatin renders the gels more elastic even at low concentrations. Furthermore correlation between a sensory perceived attribute, springiness, and stress relaxation parameters has been reported in Winwood et al. (1985) which enhances the significance of these

parameters. Similar experiments were carried out on κ-carrageenan and LBG mixtures with addition of 1% gelatin (data not shown) and the results were similar. Compression tests and dynamic rheology failed to pick up differences on the texture when 1-2% of gelatin was added whereas stress relaxation experiments modelled as described by Peleg and Normand (1983) are able to distinguish between the gels .

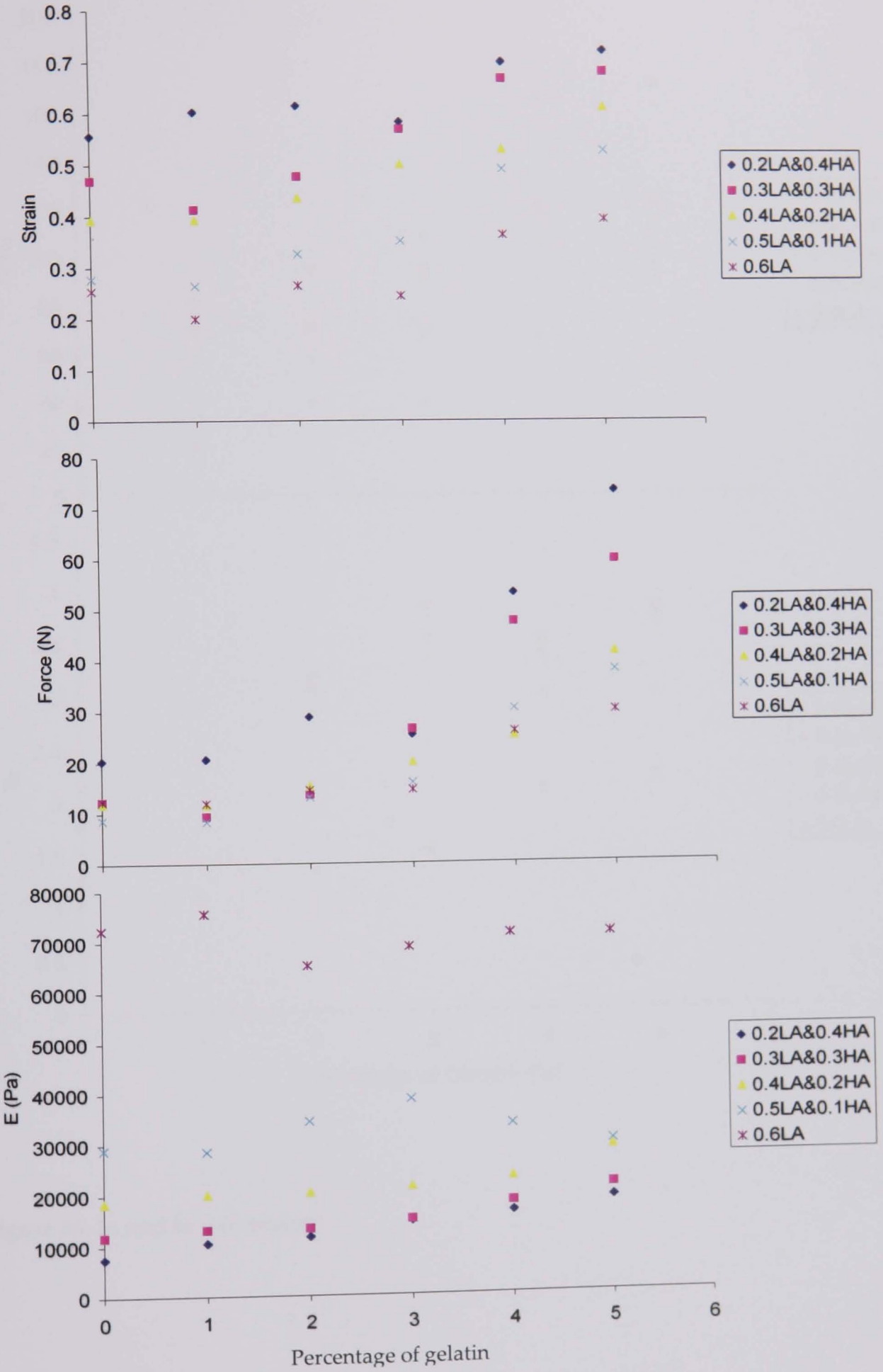


Figure 37. Rheological parameters after addition of gelatin up to 5%

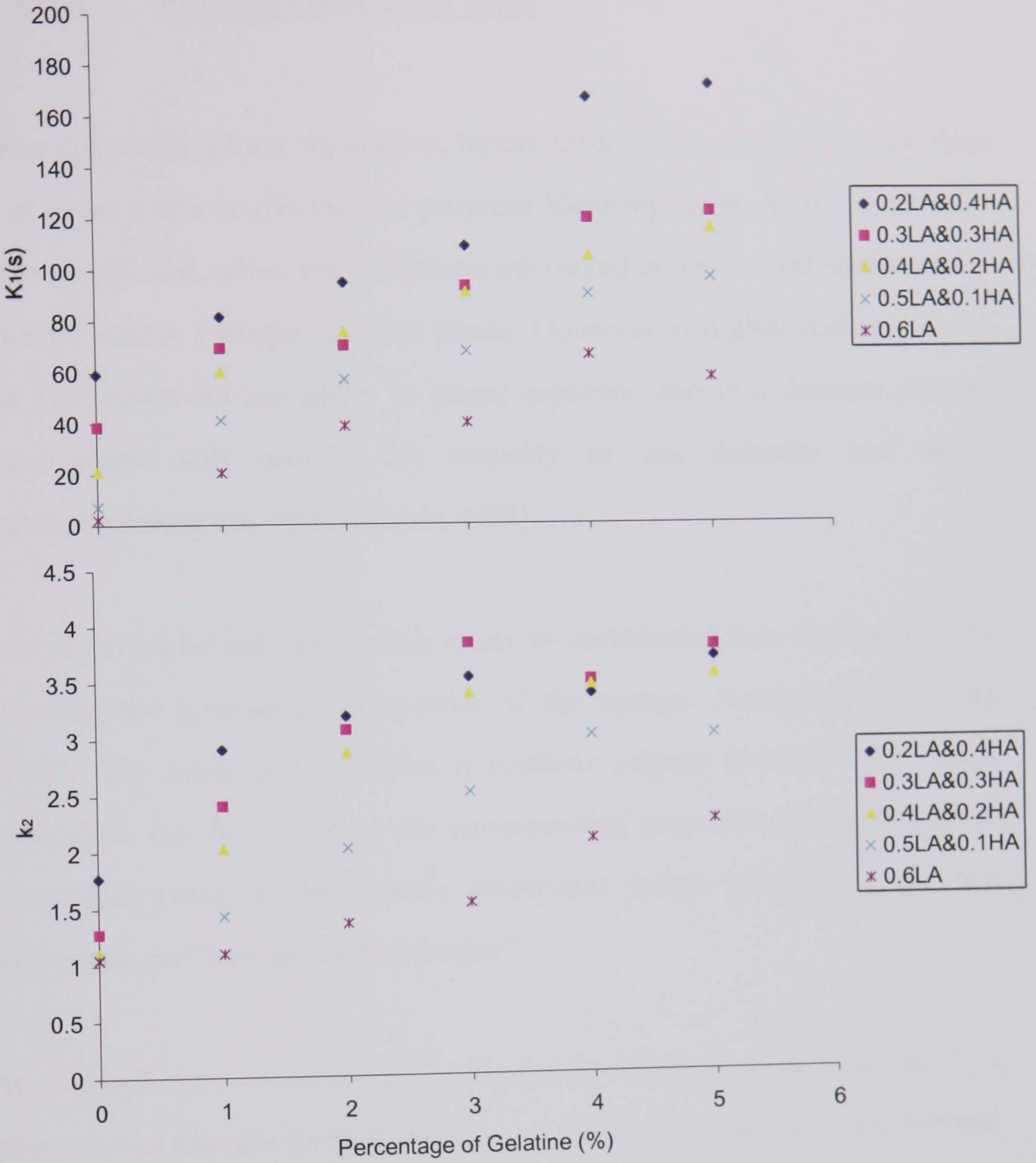


Figure 38. k_1 and k_2 parameters

5.3.1 Polymer blending laws

After discussing phase separation, before interpreting the rheological data is of great value to discuss the polymer blending laws. As it has already been mentioned, when two polymers are mixed at low concentrations they co-exist within a single aqueous phase. However at higher concentrations the two polymers are likely to phase separate into two discrete phases. Each phase will contain the majority of one polymer and small concentration of the other (Morris, 1992).

It is of particular interest for this study to understand how the blending is affecting the mechanical properties of the system. According to Morris (1992) *"The mechanical properties of synthetic polymer blends (simple binary composites) can be related to the corresponding properties of the individual components based on the relative proportions (phase volumes) of the two components and their spatial distribution"*.

An example can be observed in Figure 39 where a weak material X is dispersed as discrete particles within a continuous matrix which is formed by a stronger material Y. The overall shear modulus (G) of the blend will be related to the corresponding moduli of the component phases (Gx and Gy) by Equation 26.

$$G = Gx\varphi x + Gy\varphi y \qquad \text{(Equation 26)}$$

where G: is the overall shear modulus of the polymer blend and ϕx and ϕy denote the proportions of the total volume occupied by each component (with $\phi x+\phi y=1$).

When the system is subjected to a force the degree of deformation will be dictated by the stronger material Y and will be the same for the inclusions of material X and this is called the iso-strain model.

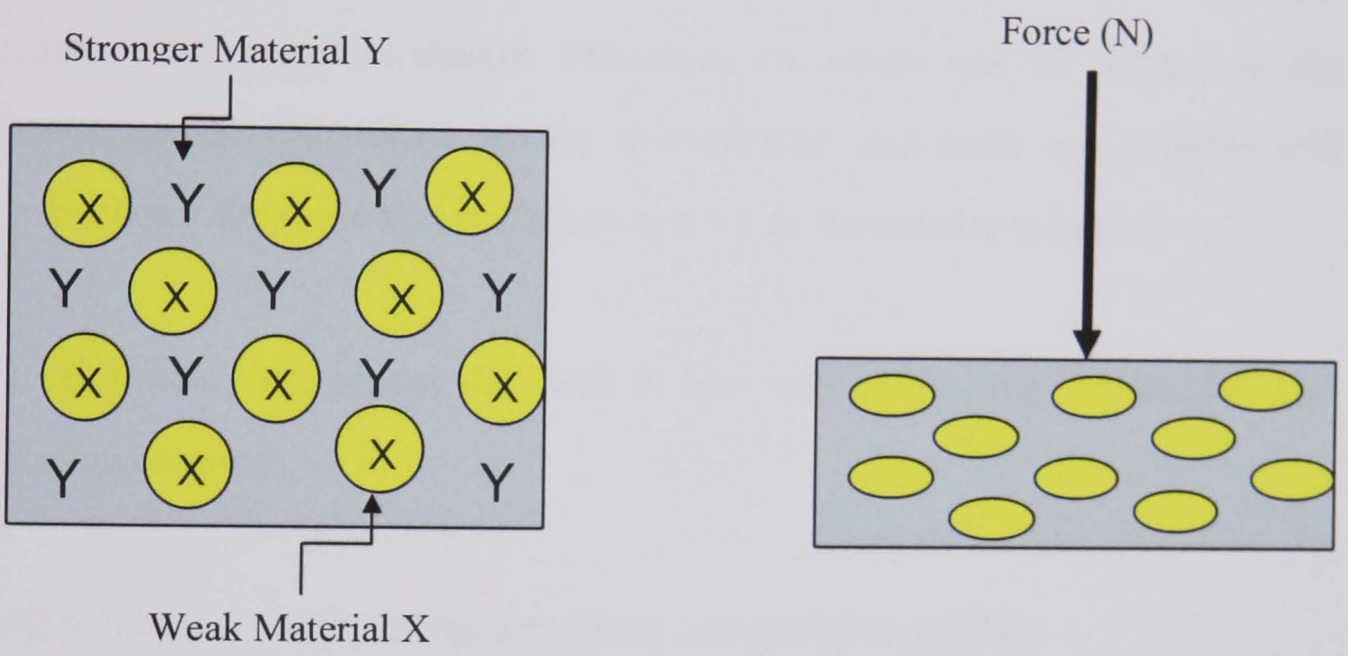


Figure 39. Biphasic polymer blend where the continuous phase is comprised by a stronger Y material and the small circular inclusions are comprised by a weaker material X. Under application of a force that deforms the gel network both materials are subjected to the same strain (Iso strain model)

In the reversed situation where the weaker material forms the continuous phase and inclusions are formed by the stronger material then the overall shear compliance (J=1/G) can be obtained as the corresponding weighted-

average of the individual compliances (Morris, 1992) as seen in Equation 27.

$$J = Jx\varphi x + Jy\varphi y \qquad \text{(Equation 27)}$$

where J: is the shear compliance of the polymer blend and ϕx and ϕy denote the proportions of the total volume occupied by each component (with $\phi x+\phi y=1$).

In this situation the filler component will be less deformable than the surrounding weaker matrix. Therefore the stress will be limited to the resistance of the matrix to the deformation and both components will experience the same stress. This is termed as the iso-stress model.

In this study the model that will fit best will be the isostrain model. The reasons being :

A) gellan is a much stronger material compared to gelatin;
B) gellan was forming the continuous matrix based on temperature sweeps performed on a CVO rheometer (Figure 40) as well as visual observations of the gels after heating them in water of 40°C (The composite gels retained their shape and integrity whereas pure gelatin gels were melted).

The Young's modulus for the weaker gels (0.2%LA-&0.4%HA-gellan, 0.3%LA-&0.3%HA-gellan and 0.4%LA-&0.2%HA-gellan) is increasing with the addition of gelatin. This is supported by the polymer blends theory since the increase of gelatin will increase the phase volume occupied by gelatin and consequently increase the concentration of gellan in its phase

(the phase volume will be reduce thus the concentration of gellan will increase). The increased concentration of gellan in the continuous phase will increase the Young's modulus of the system. For the stronger gels this doesn't hold true as the increasing gelatin concentration is not affecting the Young's modulus. Interestingly the stronger gels at addition of 4 and 5% gelatin appear to be more deformable as their strain at rupture is increasing which if we take into consideration that in the isostrain model the strain should be equal between the two phases should not be occurring. However when the continuous phase Y is at very high strains the inclusions of the weaker filler X which is a very elastic material may be absorbing some of the energy (similar to an elastic spring acting as a shock absorber) and this could account for the increase in the strain at rupture as well as for the increased force required to break the gels.

The increased deformability of the gels can be verified with the results from the stress relaxation experiment. In Figure 38 k_1 and k_2 increase with the addition of higher amounts of gelatin. This is the case for the elastic as well as the brittle gel. In order to explain this behaviour lets first consider how each of the different components will behave in a single component gel. It can already be observed how a pure 0.6%LA-gellan will behave. The k_1 and k_2 are very low and although in the present work the ability of the gels to return to their original size was not actually measured, in visual observations after the experiment it was clear that the gel cylinders had smaller height than prior to the experiment. On the contrary very elastic gels were able to regain a lot more of their original height. A pure gelatin gel for example at high concentrations (10%) will have an elastic behaviour

and will be able to return to its original shape after an application of stress. Therefore the continuous phase and the inclusions will be subjected to the same strain but will behave differently to the applied force. The weak filler in the present situation gelatin will absorb the energy and deform but upon removal of the constant stress it will be applying a stress within the network trying to regain its original shape and size, thus pushing the whole gel network back to its original height.

In the literature mixtures of gellan and gelatin have been examined, however the total polymer concentration was kept constant (1.6% of total polymer in Lau et al. (2000) or 1% in Lee et al. (2003)). In the present work the amount of gellan gum (LA+HA) added was kept constant at 0.6% but the amount of gelatin varied from 1% to 5%. This relationship between polysaccharide and gelatin can be present in certain type of gelled food products.

A typical temperature sweep is shown in Figure 40. Gels that contained high amounts of LA-gellan (0.5%) and 0.1 HA- gellan and gelatin exhibited a drop in the G′ which started at around 28°C and started to stabilize at 35°C. This drop was evident for addition of 2% gelatin and can be attributed to the melting of the gelatin included in the mixture. The question that needed to be addressed now was if that mobility of gelatin could affect the flavour release from the gels systems, but before discussing that it should first be discussed the phase composition of the system. The diffusion experiment results are presented in Figure 41 for 0.6%LA-gellan gels that contained 3% gelatin were used to monitor the

diffusion of salt at three different temperatures. At 30°C which was the point where the drop in G′ would start occurring, at 35°C which was in the middle of the dropping 'zone' and 40°C degrees which was a point where the G′ 'stabilized' (G′ was still reducing but with much slower rate). An increase in the salt measured is observed as the temperature is increasing, therefore it can be concluded that the melting of gelatin in the mixture is in fact affecting the salt release. The more mobile gelatin is, the higher the measurement of salt release.

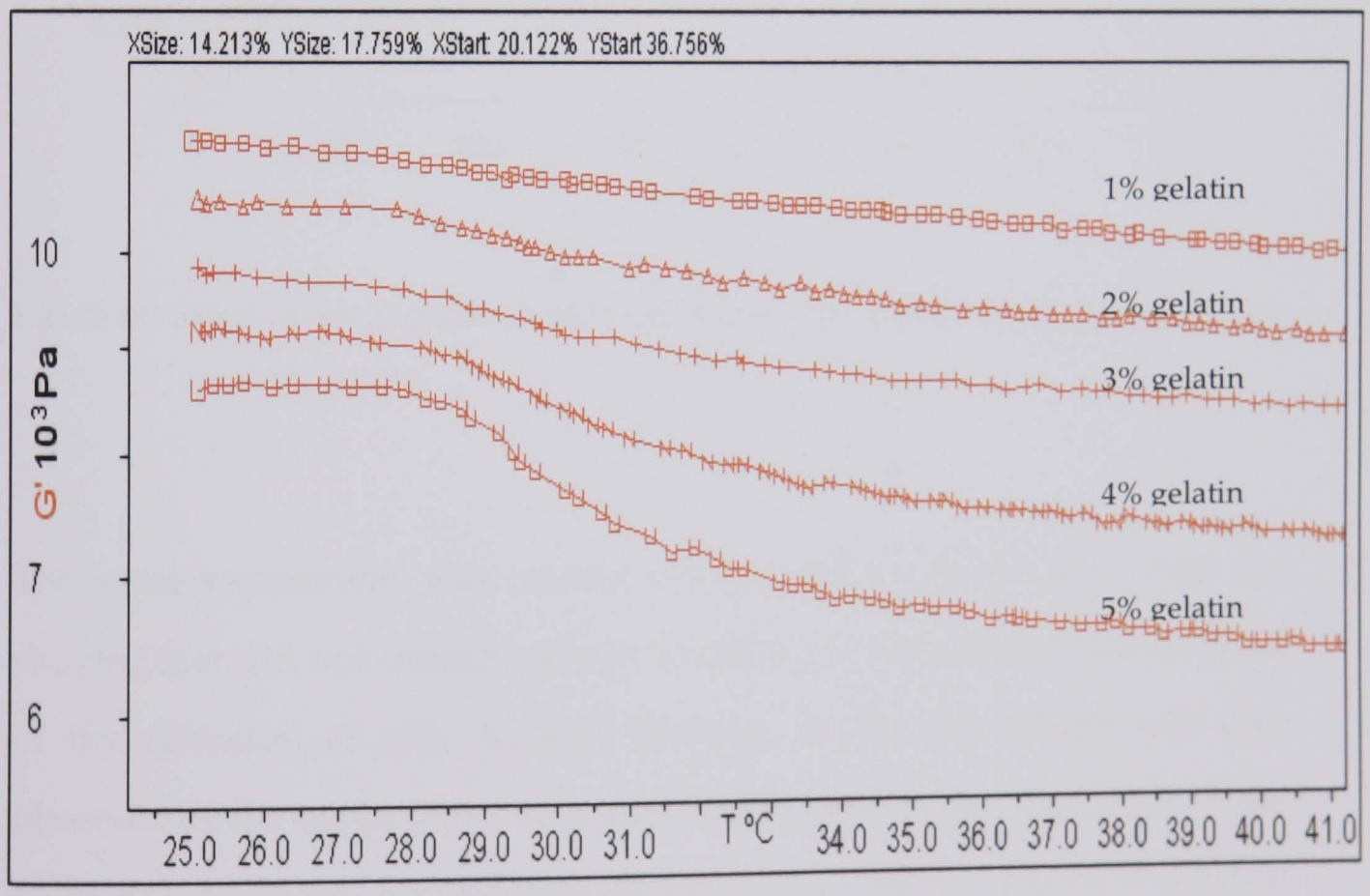


Figure 40. Temperature sweep of gels containing 0.5%LA-&0.1%HA-gellan and different amounts of gelatin (1% -5%). The drop of G′ at around 28°C is attributed to the melting of gelatin inclusions within a continuous gellan phase.

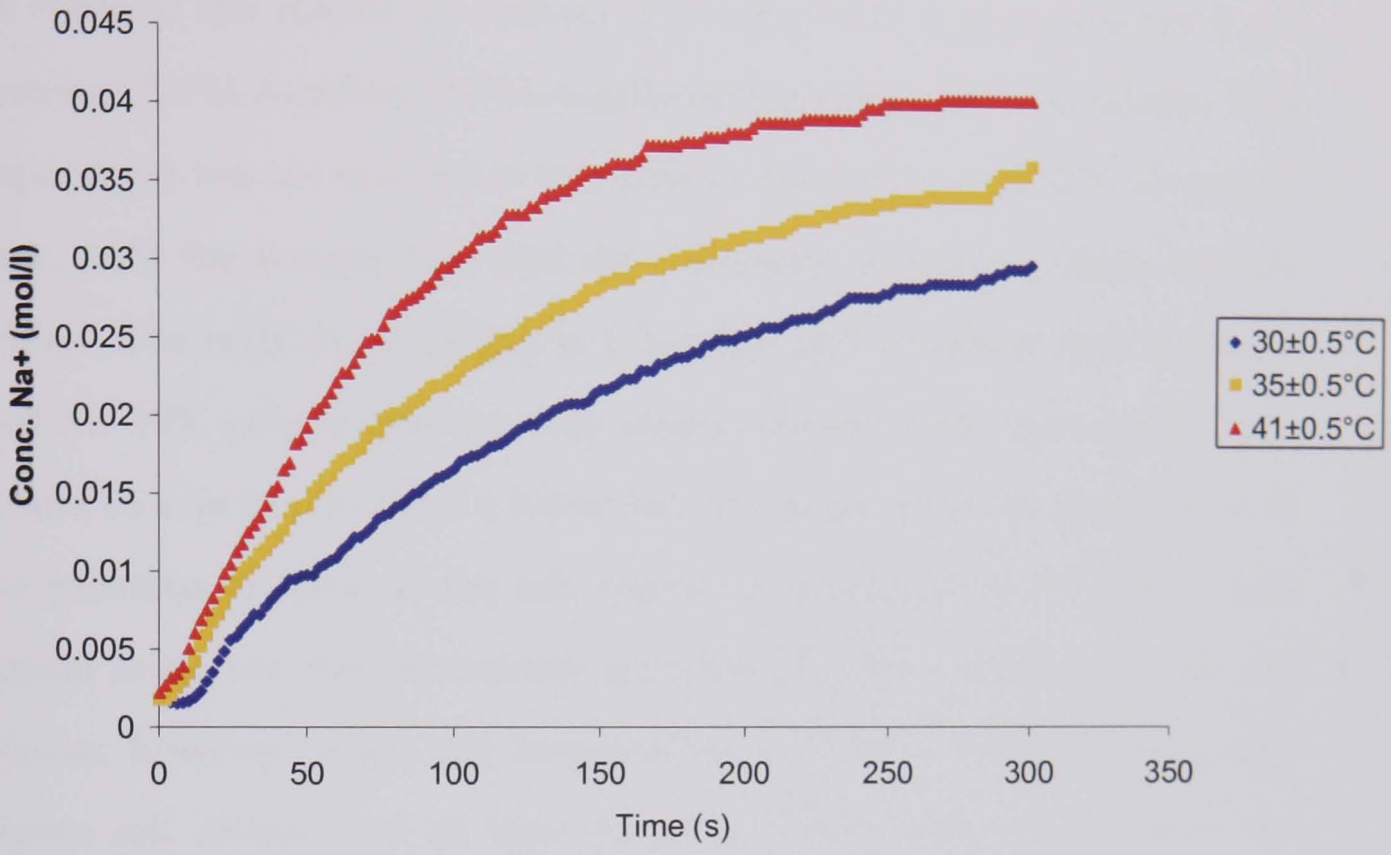


Figure 41. Effect of temperature on salt release from gels that contained 0.6%LA-gellan and 3% gelatin.

The same experiment was carried out for 0.6%LA-gellan gels (data not shown) that did not contain gelatin to check the temperature dependence of the diffusion of salt. A small increase in the salt release was also observed as the temperature was increasing however the magnitude of the increase was far less compared to the gels that contained gelatin. Following the diffusion experiments the salt release was measured after performing TPA on the gel cylinders. The release of flavour by means of diffusion may be insignificant for most food products but for others it can be very

important e.g. jelly beans or other forms of confectionary products that are not meant to be masticated.

In Figure42 the release of sodium ions after TPA is presented for 3 gel systems: 0.6%LA-gellan, 0.6%LA-gellan&3%gelatin and 10% gelatin. This experiment was carried out at two temperatures 20°C and 37°C to look at how, both the temperature and the difference in texture affects the salt release. The addition of gelatin in LA-gellan gels increased their elasticity and the 10% gelatin gels are very elastic, therefore the more elastic gels would be expected to show a lower salt release based on the conclusions of the previous chapter. If the salt release is compared at 20°C, this does appear to be true, the more elastic gel (10% gelatin) is exhibiting lower salt release; however when the temperature is at 37°C, there is markedly higher salt release and as time progresses the release of salt from the gelatin gel exceeds the release from the other two gels. The pure gelatin gels, due to the temperature of the water being higher than their melting point, are releasing more salt in the solution over time. The in-mouth conditions would favour more flavour release from gelatin gels and perhaps from the mixtures of gellan gum and gelatin. Mastication would effectively mix the gel with saliva, the temperature at 37°C would be sufficient to melt the gelatin in the network and since gelatin is mixing well with the saliva even at high viscosities the flavour perception would be expected to be superior.

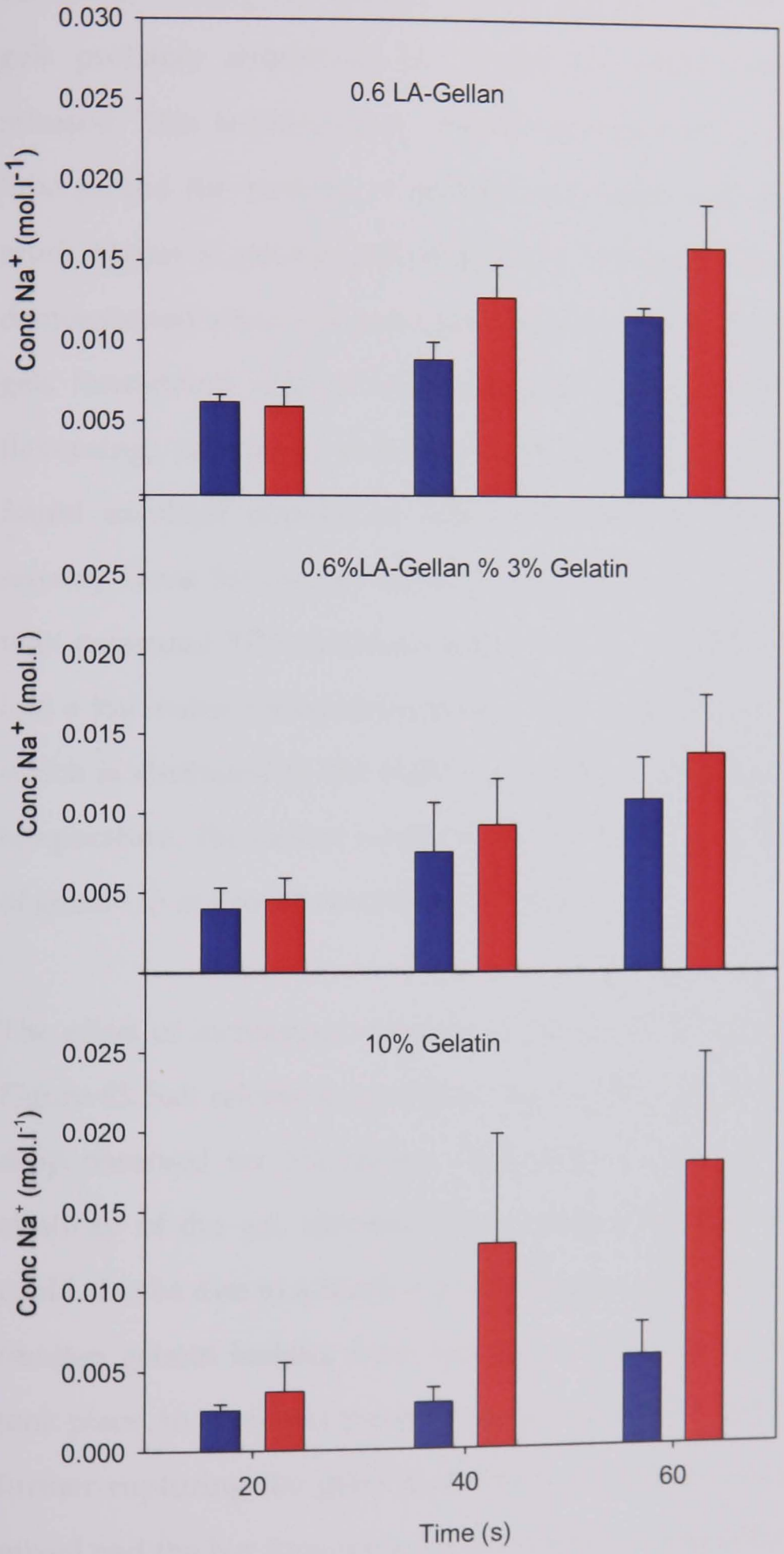


Figure42. Salt Release after TPA at 85% as recorded at 20s Intervals measured at 20°C (blue) and 37°C (red).

Texture is driving the flavour release and the fracture mechanisms of the gels probably determine the 'speed' at which tastant molecules are released. This is particularly significant over short time scale. In longer time period the melting of gelatin dominates and the tastant release is much higher in gelatin gels (Figure42). Gelatin's superiority is very well demonstrated when a sensory panel is used. Clark (2001) has tested dessert gels formulated with a wide range of hydrocolloids with addition of flavouring, colouring, sweetener and acid for overall flavour and has found excellent correlation between measured TPA hardness and the sensory score for overall flavour. Two gel systems had poor correlation with measured TPA hardness. One of them was gelatin which although it had a low value for hardness it had an excellent score for overall flavour, which is attributed to the well-known property of gelatin to melt at body temperature. The author would like to add the very well mixing behaviour of gelatin in in-mouth conditions with saliva.

The effect of increasing amounts of gelatin on measured Na^+ is shown in Figure 43. Salt release is unaffected up to concentration of 3% but there is a drop observed for 5% gelatin. This could be due to the increase in the elasticity of the gel, therefore less surfaces exposed after rupture, but it could also be due to a much higher increase in the viscosity of the solution (molten gelatin leaking from the gel). During the experiment no mixing took place, to avoid gel pieces coming into contact with the electrodes and further rupturing the gels) therefore the solution could not efficiently be mixed and the Na^+ ions were not dispersed homogenously.

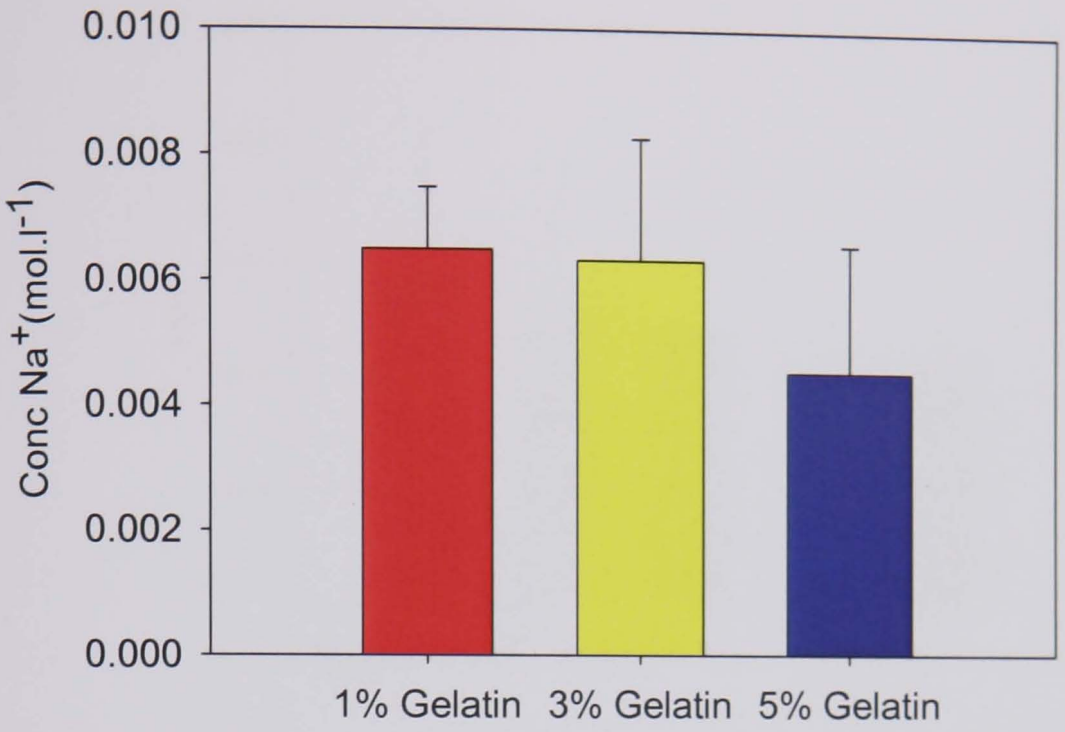


Figure 43. Effect of increasing gelatin concentration on release of Na^+ 20°C. All the gels had the same amount of polysaccharide (0.4%LA-&0.2%HA-gellan).

The aroma release, for gels that contained gelatin, as measured by APCI–MS is in agreement with the data presented in Chapter 3. The maximum intensity (Imax) is presented in Figure 44. A smaller Imax from the elastic gels and in particular gelatin gel was observed, but in general it was not able to clearly distinguish between the gels. Once more the gel that exhibited the highest Imax was the most brittle gel (0.6%LA-gellan).

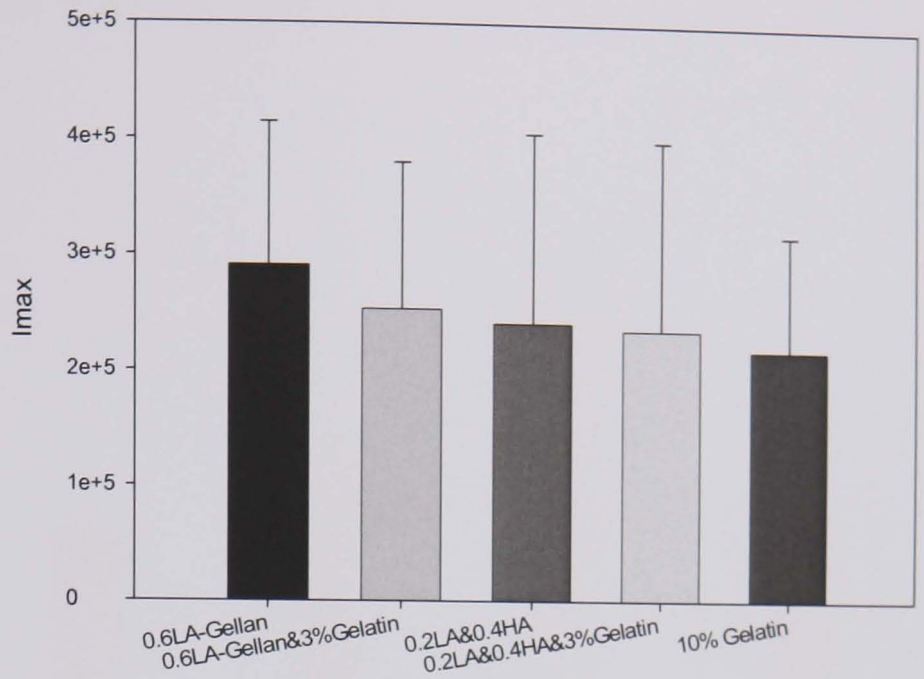


Figure 44. Comparison of Imax for gels with 0%, 3% and 10% gelatin from nosespace measurements

A clear effect of gelatin on the measured static headspace was observed (Figure 45). The headspace was measured at 2 different temperatures 20° and 37°C. The pure 10% gelatin had a significantly higher amount of volatile present in the headspace ($P<0.01$). compared to the other gels for both temperatures. Also when 3% of gelatin was added to 0.6%LA-gellan and 0.2%LA-&0.4%HA-gellan an increase in the headspace was noted for both gels, statistically ($P<0.05$) for the latter. As was expected, when the gels were heated to mouth temperature (37°C) more volatile was present in the headspace. It has already been demonstrated in Figure42 that the pure gelatin gel at 37°C showed a higher salt release than at 20°C, particularly at longer time scale. Combined with the fact that gelatin melts when present in a mixture, with gellan gum thus increasing the release of tastant

molecules these observations can contribute further insight to the superiority of gelatin with regard to flavour release.

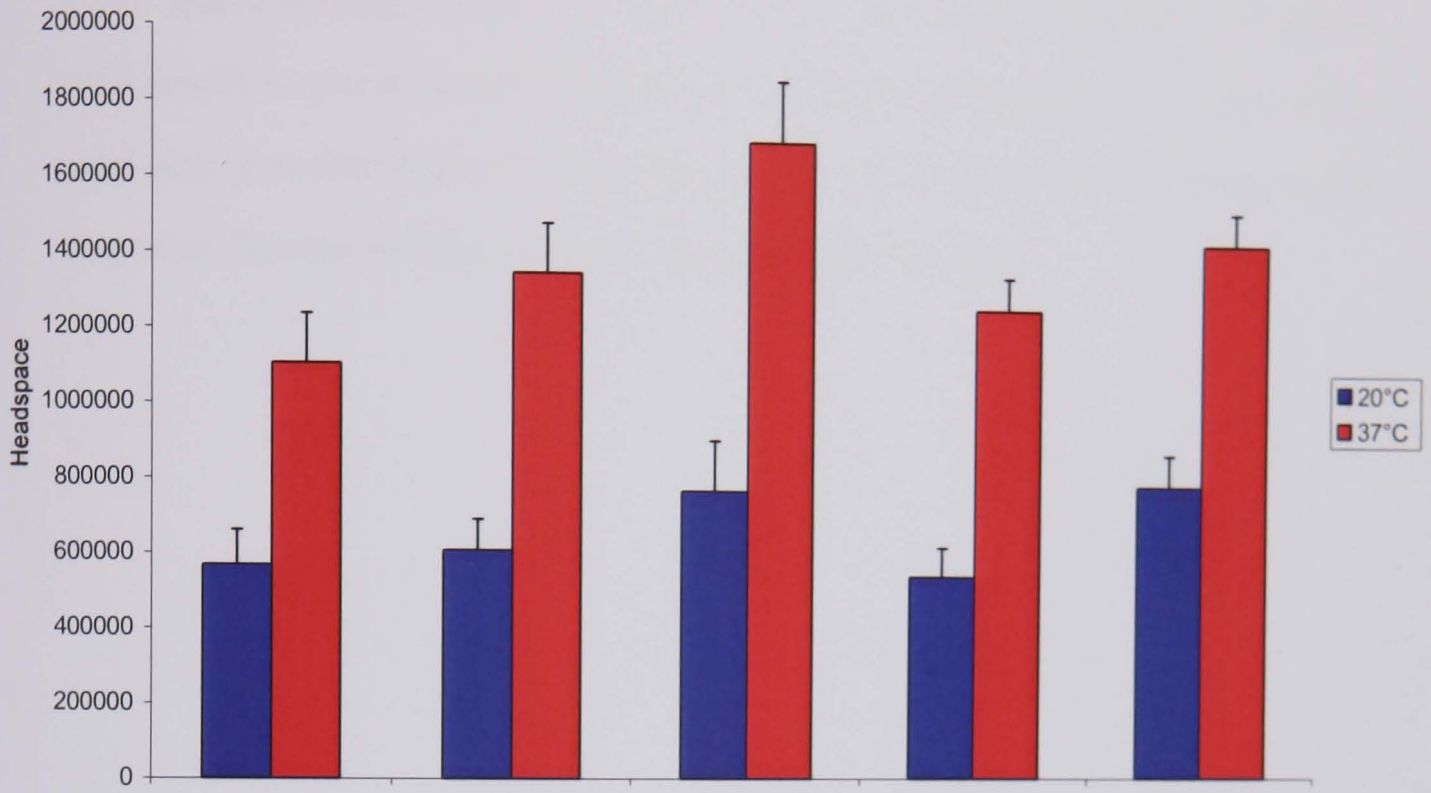


Figure 45. Static headspace concentration (arbitrary units) for gels with and without gelatin at 20°C and 37°C

5.4 Conclusions

Addition of gelatin affects the texture of the gellan system as was expected. The simple alteration of the texture is a sufficient reason to argue that gelatin has an effect on the flavour release if we accept that flavour release and perception is driven by texture. However gelatin has a different property, that has already been well documented, and that makes a significant contribution to flavour release. At 37°C gelatin is a solution rather than a gel which can potentially increase the flavour release

especially in an extremely complex 'environment' such as the human mouth where constant mixing is occurring as well as ideal conditions for gelatin melting. In addition to that, gelatin will not only melt but will also have good mixing behaviour with saliva. The above attribute of gelatin will result in more tastant molecules reaching the taste buds on the tongue and also possibly higher aroma release during that process resulting in the superior flavour release and perception of gelatin gels.

Chapter 6

Deacetylation of gellan gels

6.1 Introduction

This chapter will deal with the effect of sterilization on deacetylation of HA-gellan and on the rheological properties. Sterilization is a process that will certainly take place in the production of wet petfood, therefore its effect on the gelling agents used is very important. The behaviour of blends of carrageenan and locust bean gum in the presence of gelatin during autoclaving has been investigated by Jones (2004). So this part of project will focus on the gellan system, also because of the fact that sterilization will induce deacetylation of HA-gellan and therefore the process could start with addition of only HA-gellan which will afterwards be deacetylated during sterilization in order to give the desired texture.

As has already been discussed in Chapter 1, native gellan consists of a backbone of tetrasaccharide repeat units of glucose, glucuronic acid and rhamnose (in a 2:1:1 ratio). The repeat units are partially esterified with L-glycerate and O-acetate. Commercially available gellans are classified as either high or low acyl. The low acyl gellan gives very brittle gels and in

contrast high acyl gellan gives very elastic gels. The key difference between the two is the absence of the O-acetyl substituents of high-acyl gellan that inhibit the close packing of the helices into crystalline domains. Highest crystallinity and formation of more rigid and brittle gels is observed when the O-acetyl groups are absent (Kang and Pettitt, 1993).

In this part of the study blends of LA- and HA- gellan were compared to gellan gels and deacetylation was achieved either by thermal processing or by application of strong alkali.

6.2 Material and methods

6.2.1 Gel preparation

The gels were prepared as described in section 2.2.2 and were poured into cans (height 60mm, diameter 75mm), containing cylindrical Perspex moulds and sealed using a can seamer (Metalbox, Reading, UK). The cans were afterwards processed in a Millwall steam retort (John Fraser and Son Ltd., London, UK) for the appropriate length of time as dictated by the experiment (the standard process being 121°C/15psi for 15min). The cans after cooling, (first cooled in the retort at temperatures below 100 C and afterwards in a sink with water at temperature 20°C) were placed at 4 C for 24h.

6.2.2 Deacetylation by stong alkali

The buffer solution was placed in a conical flask and heated to 90°C. Then 6.0g of HA-gellan was added and the solution was mixed for 2min (Ultra Turax 16000revs). The pH was then adjusted to a value of 10 by the addition of 10M NaOH and the solution was stirred (magnetic stirrer) at this pH for as long as the experiment dictated in order to achieve different degrees of deacetylation. Following that the pH was readjusted to a pH of 6.5 by the addition of HCl 8.37M and the solution was stirred for another 10min. The solution was finally poured into cylindrical Perspex moulds, was allowed to cool and then placed at 4°C for 24h prior to rheological characterisation.

6.2.3 Gel characterisation

Upon removal from the fridge the cans were opened and the excess of gel surrounding the perspex mould was removed carefully with a scalpel, so that a gel cylinder was obtained with the dimension of 20mm in height and 20mm of diameter. The methodology used in the previous chapters was employed for rheological characterisation of the gels.

6.2.4 Acetic acid assays on processed gels

Determination of the degree of acetylation during processing was essential in order to be able to compare the deacetylated gels with blends of HA-

and LA-gellan that will have the same ratio of HA and LA-gellan present. Therefore the acetic assay was performed using a commercially available kit (item number 10 148 261 035, from Roche/R Biopharm AG, Darmstadt, Germany). By the use of the acetic assay kit the release of acetic acid caused by deacetylation during processing (retorting or application of strong alkali) was measured.

The assay is a colorimetric method, and depends upon the coordinated use of a series of pre-prepared reagents in order to form reduced nicotinamide-adenine dinucleotide (NADH), a compound which can be measured using absorbance spectrophotometry at 340nm. Basically the higher the level of deacetylation during processing, the higher the level of NADH should be. This NADH level can be converted to the amount of free acetic acid present (Newton, 2006). The method has been described in detail by Newton and she kindly provided the data on the acetic acid assay.

6.3 Results and discussion

6.3.1 Effect of process time on the deacetylation process

In the experiment regarding thermal processing of the gels, the temperature of retorting was kept constant at 121°C, but the time of processing varied in order to achieve different degrees of deacetylation (Table 6). The same principle was applied for the deacetylation with strong alkali (Table 7). The solution was kept at a value of pH 10 for different times in order to achieve different degrees of deacetylation. Based on the degree of deacetylation the data were then converted into an equivalent ratio of HA- to LA-gellan so that a comparison between the different methods could be made. The percentage of deacetylation is also presented in the 3rd column.

Table 6. Effect of processing time on ratio of LA:HA-gellan (Data courtesy of Jane Newton, 2006)

Process time/min at 121°C	Equivalent concentration of low acyl gellan(%): concentration of high acyl gellan (%) in gel (total polysaccharide content = 0.6%)	Degree of deacetylation (%)
0	0:0.6	0
7	0.12:0.48	20
15	0.19:0.41	31.66
22	0.26:0.34	43.33
30	0.29:0.31	48.33
45	0.35:0.25	58.33

Table 7. Effect of time holding at pH 10 on the ratio of LA:HA gellan (Data courtesy of Jane Newton)

Process time at pH 10/min	Equivalent concentration of low acyl gellan(%): concentration of high acyl gellan (%) in gel (total polysaccharide content = 0.6%)	Degree of deacetylation (%)
0	0:0.6	0
2	0.21:0.39	35
3	0.30:0.30	50
4	0.35:0.25	58.33
5	0.51:0.09	85
6	0.53:0.07	88.33

The gel that was heat processed for 30min has a total acetyl group composition close to a 50:50 blend of high and low acyl gellan. Similarly the gel subjected to strong alkali for 3 min had the same acyl composition as a 50:50 blend of high and low acyl gellan.

Figure 46 compares the Young's modulus for blends of HA- and LA-gellan with gels of 0.6%HA-gellan that were deacetylated to different degrees, by heat processing or by the application of strong alkali, and finally gels that contained only LA-gellan. It can be seen that the addition of quite small amounts of HA-gellan to LA gellan (0.5%LA-&0.1%HA-gellan) reduces the value of Young's modulus. Therefore addition of HA-gellan distorts the close packing of LA-gellan helices and the gels are more elastic. For the gel systems that did contain both HA- and LA-gellan the modulus appears to have similar values, regardless of whether the gels were unprocessed blends of HA- and LA- gellan or HA-gellan deacetylated to different extent. However despite the gels being of identical firmness they may have a different overall texture.

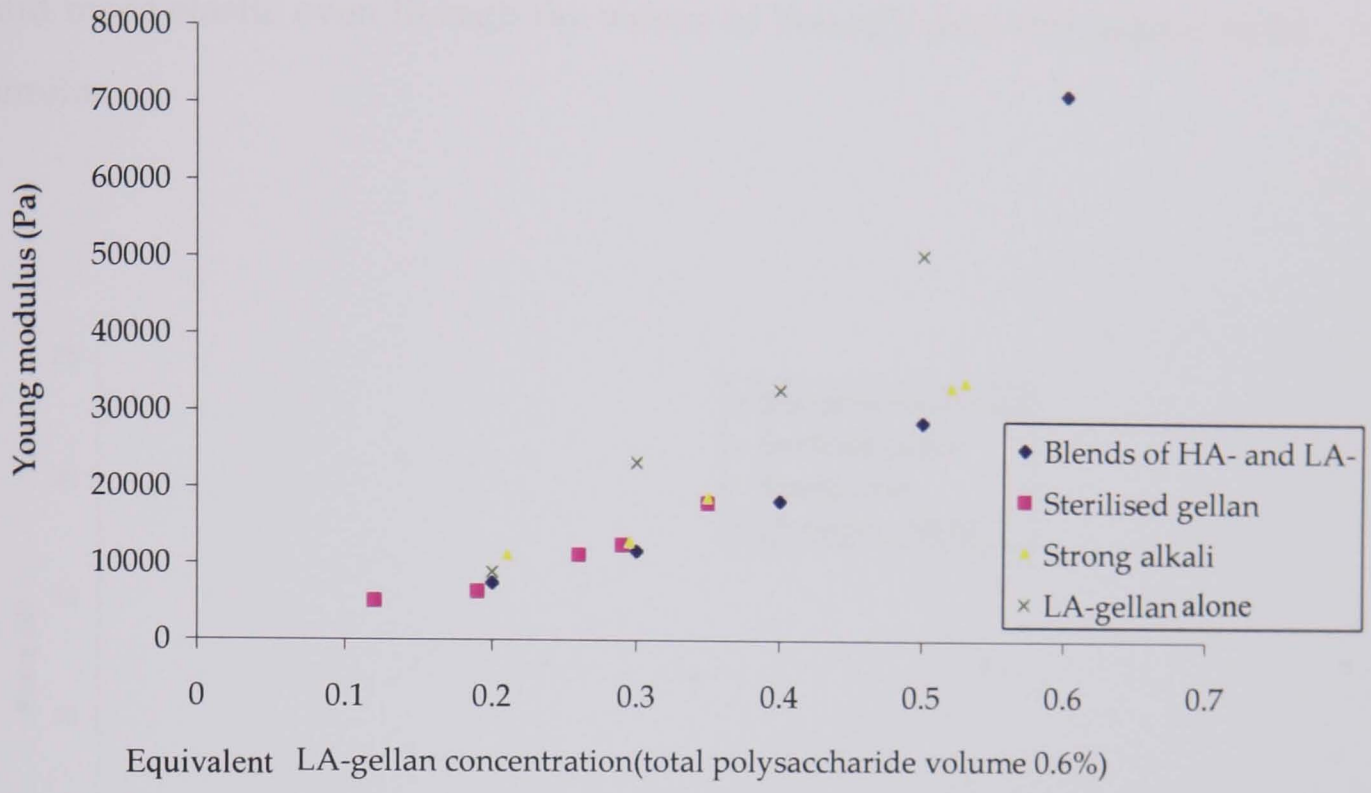


Figure 46. Young's modulus of blends of high and low acyl gellan, gels that were sterilised (equivalent LA-gellan concentration), gels that were subjected to strong alkali (equivalent LA-gellan concentration), and gels that contained increasing concentrations of LA-gellan (alone)

The difference in texture between the gels is clear in Figure 47 and Figure 48. Gels that had almost identical values of Young's modulus have quite different textures. The force required to rupture the gels is significantly higher for the blended mixtures compared to the gels where deacetylation was achieved by heat processing. Furthermore the distance to rupture was also lower for the samples that were subjected to thermal processing. From the above two parameters it can be concluded that although blended gels and gels for which deacetylation was achieved by heat processing may have the same equivalent ratio of HA: LA-gellan their texture is different

as that is measured in term of the force required to break them and also how deformable the gels are. The blended gels appear more deformable and more elastic even though the values of Young's modulus appear to be similar.

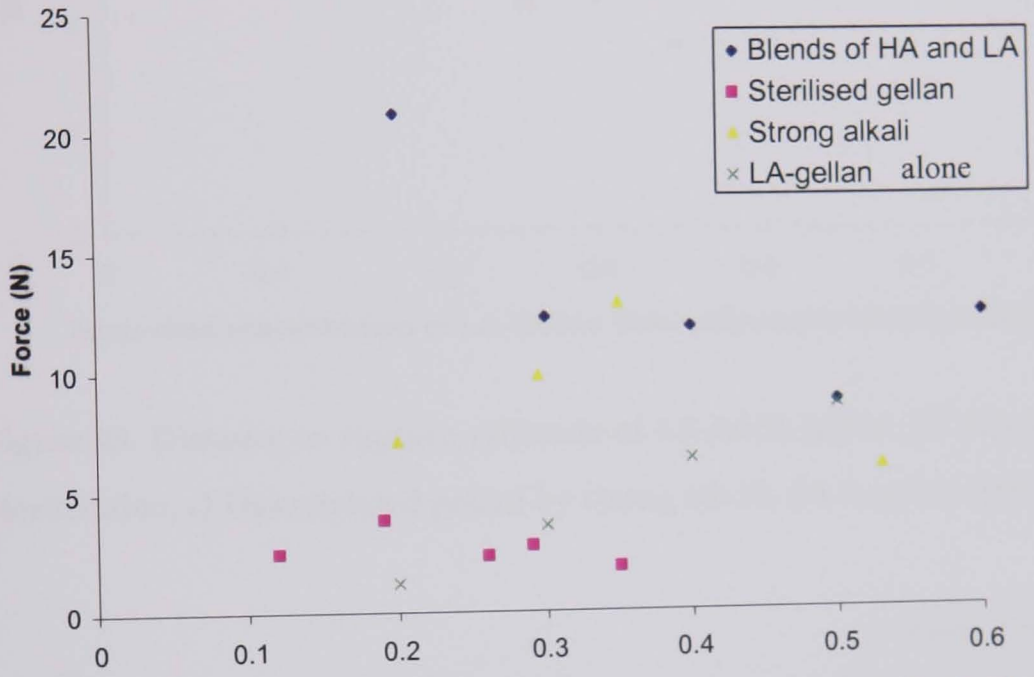


Figure 47. Force to rupture for a)Blends of LA-&HA-gellan, b) Deacetylated gellan by sterilization (equivalent LA-Gellan concentration), c) Deacetylated gellan by strong alkali (equivalent LA-Gellan concentration), d)LA-gellan(alone) gels

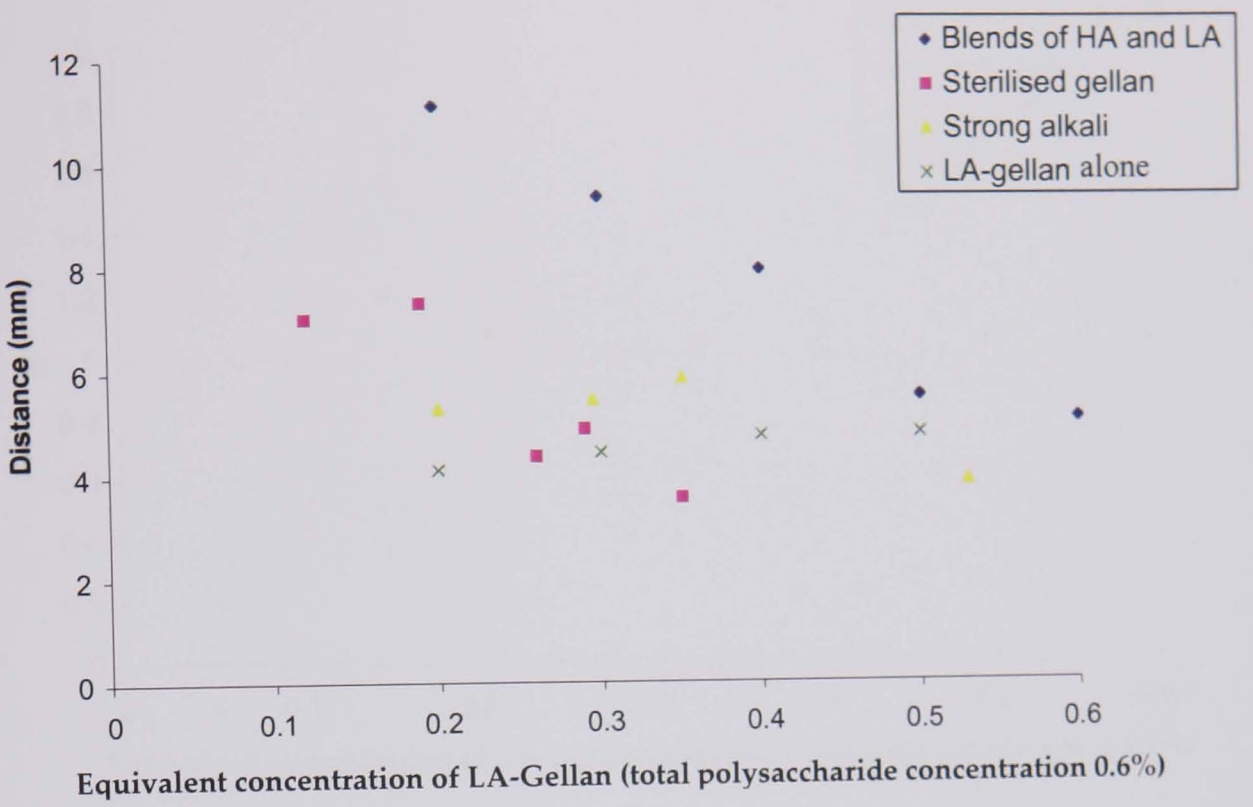


Figure 48. Distance to rupture a)Blends of LA-&HA-gellan, b) Deacetylated gellan by sterilization, c) Deacetylated gellan by strong alkali, d)LA-gellan gels

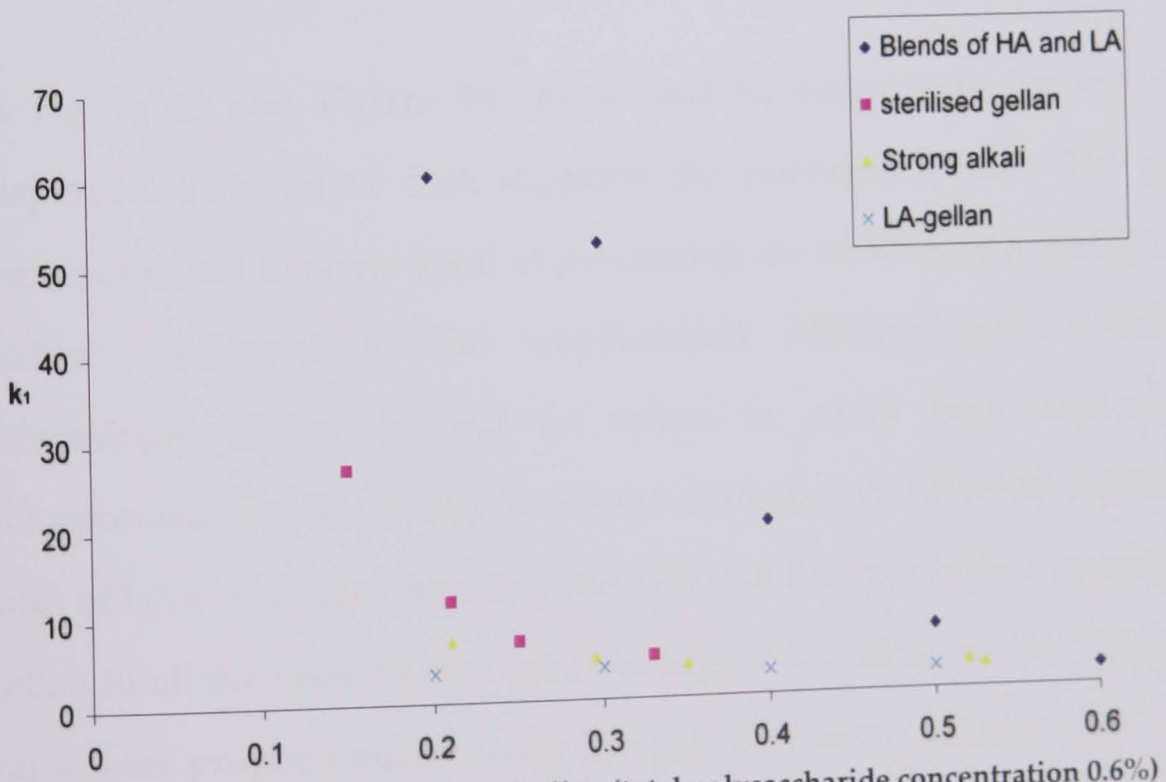


Figure 49. k_1 for: a) Blends of LA-&HA-gellan, b) sterilised gellan, c) deacetylated gellan by application of strong alkali, d)LA-gellan

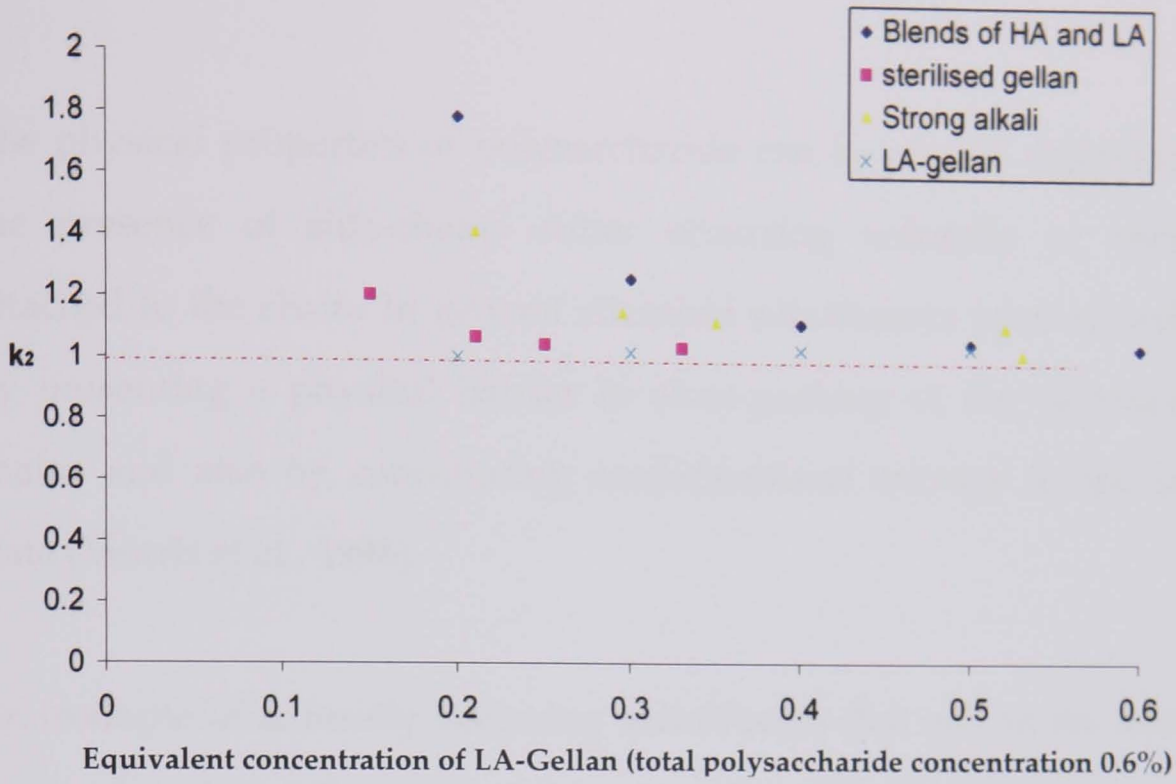


Figure 50. k_2 for: a) Blends of LA-&HA-gellan, b) sterilised gellan, c) deacetylated gellan by application of strong alkali, d)LA-gellan

In Figure 49 and Figure 50, the k_1 and k_2 parameters for the gels are displayed. This set of data supports the previous results. The gels that were subjected to some form of processing are exhibiting a change in their texture compared to the unprocessed blended gels which have increasingly higher k_1 and k_2 values as more high acyl gellan is incorporated into the blend. The same curvature is observed regarding the ratio of LA:HA gellan, meaning the gels that had the higher amount of HA gellan in all the systems did have the higher k_1 and k_2 values, however this value was proportionally lower for gels that were deacetylated either by heat processing or by deacetylation by strong alkali.

6.3.2 Role of deacetylation in gel texture

The physical properties of polysaccharide can be altered significantly by the presence of side-chains either occurring naturally or chemically attached to the chain. In general chemical substituents promote solubility by presenting a physical barrier to close-packing of the polysaccharide chains and also by contributing conformational entropy in the solution state (Morris et al., 1996).

An example of naturally occurring substituents that acts in the same way can be found in galactomannans such as locust bean gum, tara gum and guar gum. Their solubility is conferred by irregularly-spaced single-sugar sidechains of α-D-galactose linked (1-6) to a proportion of the mannan backbone (Dea and Morrison, 1975). In the absence of the sidechains the mannan backbone would have been insoluble. Native gellan is biosynthesised with an L-glyceryl substituent on O(2) of the 3-linked glucose and, in at least a proportion of the repeat units, an acetyl group on O(6) of the same residue (Kuo et al., 1986). Therefore there are two ways that the suppression of helix-helix aggregation could potentially take place. Either the acetyl groups on the surface of the helix would promote solvation and prevent close packing of the helices or the glyceryl substituents could alter the stereochemical structure in the vicinity of the carboxyl groups to a form that would be incapable of accommodating site-bound counterions (Morris et al., 1996). These two possibilities were investigated by Morris et al. (1996), where gellan samples were prepared with either the glycerate or the acetate substituents selectively removed.

This was achieved by heating the gellan until it was in its disordered conformation and then carrying out alkaline treatment. This allowed the glyceryl substituent to be liberated more rapidly than the acyl ones. The deacetylated gellans were subjected to gel melting/setting experiments. By comparing between the samples that had different degrees of acetate content but similar degrees of glycerate, it was found that the higher the content of acetate the less thermal hysteresis there was. This suggested that the dominant preventer of helix-helix aggregation was the peripheral acetates. Removal of the glyceryl groups alters the conformation of the helix itself such that it would be less thermally stable but will form more brittle gels.

During this study the two methods used had most probably an effect on both the acetate and the glycerate substituents of HA-gellan. Baird et al. (1992) have shown that native gellan samples that were treated with hot alkali for 4min at 90°C had no glycerate, or acetate determinable. Therefore during the deacetylation method applied in this study in some of the samples a total removal of glycerate and acetate subsitutents was achieved. During the thermal processing at 121°C the glycerate substituent would be very susceptible to removal, even at pH 6.5 (due to high temperature). It is also probably not very appropriate to compare these data with data obtained from blends of HA and LA-gellan. However some interesting observations have arisen. First of all by performing the experiments on gels that contained LA-gellan alone (0.6%LA-gellan and 0% HA-gellan), the important effect that HA-gellan has on the texture has been apparent. Even at low concentrations, as low as 0.1%HA and 0.5%LA-gellan a drop of

22000 Pa was observed on the moduli when compared to a 0.5%LA-gellan. Secondly Young's modulus does not differentiate between gels that appear to have a similar ratio of deacetylation, but all the other rheological parameters measured have shown that gels that were subjected to a harsh deacetylation process were more brittle when compared to blends of HA and LA gellan. In addition to that when comparing the two deacetylation processes between them it can be argued that heat processing (even at a pH 6.5) is a much more harsh procedure. Degradation (depolymerisation) of the molecule during the heating process is probable. The gels that were heat treated have lower force to rupture, distance to rupture as well as k_1 and k_2 parameters, meaning that they were more brittle.

6.4 Conclusions

The gellan system offers a great diversity of textures. Various ways to achieve a big variety of textures have been demonstrated. Addition of small amounts of HA-gellan to LA-gellan reduces the brittleness and makes the gels more elastic. High acyl commercial gellan gum can be deacetylated during thermal processing. Due to the high pressure conditions that are present during retorting deacetylation occurs at much lower pH that would normally be needed in a typical deacetylation process (Newton, 2006). Also during this process not only the acetate , but also the glycerate substituents are removed and probably degradation of the gellan molecule also takes place. The degradation of the molecule would explain the more brittle textures obtained. Therefore we could conclude that new textures can be achieved by deacetylation through heat

processing and thus in potential petfood application it could be possible to start with HA-gellan in the mixture prior to sterilization. However comparing gels that were prepared by processing to give an acyl content equivalent to blended mixtures gave different textures opt the blended mixtures. Even though the Young's modulus gave similar results the difference in texture is evident. This is because in blended gels the high and low acyl components behave independently from each other probably giving phase separated mixtures and there are no evidence to support formation of hybrid double helices. Finally although the flavour release from deacetylated gels by heat processing has not been investigated the fact that they appear more brittle than the blends indicates that they could potentially exhibit higher flavour release.

Chapter 7

Final Discussion-Conclusions

7.1 Discussion

For companies that wish to produce attractive gelled food products it is of increasing importance to understand the relationships between flavour release and the food gel texture. The knowledge of this interaction is important not only for improving the quality of an existing product but also for replacing key ingredients without experiencing inferior quality in terms of texture and flavour. The structure, texture and flavour release and perception are all linked together and what affects one will most probably have an effect on the others.

The hypothesis that was driving this project was related to a textural parameter, brittleness and was: ''Do brittle gels give a better flavour release?'' and one of the outcomes of this work was that indeed brittle gels will give a better flavour release. Other studies, dealing with sensory panels have concluded that brittle gels will give a higher flavour perception. Many of them have argued that it is the texture that drives the enhanced flavour perception through psycho-physiological routes. As discussed earlier, when a food is consumed there are many different signals accumulating and being processed in the central nervous system.

The psycho-physiological route implies that the texture of a food by stimulating the mechanical receptors present in and around the mouth could be driving flavour perception. Weel et al. (2002) have concluded that the texture of gels (whey protein) determines perception of flavour intensity rather than the in-nose flavour concentration. Many other authors have produced result regarding in-nose flavour concentration on a large variety of systems. None of the results were really conclusive as for the effect of texture on in-nose aroma-volatile concentration. What has been not been investigated in depth is the effect that texture may have on the release of the tastant. In a study that is not often referred to Morris (1994) reported a clear inverse relationship between strain at break and the sensory perception of flavour from gels and suggested that this was a consequence of the greater exposure of surface on chewing for the more brittle gel. The work reported in this thesis strongly suggests that the release of the tastant rather than aroma release is the key driving factor in this relationship and it is the release of the tastant in gelled products and not the aroma that is driven by the texture. Very recent studies that were published at the same time as some of this work was published (Koliandris et al., 2007), corroborate the results presented in this work. Bayari et al. (2006) have reported that for gelled systems of κ-carrageenan & LBG and gellan gels, the aspartame needed to deliver a sweetness intensity equivalent to that of gels with 20% sucrose were higher for soft gels than for hard gels. This data implies that the harder, more brittle gels were more efficient in delivering the aspartame, thus lesser amount was needed to reach the required value. In another study Bayarri et al. (2007), by using a sensory panel to assess maximum intensity for sweetness, have found that

brittle gels gave higher values and furthermore that gellan gels (which are more brittle) gave higher values than κ-carrageenan&LBG.

Another important aspect of this work involves gelatin. There is an ongoing search to replace gelatin in food products for various reasons. These include: need to produce products for vegetarians (growing market), animal diseases (BSE, Bovine Spongiform Encephalopathy), cost reduction, better manipulation of texture etc. However in order to replace a key ingredient of a product with an alternative without actually experiencing a loss of quality, it must be fully understood how the ingredient works. In this case, gelatin, has the well known and unique ability of melting in mouth temperature. Based on this the superior flavour release over other gelling agents was explained. This study has demonstrated that the melt-in-mouth ability of gelatin is not the only reason behind its superiority in flavour release and perception, but also the fact that gelatin is a very efficient mixer comes to complement the melting in mouth, by efficient mixing with saliva that will help to bring more tastant molecules to the taste buds. Furthermore during this study the role of gelatin in mixed gel systems has been investigated which could be important for gelled petfoods and other meat based systems.

7.2 Applications for the industry

7.2.1 Solutions

Possible applications for the industry will be described, dealing first with the study on solutions. Products that exist in liquid form in the food industry cover a major part of the product range available. A few examples are: marinades, salad dressings, various sauces, soups etc. In most of these products some sort of thickener is used to give the desired texture, however based on the work presented here hydrocolloids such as LBG, when used in concentration above c* will show a decrease in flavour. Therefore alternative thickeners, such as gelatin, that exhibit good mixing behaviour even at concentrations above c* should be sought. Often in these products starch is used as a thickening agent. Work from Ferry et al. (2006a, 2006b) has shown that the type of starch used is also impacting on flavour perception.

Also although it did not fall into the scope of this study, there is considerable current interest in reducing NaCl in foods. Means of reducing it are constantly being sought. This part of the study may also help this attempt on salt reduction by providing information on the correct use of thickener in order to have better flavour release and thus using less salt without losing any flavour. A final comment on the solutions is that industries that seek to replace gelatin with an alternative hydrocolloid

should pay particular attention to matching the special attributes of gelatin (melt in mouth and good mixing behaviour). A recent study by Agoub et al. (2007) has proposed a mixture of xanthan and konjac glucomannan which could offer a melt in mouth property similar to gelatin, however these hydrocolloids in gelling concentrations are possibly not very efficient mixers therefore the flavour release of the products produce by this mixture could be poor when contrasted with gelatin.

7.2.2 Gels

This project was primarily concerned with the texture of petfood gels and flavour release, however from the results obtained the applications cover a much wider range of products, like gelled deserts, jelly beans, savoury gourmet dishes where a gelling agent is used. The apparent effect of the gelling agent on not only the texture but also the flavour release and quite possibly perception could be applied in all of the above mentioned products. By manipulation of the concentration used a wide range of textures may be achieved and where possible more brittle textures should be chosen in order to improve the flavour attributes of the product.

Focusing on the gelled petfood products, the outcome of this work has a number of possible applications. First of all the current mixture that is being used in the industry, κ-carrageenan with galactomannans could be optimised to improve the flavour release. For example reducing the amount of galactomannan added would render the gels more brittle thus a possible improvement on the quality of the product could be made. Also

the protocols for the rheological characterisation could allow to determine if a possible replacement of the gelling agent would give the same characteristics to the product.

Finally this project has demonstrated a possible alternative gelling agent to the one currently used; gellan gum which possesses the following advantages over the κ-carrageenan and LBG system:

- Wider range of texture
- Much more brittle textures at the same concentration of polysaccharide
- Better flavour release due to the highest brittleness
- Potentially cheaper cost if its produced on site (by fermentation)
- No dependency on third parties for provision of gelling agent
- HA-gellan can be deacetylated during heat processing
- Use of fermentation broth to produce gelled petfood is feasible (Newton, 2006)

Therefore the gellan system should be investigated further in order to validate that an advantage exists over the current mixtures, which leads this document to its final section of future studies.

7.3 Future studies

In terms of petfood, the next step before possible application of the gellan system in gelled petfood is validating that indeed there is a flavour advantage over the current system used by carrying out palatability trials with trained cat and dog panels. Furthermore validating the conclusion that brittle texture does give a better flavour release and flavour perception is also very important.

As for gelled products in general, further studies should be carried out with sensory panel to verify the possible effect of texture on flavour perception. The investigation should be two fold. One part of the study could be looking into different textures to which the same amount of flavour has been added and another part could be investigating if a particular texture even with less amount of added flavourants will give an equal flavour perception meaning that the stimuli that arise from masticating a particulate texture compensate for the lack of actual flavour present.

Furthermore thermal analysis experiments on the gel samples via differential scanning calorimetry (DSC) will reveal useful information regarding gelling and melting temperatures of the different gelling systems. DSC experiments can also yield information regarding the behaviour of gelatin in the system, as well as provide information regarding deacetylation of high acetyl gellan gum. In addition to that

microscopy could also help in identifying the structure of the network and aid with interpretation of flavour release experiments.

Finally it will be of interest to investigate 3 phase systems in order to understand the phase behaviour of such systems and how this will impact on texture, flavour release and sensory perception. The techniques mentioned in the previous paragraph with a focus on microscopy will have to be implemented.

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