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Mixed fluid gels formation, structure and rheological properties

By

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Abstract

The World Health Organisation in its Global Status Report on non-communicable diseases from 2014 warned that worldwide obesity levels had nearly doubled since 1980 and due to the concern surrounding this figure, one of the main global targets is to half this number by 2020. It has been agreed that the best way to reach this goal is to promote physical activity and consumption of a healthy diet. However, up to 85 % of foodstuffs consumed in developed countries are processed products. Therefore, principal route to obtain more of healthy and nutritious food is to decrease the amounts of fat, sugar or salt in existing mass-market products.

Changing product formulation is a very challenging task as every component and their correlations have an impact on food taste, texture, release of flavours and most importantly on consumer acceptance. Reformulated products in comparison to their original version require different process parameters and 'clean label' ingredients. As a result, there has been a demand for natural and novel structures, precisely designed to closely mimic the properties of replaced component, without compromise on sensory perception of taste and texture.

The food industry has been using polysaccharides derived from red seaweed as thickening, gelling and stabilizing agents for many years. However, a relatively new approach is the application of shearing force to polysaccharides undergoing gelation in order to obtain a structure with bulk rheological behaviour similar to emulsions used in soft solid food products. This structure called 'fluid gel' consists of gel particles in a non-gelling medium and its particles are able to mimic fat particles found in full-fat mayonnaise duplicating their rheological behaviour. This feature makes the fluid gel structure a potential fat replacer in foods requiring this consistency. The formation of single polysaccharide fluid gel structure

has been well described in the literature. However, mixed fluid gels have a greater potential of control over texture and in use performance but mechanisms governing their structure formation are not very well understood yet. It is therefore of the utmost importance to attain a better understanding of the relationship between formulation, process conditions, structure and rheological properties in mixed fluid gels to maximise the potential of these versatile structures and contribute to a healthier food products.

This research aims to contribute to this understanding by focussing on the investigation of sheared gel mixtures formed from different charge density, red algal polysaccharides (ι-carrageenan, κ-carrageenan, furcellaran and agarose) and starch and its derivatives (pregelatinized cross-linked waxy maize starch and maltodextrin Paselli SA2). A combination of rheological and optical microscopy analysis were used to investigate how presence of an additional co-solute and manipulation of process parameters during gelation (shear rate and the cooling rate) impact on mixed fluid gel structure and rheological properties.

Investigations revealed that mixed fluid gels have a potential as more effective viscosity and viscoelasticity modifiers compare to single fluid gel structures. It was observed that mixed fluid gel structures were formed as a result of weak physical interactions rather than chemical interactions. The addition of starch or maltodextrin to red algal polysaccharide can significantly influence final structure. If gelation of the red algal polysaccharide in the complex system occurred, the size of mixed fluid gel aggregates and interactions between them decreased with a decrease in red algal polysaccharide charge density. In terms of process parameters, applied shear rate was found to be more effective structure modifier in mixed systems with addition of starch, whereas cooling rate in mixtures containing maltodextrin.

Acknowledgements

I would like to express my enormous gratitude to my academic supervisor Prof Ian Norton for affording me this unique opportunity of Ph.D. studentship within The Microstructure Engineering Research Group at the University of Birmingham. Additionally, I would also like to thank Dr Ian Hamilton, the research fellow who assisted me throughout my project.

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Thank you.

Mamie, Tacie i Siostrze

(to my Mum, Dad and Sister)

„Niczego w życiu nie trzeba się obawiać, Trzeba to tylko zrozumieć.

Nadszedł czas aby więcej rozumieć, by moc się mniej bać”

‘Nothing in life is to be feared, it is only to be understood.

Now is the time to understand more, so that we may fear less’

- Maria Skłodowska-Curie (1867 – 1934)

Table of Contents

List of Figures

List of Tables

Nomenclature

η	Apparent viscosity
η_0	Zero shear viscosity
$[\eta]$	Intrinsic viscosity
$\Delta\,\eta$	Viscosity change
C	Concentration
C^*	Critical concentration
Mw	Molecular weight
K	Mark-Houwink constant
a	Mark-Houwink exponent
B, K	Consistency coefficient
c,n	Flow index
$\dot{\gamma}$	Shear rate
σ	Shear stress
γ	Cooling rate
T	Temperature
T_{gel}	Gelation temperature (sol to gel phase transition temperature)

T_m	Melting temperature (gel to sol phase transition temperature)
ΔH	Enthalpy change
G	Shear modulus
G'	Elastic (storage) modulus
G''	Viscous (loss) modulus
G^*	Complex modulus
$Tan\ (\delta)$	Loss tangent, damping factor
γ	Linear viscoelastic region
F	Applied force
A	Area
Δy	Distance between plates
s	Deflection path or Extension
ΔV	Velocity difference
C_H	Spring constant

Abbreviations

DE	Dextrose equivalent
G	Quiescently cooled gel
SG/FG	Sheared gel, fluid gel
LVR	Linear viscoelastic region
κC	κ-carrageenan
ιC	ι-carrageenan
Ag	agarose
Fu	furcellaran
CLWM	Cross-linked waxy maize starch
SA2	Maltodextrin Paselli SA2

Chapter 1. INTRODUCTION

1.1. Background

In the Global Status Report on non-communicable diseases published in 2014, the World Health Organisation (WHO) warned that worldwide obesity levels had nearly doubled since 1980, and their aim is to half this increase by 2020. (WHO, 2014)

A key factor responsible for this situation is an unhealthy diet rich in calorie dense, processed foods. The WHO reported that: 'Obesity can be prevented through multisectoral population-based interventions that promote physical activity and consumption of a healthy diet, throughout the life-course'. (Mendis, Davis, and Norrving; 2015) To reach this target, the cooperation of the food industry in areas such as product reformulation is essential. However, due to the complexity of a food structure, product reformulation is a challenging task. It is extremely dificult to successfully replace fat, sugar or salt, without affecting material properties such as texture or mouthfeel, which play a significant role in consumer perception and acceptance of a food product. A new, promising way to achieve this aim is a microstructural engineering approach presented in **Fig 1.1**.

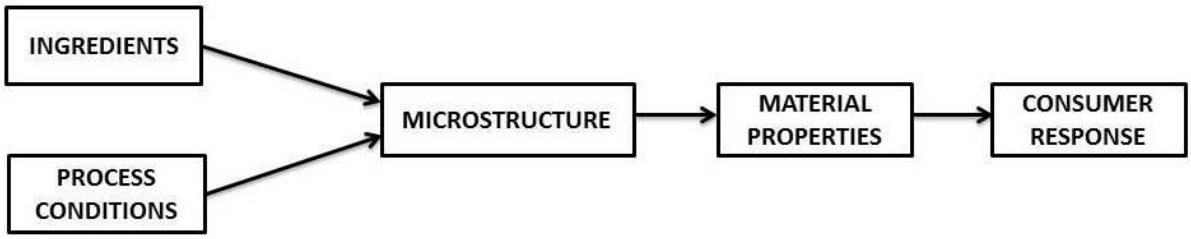

Figure 1.1 Microstructural engineering approach to novel food microstructure design

This approach enables the design of novel food microstructures with similar or identical rheological behaviours to the fats, sugars and salts they will replace, while maintaining the same oral response as the primary product. This could be achieved by manipulation of the

formulation and process conditions and understanding correlations between ingredients, process conditions, structure and material properties. (Aguilera 2005; Norton, Frith, and Ablett 2006; Aryana and Haque 2001; Gidley 2013; Kavas et al. 2004; Koutsopoulos, Koutsimanis, and Bloukas 2008; McMahon et al. 1996; Norton and Norton 2010; Passos and Ribeiro 2010; Prindiville, Marshall, and Heymann 2000; Totosaus, Alfaro-Rodriguez, and Perez-Chabela 2004)

Fat is a great contributor to the experience of food consumption as it is a carrier of aroma and flavour. It also impacts on palatability, creaminess, crispiness and flakiness. Discrete particles of fat act as filler and contribute to the sensation of thickness and perception of smoothness which significantly influence food texture and mouthfeel. The Organisation of International Standards describes the texture as: 'all the mechanical, geometrical and surface attributes of the product perceptible using mechanical, tactile, visual and auditory receptors'. (Szczesniak, 2002) Mouthfeel is described by Stokes *et al.* as a perception of texture during consumption. (Stokes, 2013) These features strongly determine product acceptance by the consumer which is why when fat is removed from the product, is it crucial to replace it with a constituent that is less calorie dense but provides identical texture and mouthfeel.

Food microstructure significantly influences texture perception as well as many aspects of human health. (Norton, Frith, and Ablett 2006) Sensory studies have revealed that a direct relationship exists between particle size and texture perception. Particle sizes below 0.1 μm resulted in a watery sensation and sizes between 0.1-3 μm were related to the sensation of *creaminess*, while particles above 3 μm provided a gritty sensation. Research by Tyle *et al*. (2013) reported that in-mouth particle detection was related to particle size, shape, and hardness. (Tyle 1993) Kokini *et al*. (1987) also demonstrated that *creaminess* is related to perceptions of thickness, smoothness, and slipperiness. (Kokini and Plutchok 1987; Garrec,

Guthrie, and Norton 2013) and Guinard & Mazzucchelli have shown that smoothness depends on particle size within the dispersion. Research carried out by Richardson *et al.* (1989) and Shama *et al.* (1973) have indicated that thickness perception can be related to the viscosity. (Shama 1973; Richardson 1989)

Red algal polysaccharides have high water absorption capacity and therefore shown great potential as thickening, gelling and stabilizing agents which are able to influence rheological properties such as the viscosity and elasticity in the similar way as fat. (Saha, 2010) However, the discovery that seaweed polysaccharides can be sheared during gelation to produce an emulsion-like 'fluid gel' structure, that is, gel particles in a non-gelling medium has widened the potential application of these gelling agents in emulsion based soft solid food products. Norton *et al.* found that spherical particles of agar fluid gel can mimic the rheological behaviour of full-fat mayonnaise. (Norton and Norton, 2010) The main advantage of a fluid gel over a quiescently cooled gel is the possibility of obtaining various, rheologically different structures from the same material by manipulation of the processing conditions.

Single component fluid gel formation has been described in detail in previous studies carried out by Norton *et al.* (1999), Gabriele *et al.* (2009) and Garrec *et al.* (2012,2013). (Garrec, Guthrie, and Norton 2013; Garrec and Norton 2012; Gabriele, Spyropoulos, and Norton 2009; Norton, Jarvis, and Foster 1999) Their results have shown that the rheological properties of fluid gels depend greatly on the polymer density within particles as well as particle size and the interactions between them. Junction zone density can be affected by biopolymer and gel promoting ion concentration. Particle size and interactions are related to the shear, and cooling rate applied during the structure formation process.

Nevertheless, it is very difficult to mimic fat using a fluid gel based only on one ingredient. Hydrocolloid mixtures give versatility of texture and in use performance. Introducing another component in the fluid gel system can lead to superior functional properties (viscosity and viscoelasticity) and reduction in cost of the ingredients. Desired microstructures of mixed fluid gels can be achieved not only by altering the processing conditions but also by modifying the formulation, especially concentration of co-solvent. As a result, replaced ingredients in reformulated application can be more precisely matched without compromising on texture or mouthfeel.

Although there have been a number of publications, where a brief description of mixed fluid gels was included (Norton, 2000; Norton, 2006), prior to this study there has been no systematic study on the effect of the formulation and process conditions on mixed fluid gel structure and rheological properties, which could resolve the shortcomings of the use of a single component fluid gel as a fat replacer.

1.2. Aim of research – thesis objectives

The aim of this thesis is to gain a better understanding of the formation of mixed fluid gel structures and the correlation between blend formulations, process conditions, and rheological properties. To this end, the research was focussed on characterizing sheared gel mixtures formed using red algal polysaccharides (ι-carrageenan, κ-carrageenan, furcellaran and agarose), pregelatinized cross-linked waxy maize and maltodextrin Paselli SA2. A combination of rheological investigations and microscopic analysis has been used to examine the effects of varying the shear rate and cooling rate on the gelation of these mixtures. The aim of this thesis is to gain better understanding of the formation of mixed fluid gel structures and the correlation between blended formulations, process conditions and rheological

properties. To achieve these objectives mixtures of red algal polysaccharides with pregelatinised cross linked waxy maize starch or maltodextrin Paselli SA2 were investigated. Rheological measurements of mixtures were made using a Kinexus Pro rheometer equipped in vane geometry. The Kinexus Pro rheometer was chosen as it enables the researcher to precisely control the applied shear and cooling rate which are crucial variables in understanding fluid gel production.

In the first part of this study, the mixture under investigation was κ-carrageenan with pregelatinized cross-linked waxy maize starch. Variables included the shear rate, cooling rate, and concentration of components; their effects on fluid gel structure formation and viscosity were investigated. κ-carrageenan was chosen as a primary gelling agent because single fluid gel structure formed from this polysaccharide has been well described in the literature. (Gabriele, 2009) Pregelatinized cross-linked waxy maize starch was selected as an additional component as it is known to have a high molecular weight thickening ingredient with low DE, which significantly influences the viscosity. It is also widely and cheaply available, and has well-defined chemical structure (>95 % amylopectin), which provides a clean label in food products. In the second part of the study, κ-carrageenan was instead mixed with maltodextrin Paselli SA2 and the fluid gel structure formation was investigated. Maltodextrin Paselli SA2 is a potato starch hydrolyzate formed of intermediate-length chain of D-glucose. The advantage of the Paselli SA2 over starch is providing fat-like texture, mouthfeel and lack of starchy aftertaste. Fat-like properties are believed to originate from the three-dimensional gel network formation. Lower molecular weight of this component is also believed to enable formation of smaller mixed fluid gel aggregates, providing creaminess sensation. In the third part of this study, investigated mixtures contained different charge density polysaccharide derivatives from the same family of red algal seaweed (ι-carrageenan, furcellaran, and

agarose) and forming a gel by the same mechanism as κ-carrageenan. Polysaccharides were mixed with 8 % (sol) and 12 % (gel) maltodextrin Paselli SA2. The aim of this section was to observe the effects of polysaccharide charge density on mixed fluid gel aggregate formation. The effects observed during red algal polysaccharide/maltodextrin sheared structure formation are compared to those seen in κ-carrageenan/maltodextrin fluid gel formation under the same process conditions.

The reason for the detailed investigation of mixed fluid gels is that the material properties are expected to be influenced by their structures which consist of aggregates and non-gelling medium (**Fig 1.2**) Structure rheology is expected to depend on applied process parameters which define the size and shape of the aggregates and as a result the dispersion viscosity. The concentration of the components is also expected to influence aggregates elasticity as well as the amount and quality of the non-gelling medium which would have an impact on fluid gel structure viscosity and viscoelasticity.

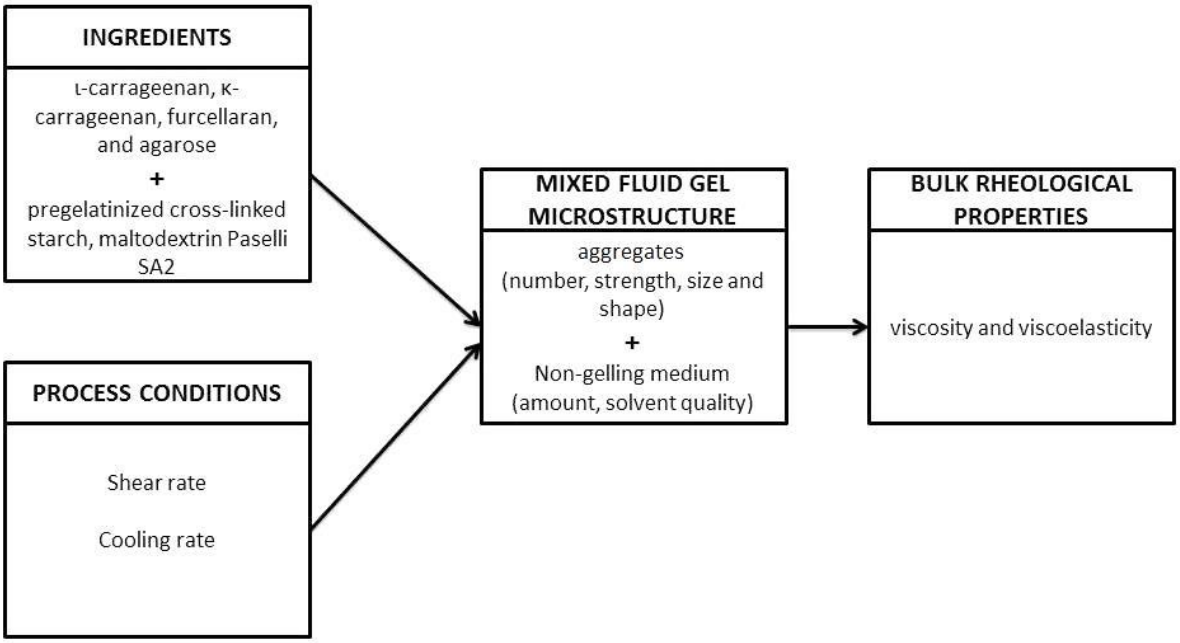

Figure 1.2 Microstructural engineering approach to mixed fluid gel structure design

1.3. Thesis layout

This manuscript consists of six chapters, an introduction, a literature review, three results' chapters, conclusions and future work.

- Chapter 1 is an introduction outlining background information and aim of the work.
- Chapter 2 is a literature review describing in detail the state of the art of single and mixed red algal polysaccharides structure formation and the fundamentals of fluid gel structure formation and characterization.
- Chapter 3 is the first results section. The aim of this chapter is to investigate the correlation between formulation, process conditions, structure and material properties in sheared gel mixtures formed from κ-carrageenan and pregelatinized cross-linked waxy maize starch.
- Chapter 4 is the second results chapter, correlations between formulation, process conditions, structure and rheological properties in sheared gel mixtures formed from κ-carrageenan and maltodextrin Paselli SA2 we investigated.
- Chapter 5, the final results chapter, investigates the formation, and viscoelastic properties of sheared gel mixtures made from various red algal polysaccharides with a different number and position of ester sulfate groups present on repeating disaccharide units (ι-carrageenan, k-carrageenan, furcellaran, agarose) together with maltodextrin Paselli SA2
- Chapter 6 presents conclusions drawn from this research and provides recommendations for future work.

1.4. Publications and presentations

Research results obtained throughout this study have been published and presented as follows:

Publications:

Gladkowska-Balewicz, I., Norton, I. T., & Hamilton, I. E. 2014, Food Hydrocolloids 42, 355-361, 'Effect of process conditions and component concentrations on the viscosity of kappa-carrageenan and pregelatinized cross-linked waxy maize starch mixed fluid gels'.

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T. 2016, 'Relationship between formulation, process parameters, structure and rheological properties of κ-carrageenan/maltodextrin Paselli SA2 gel mixtures produced under shear'. (in preparation)

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T. 2016, 'Rheological studies of the fluid gel structure formation in mixtures of different charge density red algal polysaccharide and maltodextrin Paselli SA2'. (in preparation)

Poster presentation (presenter underlined):

Gladkowska-Balewicz, I., Norton, I.T. 'Effect of process conditions, components concentration and ionic environment on rheological properties of κ-carrageenan and pregelatinized cross-linked waxy maize starch fluid gels'. 1st UK Hydrocolloid Symposium, Huddersfield, UK, 2013

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T. 'The effect of formulation and process conditions on the structure and rheological properties of hydrocolloid sheared mixtures'. 3rd International Conference on Food Structures, Digestion and Health, Wellington, New Zealand, 2015

Gladkowska-Balewicz, I., Norton, I.T. 'Science behind our everyday food choices', Food Matters Live, London, United Kingdom, 2016 - Winner of SCI Agri-Food Poster Competition

Oral presentation (speaker underlined):

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T. 'Characterisation and comparison of κ-carrageenan mixed fluid gels formation and rheological properties', 12th International Hydrocolloids Conference, Taipei, Taiwan, 2014

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T. 'Changes in viscoelastic properties as an effect of Maltodextrin Paselli SA2 addition on Red Algal polysaccharides helix aggregation in sheared gel mixtures' The 7th International Symposium on Food Rheology and Structure, Zurich, Switzerland, 2015

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T., 'The production and material properties of Red Algae polysaccharides/ Maltodextrin Paselli SA2 sheared gel mixtures' 18th Gums and Stabilisers for the Food Industry Conference, Wrexham, UK, 2015

Gladkowska-Balewicz, I., Hamilton, I.E., Norton, I.T., 'Correlation between process conditions, material structure and rheological properties in hydrocolloid sheared mixtures'. 2nd UK Hydrocolloid Symposium, Birmingham, UK, 2015

Additional activities related to this Ph.D. project

Popular Science article:

Gladkowska-Balewicz, I, 2015, Getty Science vol 2, 'Mixed Fluid Gel: Identity thief in fight against obesity.'

Gladkowska-Balewicz, I, 2016, Getty Science vol 7, 'Food structure: why we like what we bite.'

Newspaper articles:

On 30.10.2015 a major Polish newspaper, Gazeta Wyborcza published an article about this research project titled: Bedzie mozna jesc tlusto i do woli i nie przytyc (pol) / It will be possible to eat fatty foods without gaining weight (eng).

On 16.11.2015 a major regional Polish newspaper, Gazeta Lidzbarska published an article about this research project titled: Lidzbarczanka odchudza jedzenie (pol) / The girl from Lidzbark is slimming the food (eng)

TV interview:

On 23.11.2015 Poland's largest private television station, TVN broadcasted an interview with me, in the breakfast TV show 'Dzien Dobry TVN' as a part of the topic: Dlaczego tluszcz jest niezbedny w diecie? (pol) / Why is fat essential in our everyday diet ? (eng). The interview was in relation to this research project.

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Chapter 2. LITERATURE REVIEW

2.1. Background

The aim of this chapter is to present a comprehensive survey of the relevant literature, including an overview of hydrocolloids, polysaccharide structure, and association in aqueous solutions. Then polysaccharide functional properties and role in food microstructure creation, viscosity enhancement and gelation, multicomponent gel structure and rheology are discussed. Fluid gel theory is then examined regarding terminology, formation mechanism, and properties.

2.2. Hydrocolloids and food microstructure

In the food industry, the term 'hydrocolloids' refers to long chain polymers (polysaccharides and proteins) able to enhance viscosity or form gels in aqueous solutions. (Phillips & Williams, 2000; Syed K. H. Gulrez, Saphwan Al-Assaf and Glyn O Phillips, 2011)

Because of their relevance to the research presented in this thesis, only polysaccharides will be discussed in detail.

Polysaccharides used in foods can be split into two main groups. The first one includes starch and its derivatives while the second group consists of non-starch polysaccharides often referred to as gums. They are built of many sugar monomers linked together by glycosidic bonds. They also contain a large number of hydroxyl groups (-OH) which significantly increase their ability to bind water molecules and form dispersions thereby exhibiting colloidal behaviour. The viscosity of the solution depends on the volume occupied by polysaccharide. The occupied volume is related to polysaccharide shape, molecular weight and for polyelectrolytes also to the ionic strength of the aqueous medium. (Morris, 2007)

Polysaccharides functionality results from interactions which lead to viscosity enhancement, aggregation and network formation. These features make them desirable to the food industry as thickening, gelling and stabilizing agents which can influence the product's rheology:

resistance to flow (viscosity) and structure strength and stability (viscoelasticity). (Saha and Bhattacharya 2010)

The contribution of a polymeric solute to the solution viscosity is expressed by its intrinsic viscosity [η]. Intrinsic viscosity is determined mostly by polymer geometry (shape) and hydrodynamic volume (size). (Harding, 1997) The Mark-Houwink equation describes this relationship and is presented in **Equation 1**, where M_w is the polymer molecular weight, K is the Mark-Houwink constant, and α is the Mark-Houwink exponent, both related to the polymer geometry in solution.

$$[\eta] = K{M_w}^{\alpha} \quad (1)$$

Exponent α values range between 0 and 1.8, where 0 represents a spherical conformation, values between 0.5 – 0.8 characterize polymers with random coil conformation and values greater than 0.8 indicate increasing polymer stiffness and decreasing flexibility. Values rise to around 1.8 for rigid rod like structures. (Tombs 1998)

Polysaccharide solution viscosity depends not only on the polymer's intrinsic viscosity but also on its concentration and interactions between chains. The influence of polysaccharide concentration on the solution viscosity and chain interaction is presented in **Fig 2.1.**

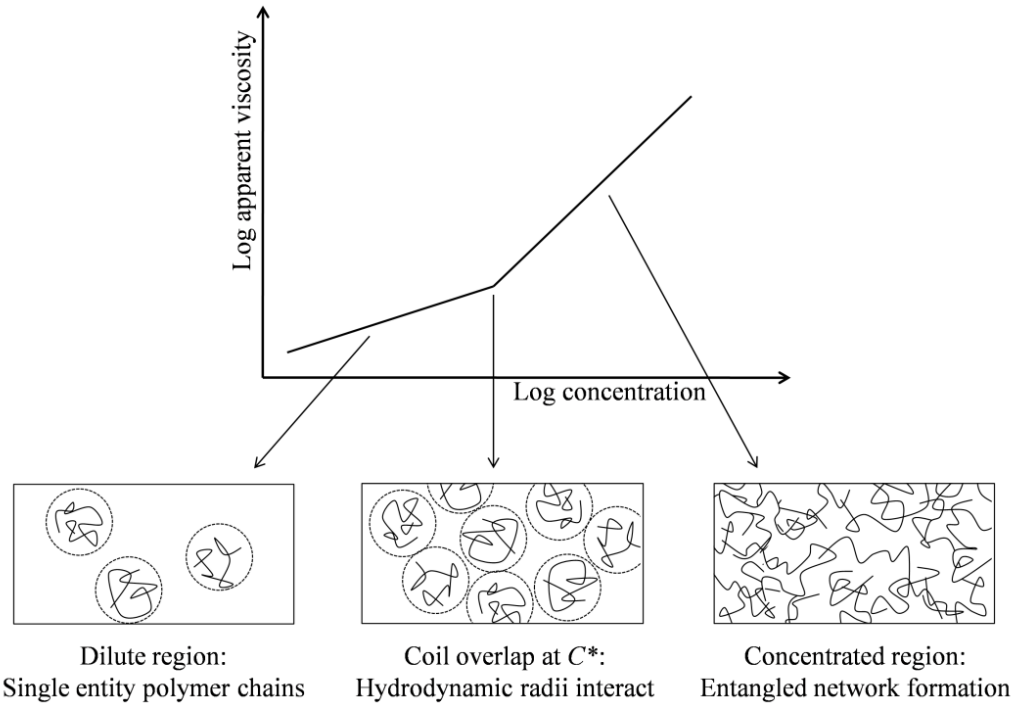

Figure 2.1 Influence of polysaccharide concentration on the solution viscosity and chain interactions in dilute region < C^* in semi-dilute region = C^* and concentrated region > C^* (Image adapted from Clegg 1995)

Low polysaccharide concentrations create dilute solutions where sufficient space exists between chains and prevent any interactions. The viscoelasticity of a dilute solution is measured by the sum of individual coil viscoelasticity and the total number of coils present in the solution. Increasing the polysaccharide concentration to the critical concentration (C^*) results in individual coils overlapping, at which point the solution is said to be a semi-dilute solution. In this region the size of the coil and the average distance between entanglements decrease with increasing concentration. Above this critical concentration, any further increase in polysaccharide concentration results in an entangled network formation where the individual character of the coils is lost. Rheological properties in concentrated solutions depend mainly on interactions between coils. The association of polysaccharide chains and

junction zones formation results in rapid increase in viscosity and gel network formation. (Morris, 1981)

2.3. Gelation and gel structure

The term ‘gel’ comes from the Latin ‘gelatus’ which means frozen, immobile. Gelation describes the ability of polysaccharides to rearrange their molecular conformation and form a network structure. Polysaccharide network formation is a thermodynamic transition from sol to gel phase where loss of conformational freedom and hydration energy is reimbursed by chain-chain interactions. Sol to gel phase transition can be achieved by changing conditions such as temperature, pH or concentration of ions which decrease intramolecular interactions and instead promote intermolecular interactions. (Rinaudo, 1993; Phillips and Williams, 2000)

The gel is a unique type of material which, in the words of Dorothy Jordan Lloyd, is easier to recognize than define. It possesses interesting rheological properties, such as solid-like behaviour, despite the fact that it is composed mostly of liquid. (Jordan Lloyd, 1926; Pierre, 1998) Several researchers have undertaken the challenge to define gel structure. Ferry *et al.* has suggested that it is a swollen polymeric system which if subjected to steady shear deformation will either fracture or rupture. However, this definition is not true of every gel structure. (Ferry, 1980) The rheological definition of the term ‘gel’ introduced later was based on the concept of dynamic viscometry, which states that ‘gel’ is a viscoelastic system with a ‘storage modulus’ larger than its ‘loss modulus’ and independent of oscillatory frequency. (de Vries, 2004a; Phillips and Williams, 2000; Burchard and Ross-Murphy, 1990)

This definition was then extended to the statement that a ‘gel’ is a solid-like material consisting of at least two components, of which one, in the vast majority, is a liquid. The

previously proposed definition of a gel as a swollen polymer network was generally accepted as long as the system under investigation also exhibits a flat mechanical spectrum under oscillatory shear (*G'* values plateau, extending in time and considerably smaller *G"* values in the same region). (Clark, 2009)

A gel structure can be obtained via various physical or chemical mechanisms. The different types of gelation mechanism are compared in the diagram in **Fig 2.2.**

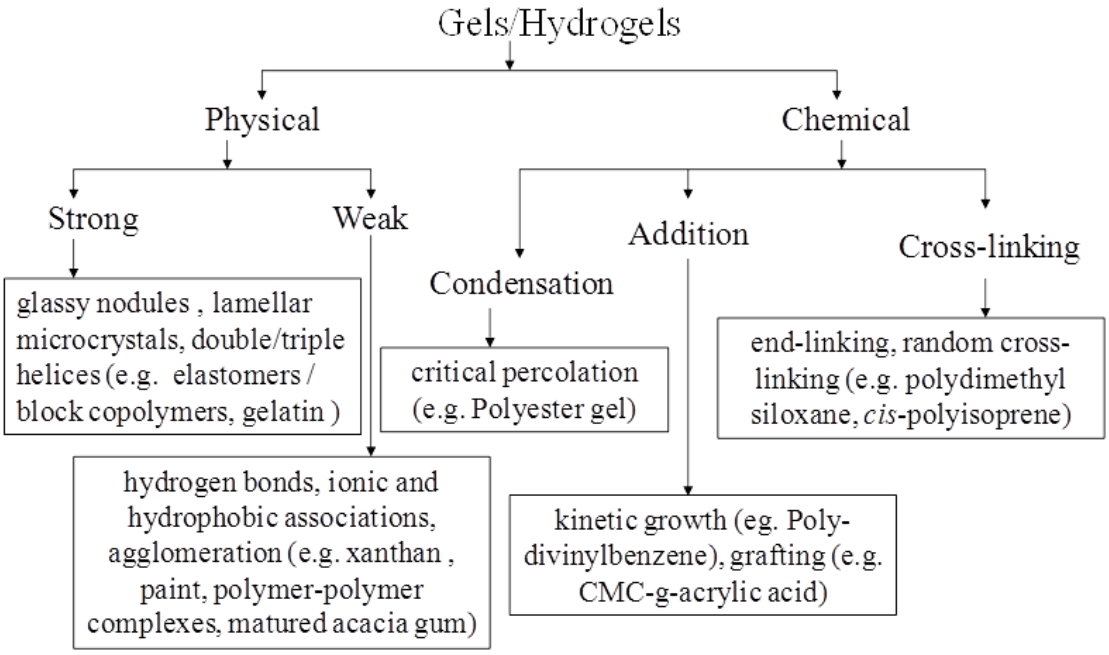

Figure 2.2 Gelation mechanisms comparison (Image adapted from Gulrez 2011)

Only the physical mechanism will be described in this chapter as this one is the most relevant to the polysaccharide gel structure formation investigated in this research.

Polysaccharide gel structures investigated in this research arise as a result of the physical mechanism which involves the formation of double helices and their further aggregation (which includes stabilization by hydrogen bonds, ionic and hydrophobic interactions). Polysaccharide molecules are able to form ordered structures with themselves and with other

molecules. Structures which contain two or more polysaccharide molecules are called ‘junction zones.' Research undertaken by Rees *et al.* (1969) underlined the significant impact of polysaccharide chemical structure on the molecular structure of junction zones in the gel network. (Rees 1969) The number of molecules which form a junction zone is an important characteristic which determinates gel properties. For example, κ-carrageenan gel structures are less flexible than those made of ι-carrageenan due to the presence of six to ten molecules at the multi-molecule junction zones whereas ι-carrageenan junction zones are made of only two molecules. (de Vries, 2004a) The high number of molecules in κ-carrageenan results in a rigid gel structure, not easily rebuilt when disturbed by shear. In comparison ι-carrageenan forms an elastic and flexible structure that is less sensitive to shear. It is not only the number of junction zones but also their flexibility and the flexibility of the interrupting segments that play important roles in the mechanical properties of gel structures. The effect of junction zones on gel properties is presented in **Table 2-1.**

Table 2-1 The effect of junction zones on gel properties (adapted from de Vries 2004a)

Structural feature	**Main effect on gel properties**
Number of molecules in junction zone	Rigidity
Strength of bonds in junction zones (calcium bridging, hydrogen bonds, hydrophobic bonds)	Melting/setting temperature
Number of junction zones per unit gel volume. Flexibility of junction zones and interrupting chain segments	Rigidity, gel strength
Number of junction zones per molecule	Rupture gel strength

The strength of the bonds holding the junction zones together determinates gel thermal behaviour and depends on:

- the solvent quality, especially the presence of sugars or salts
- junction zone length, as bond strength increases more than proportionally with junction zone length

Polysaccharide structures and their properties

The non-starch polysaccharides used in this research are high molecular weight linear algal galactans derived from *Rhodophyceae* seaweeds: κ-carrageenan, ι-carrageenan, furcellaran and agarose.

Carrageenans are negatively charged due to the presence of sulphate groups on the disaccharide units. Their galactose unit is present in the D form and sulphate group content can vary between 15–40 %. Agarose is a neutral polysaccharide and contains an anhydrogalactose unit in the L form. The number and position of the ester sulphate groups on the carbohydrate ring as well as the composition of 3,6 anhydrogalactose affect various properties such as gel strength which decreases with increasing number of SO_3^- ions. Commercially available carrageenans are not homogenous κ or ι chains but hybrids with a certain amount of other carrageenan fractions.

Despite differences in chemical structures, they share the same gelation mechanism, which involves double-helix formation and aggregation facilitated by repetitive sequences of galactose units in positions $^4C_1$ and $^1C_4$. Gel structures that form are thermoreversible. Polymers can form thermoreversible networks when low energy interactions stabilize the gel. Once heated above a critical temperature (melting point) physical bonds are broken, and the

aggregated helix structure is lost. The gel structure is melted and returns to a solution containing random coils. (Tosh, 2004)

The main advantage of red algal polysaccharides is that they significantly increase the viscosity of the aqueous solution and form gels at very low concentrations less than 1.0 %. (Rinaudo, 1993) Linear polysaccharides are more efficient viscosity enhancers compared to branched polysaccharides because their extended charged structure requires more space to gyrate and therefore presents more resistance to flow. In food products, they are used mainly as thickening, gelling and stabilizing agents. (de Vries, 2004)

κ-carrageenan is a sulphated polysaccharide. This polysaccharide structure is made of repeating D-galactose-4-sulphate and 3.6 anhydro-galactose (3.6-AG) units, joined by α-1,3 and β-1,4 -glycosidic bonds. It contains one sulphate group (SO_3^-) per disaccharide unit. The κ-carrageenan chemical structure unit is presented in **Fig 2.3.**

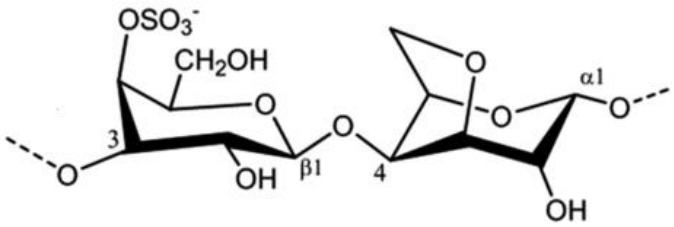

Figure 2.3 κ-carrageenan chemical structure repeating units of D-galactose and 3.6 anhydro-galactose (3.6-AG) (Image adapted from Rhein-Knudsen, Ale, and Meyer 2015)

Before the formation of a gel structure, κ-carrageenan needs to hydrate in the aqueous solution. Hydration occurs through electrostatic interactions of water molecules with negatively charged sulphate groups on κ-carrageenan and through hydrogen bonding between water molecules and OH^- groups on the κ-carrageenan polymer chain. Complete hydration

and solubilization are critical for κ-carrageenan gelation, and it is possible only when the solution is heated to around 80 ºC. After complete hydration and solubilization, in hot aqueous solution, κ-carrageenan is in a colloidal state (random coils).

The process of κ-carrageenan network formation is defined by the dynamic equilibrium between polymer-polymer and polymer-solvent interactions. The number of chains involved in helix formation was extensively investigated in the past until the double helix formation mechanism was proposed by Morris *et al*. (1980). (Morris, Rees, and Robinson, 1980) The domain model, illustrating stages of the κ-carrageenan gel network formation, is presented in **Fig 2.4.**

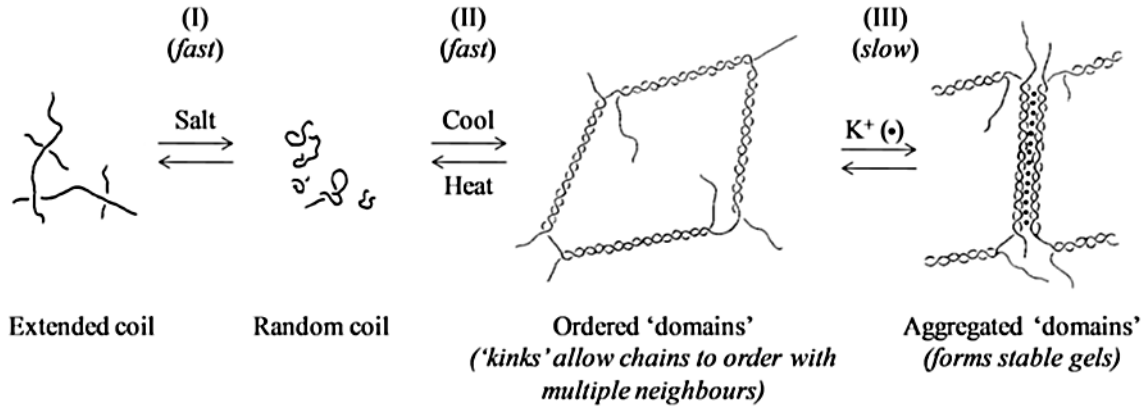

Figure 2.4 Domain model of κ-carrageenan gel network formation (Image adapted from Morris, Rees, and Robinson 1980)

In hot solution, above the melting point, κ-carrageenan exists as expanded coils. It is an effect of the excluded volume and electrostatic repulsions between chain segments (Snoeren and Payens, 1976) with a high water absorption capacity. In this conformation, the chains are flexible and sensitive to the presence of ions. (Tecante, 2012; Harding, 1996) The addition of ions such as potassium or sodium salts reduces electrostatic repulsion between sulphate groups and as a result, less expanded random coils are formed. When the κ-carrageenan solution is in a cooling environment, random coils start their conformational transformation

into ordered, double helixes. Further, decrease in temperature results in double helix aggregation and junction zone formation. The κ-carrageenan gel structure is brittle due to number of the molecules forming the junction zones. κ-carrageenan junction zones are stabilised by hydrogen bonds, as well as hydrophobic and electrostatic forces, which are reversible and can be disrupted by changes in physical condition such as pH, ionic strength, temperature, application of stress, the addition of co-solutes which compete for water. As a result κ-carrageenan structure is also thermally reversible. A model of aggregated helixes in the presence of potassium ions is presented in **Fig 2.5.**

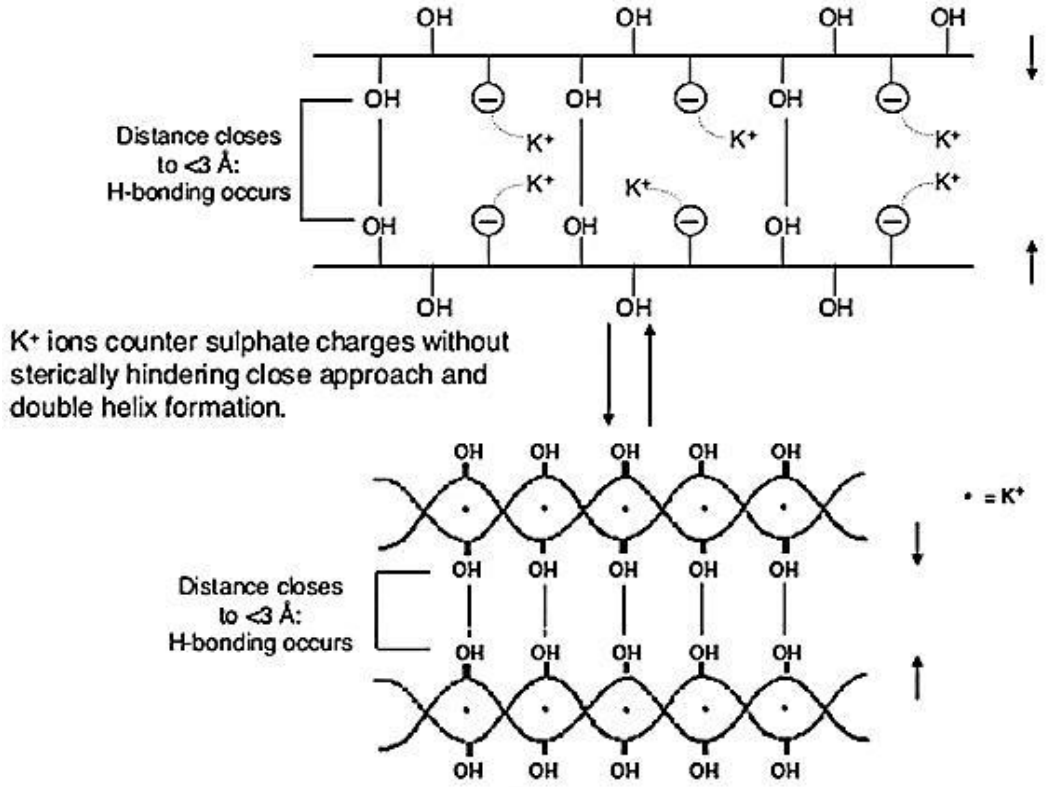

Figure 2.5 κ-carrageenan helixes aggregated in a junction zone in the presence of potassium ions (Image adapted from Imeson 2010)

The κ-carrageenan helix conformation is stabilized by intramolecular hydrogen bonding in an individual chain. Structure aggregation is stabilized by intermolecular hydrogen bonds between helices. Rates of κ-carrageenan ordering and aggregation as well as transition

temperatures are influenced primarily by the type and total ionic concentration as well as hydrocolloid concentration. (Morris *et al.* 1980; Norton *et al.* 1978; Rochas and Rinaudo, 1980) Two temperatures characterize phase transitions in thermoreversible polysaccharide systems. The first is sol to gel transition temperature (T_{gel}) at which random coils form a double helix and aggregate. The sol to gel transition temperature can be obtained using **Equation 2** where T_{gel} is the gelation temperature, a and b are constants and c is the concentration of gelation promoting cations.

$$T_{gel} = a + b\sqrt{c} \tag{2}$$

The gelation temperature depends on the square root of the concentration of gel promoting ion. The increase in ion concentration results in an increase in the gelling temperature (Stanley, 1987). The second temperature is a gel to sol transition temperature (T_m) and represents melting aggregated helices and reverse to random coils phase. The κ-carrageenan melting temperature (T_m) is higher than the gelling temperature (T_{gel}) as more energy is required to melt aggregated helices than for coils to form helices. This phenomenon is known as thermal hysteresis, and it is related to ion concentration in the solution. The larger the hysteresis between gelation and melting temperatures, the stronger, and more aggregated the gel network that formed. (Rinaudo, 1993) Below the critical potassium salt concentration (0.007 mmol/dm^3) thermal hysteresis does not occur. (Tolstoguzov, 2008)

The affinity of κ-carrageenan varies for different ions. (Rochas and Rinaudo, 1980; Rinaudo, 1993; Stephen, Phillips, and Williams, 2006) Taking into account the ability of ions to stabilize the ordered κ-carrageenan conformation, ions promoting gelation can be divided into three groups:

- 'nonspecific' monovalent cations: $(CH_3)_4N^+$, $Na^+ > Li^+$

- 'specific' monovalent cations: $Rb^+ > K^+$, $Cs^+ > NH_4^+$

- divalent cations: $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+} > Zn^{2+} > Co^{2+}$

It was observed that all of the investigated ions have the ability to stabilise helixes by shielding the anionic charge on the polysaccharide. The electrostatic repulsions between helixes decrease with increasing ionic strength of the solution due to the accumulation of counter-ions between surfaces. The 'specific' monovalent cations not only reduce the net charge of the single coil but also impact on double helix stabilization and aggregation due to the decrease in electrostatic repulsion which promotes cooperative hydrogen bond attractions. Ions selectivity was attributed to cation radius and ease of incorporation into the junction zone structure.

ι-carrageenan is sulphated polysaccharide with a structure made of repeating D-galactose-4-sulphate and 3.6 anhydro-galactose-2-sulphate joined by α-1,3 and β-1,4 -glycosidic bonds. It contains two sulphate groups (SO_3^-) per disaccharide unit. The ι -carrageenan disaccharide repeating unit is presented in **Fig 2.6.**

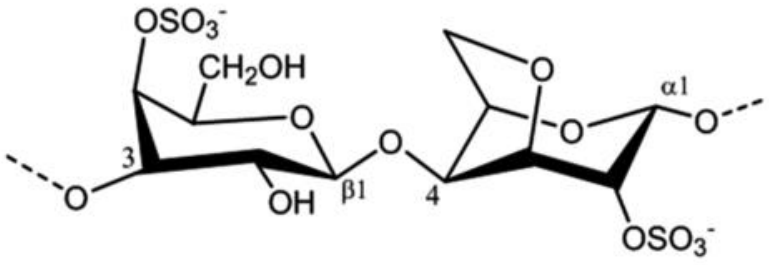

Figure 2.6 ι -carrageenan chemical structure repeating units of D-galactose-4-sulphate and 3.6 anhydro-galactose-2-sulphate (Image adapted from (Rhein-Knudsen, Ale, and Meyer 2015)

Under cooling conditions an ι-carrageenan aqueous solution forms a thermoreversible gel. The gel structure is promoted primarily in the presence of calcium ions which were found to be 'specific' for this type of carrageenan. The two sulphate groups on the outside of the ι-carrageenan molecule do not permit the helices to aggregate to the same extent as κ-carrageenan and as a consequence hydrogen bonding is prevented. However, interactions with calcium cations result in the additional divalent bridging formation, which supports carrageenan molecules in helices formation and aggregation. The mechanism of ι-carrageenan junction zones formation is illustrated in **Fig 2.7.**

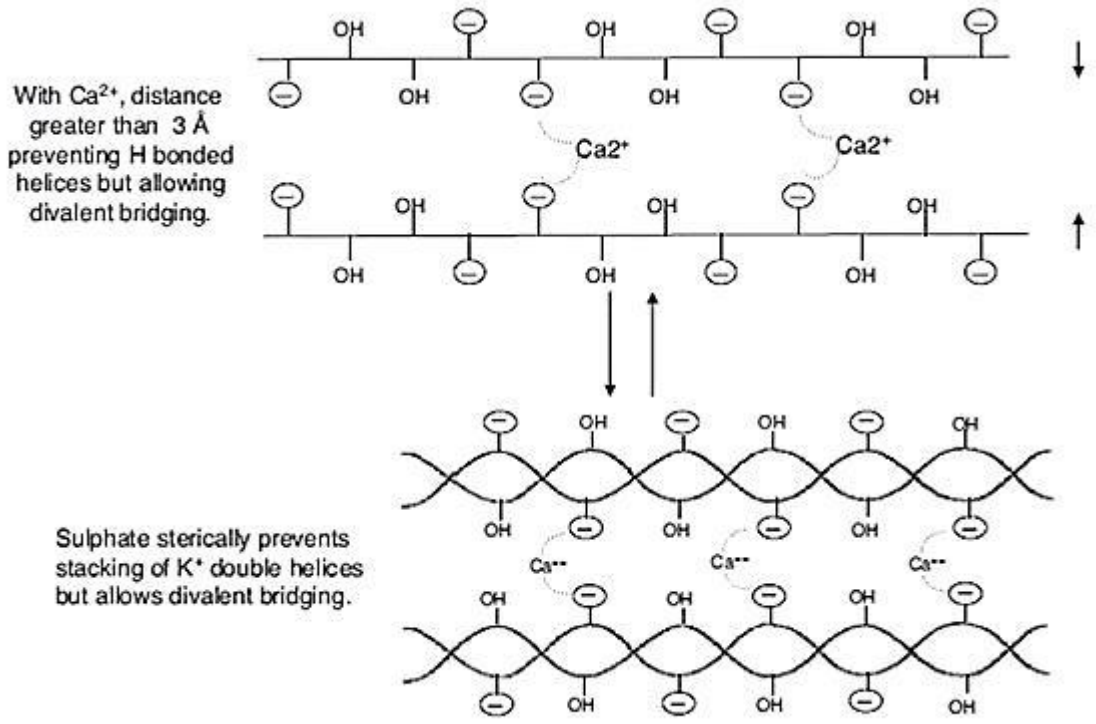

Figure 2.7 Mechanism of ι-carrageenan junction zones formation in presence of calcium cations (Image adapted from Imeson 2010)

Junction zones containing calcium bridges result in a more elastic, softer gel structure which has an unique ability to recover (re-gel) after being subjected to deformation under shear.

Nevertheless, gel structure strength is expected to be lower in comparison to κ-carrageenan as a result of a less aggregated network. (Whistler and BeMiller, 1973)

Furcellaran used in this study was extracted from *Furcellaria lumbricalis*. It is partially sulphated polysaccharide composed of D-galactose, 3,6-anhydro-D-galactose, and D-galactose-4-sulphate. Structurally it is similar to κ-carrageenan. However instead of one sulphate group (SO_3^-) on every disaccharide unit, it contains one on each alternate disaccharide unit. The repeating units which form the furcellaran chemical structure are illustrated in **Fig 2.8.**

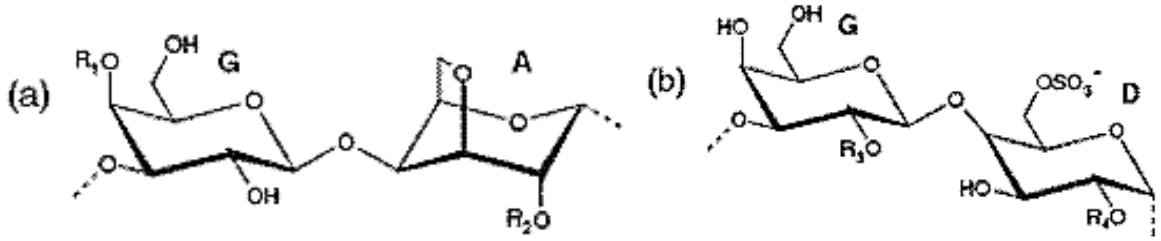

Figure 2.8 Furcellaran chemical structure comprising repeating units of (a) D-galactose, 3,6-anhydro-D-galactose and (b) D-galactose- 4-sulphate (Image adapted from http://www.estagar.ee)

The furcellaran gel structure is formed similarly to carrageenan, so helices form and aggregate in the presence of potassium cations. However, since it is a less sulphated structure than κ-carrageenan helices can aggregate to a greater extent and as a result a stronger gel network is expected.

Agarose is a neutral polysaccharide composed of repeating units of D-galactose and 3,6-anhydro-L-galactose linked by α-1,3 and β-1,4 -glycosidic bonds. The disaccharide unit of agarose is illustrated in **Fig 2.9.**

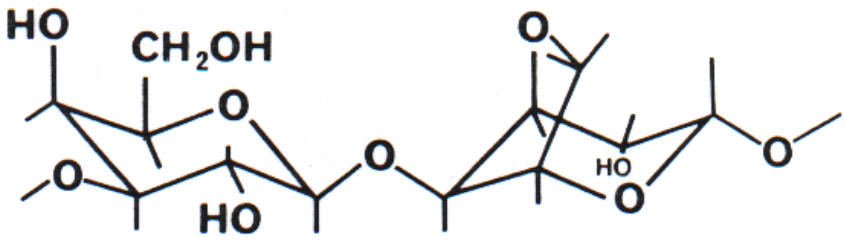

Figure 2.9 Agarose chemical structure composed of repeating units of D-galactose and 3,6-anhydro-L-galactose (Image adapted from Ghorbal 2013)

The gelation process occurs via helix formation and aggregation. The absence of sulfate groups on the disaccharide units enables agarose helices to aggregate to a greater extent and form one of the strongest gels obtained from natural polysaccharides. A simplified diagram of agarose gel structure formation is illustrated in **Fig 2.10.**

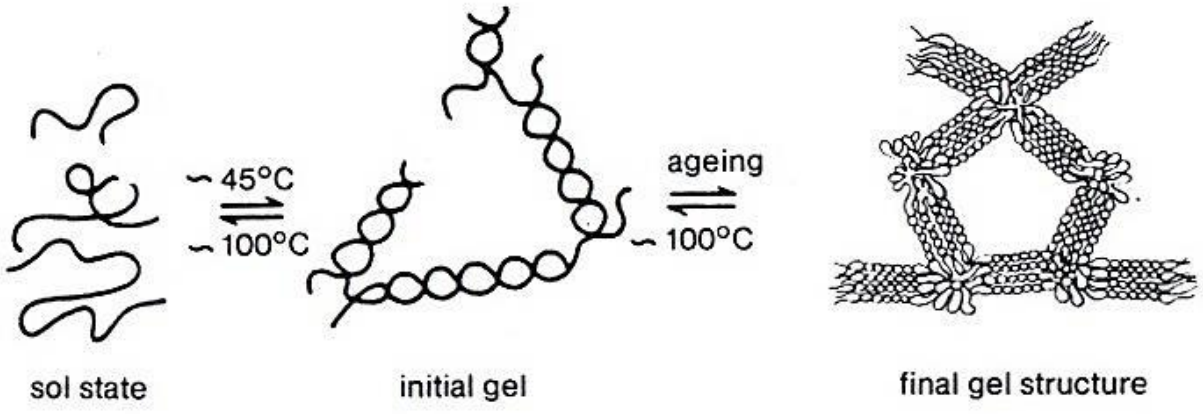

Figure 2.10 Agarose gel structure formation (Image adapted from Laas 1975)

Multiple agarose helix aggregation stabilized by extensive hydrogen bonding creates a strong gel structure (Rinaudo, 1993)

Agarose was the only non-sulphated polysaccharide in this study. Agarose neutral structure was used as comparison to the structures obtained from sulphated polysaccharides.

Starch and its derivatives

Starch and its derivatives used in this research are pregelatinized cross-linked waxy maize starch and maltodextrin Paselli SA2

Pregelatinised crosslinked waxy maize starch

Starch is a biopolymer synthesized by green plants, and it is present in a granular form. The two principal components of starch are linear amylose (α-(1–4)-linked D-glucose units) and highly branched amylopectin (α-(1–4)-linked D-glucose backbones and about 5% of α-(1–6)-linked branches). (Phillips and Williams, 2000; Perez, 2010) The basic chemical structures of glucose, amylose and amylopectin are presented in **Fig 2.11.**

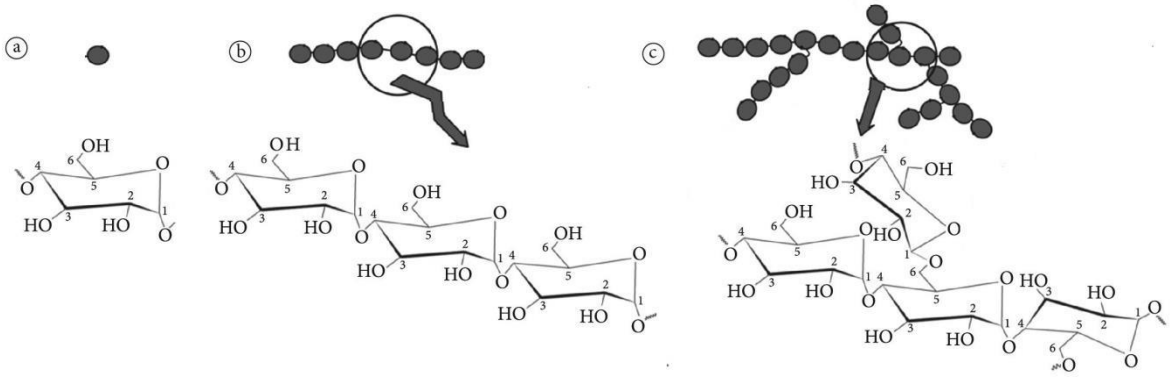

Figure 2.11 Basic chemical structure of (a) glucose, (b) amylose, (c) amylopectin (Image adapted from Pérez 2010)

In the food industry, the most widely used starch is corn (maize) starch. (Hirashima, 2005) Waxy maize starch contains < 5% of amylose, and this has a significant impact on gels produced from this type of starch. Amylose has a high tendency to retrograde which results in robust gels and strong films. In comparison amylopectin, dispersed in water, is more stable and as a consequence soft gels and weak films can be obtained. (Perez, 2010)

Cross-linking (replacing hydrogen bonding by stronger covalent bonds) increases starch granule stability to acids, heat and shear while also improving the texture of gels and pastes. Pregelatinization makes starch solutions homogeneous, helps with dissolution, even in cold water, and eliminates the necessity for cooking.

An optical microscopy image of the pregelatinized cross-linked waxy maize starch used in this study is illustrated in **Fig 2.12.** To increase the visibility of the starch granules, the sample was diluted and dyed using iodine.

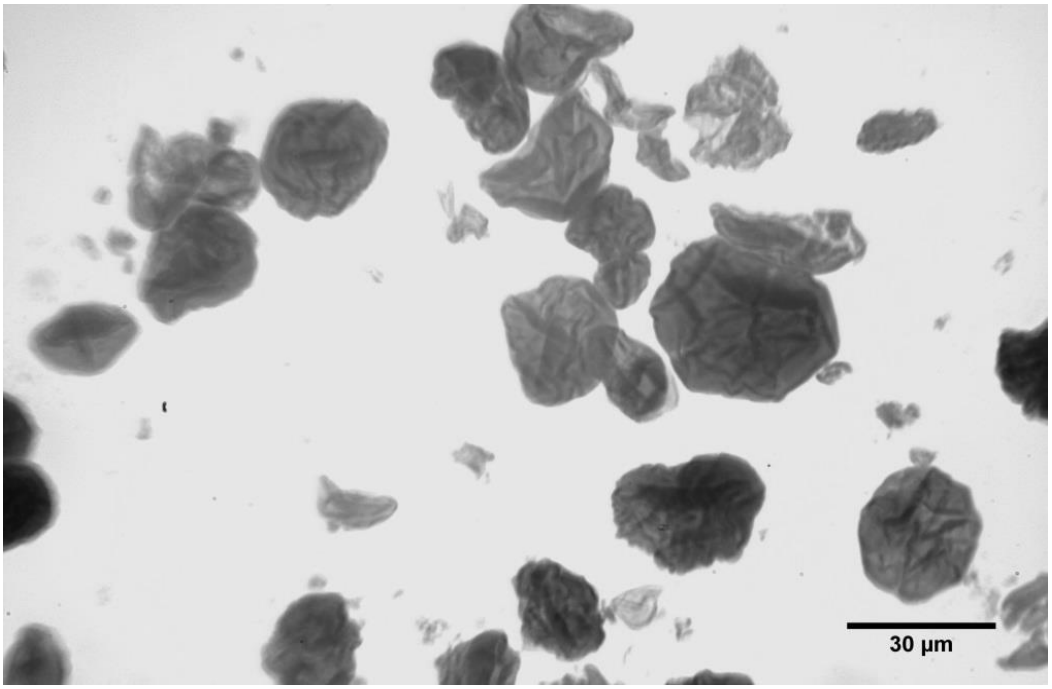

Figure 2.12 Optical microscopy image of pregelatinized cross-linked waxy maize starch granules used in this study

It was observed that starch granules are not perfectly spherical, and their average size in diameter was approximately 30 µm. Some broken granules fragments were also visible, and their size range is less than 15 µm.

The pregelatinised cross-linked waxy maize starch dextrose equivalent (*DE*) is below 1. As a comparison pure glucose dextrose equivalent is 100. The dextrose equivalent represents the

extent of hydrolysis (percentage of glycosidic bonds cleaved or reducing sugars) and at the same time is a measure of sweetness. The sweetness of the structure increases with increasing *DE* number. The determination of the dextrose equivalent value is presented in **Equation 3.**

$$DE = 100 \times \frac{Number\ of\ glicosidic\ bonds\ cleaved}{Initial\ number\ of\ glicosidic\ bonds}\ or\ \frac{Number\ of\ reducting\ sugars\ (glucose)}{Total\ carbohydrates} \quad (3)$$

Key benefits for the use of starch as a thickening agent are nutritious value, low-cost and wide availability. Additionally, the convenience of instant starches includes excellent shelf life, freeze-thaw stability, fast hydration, and smooth and creamy texture.

Food applications for this type of starch include a baked goods, convenience foods, dressings, soups, sauces and gravy mixes and dairy products. In the majority of foods products, it is not the primary gelling agent, but it is used mainly as a support to: improve mouthfeel, increase nutritional value or reduce the amounts of primary gelling agent needed.

Maltodextrin Paselli SA2

Another component used in this research is maltodextrin Paselli SA2. It is partially hydrolysed potato starch with a dextrose equivalent (*DE*) around 2. The low dextrose equivalent eliminates unpleasant sweetness and the need to mask flavour when used in food products. The chemical structure of Paselli SA2 lies somewhere between complex starch chains and simple sugar molecules and is a mixture of high and low molecular weight material with three main fractions: 150-180, 50-180, 6 kDa. (Roller and Jones, 1996) Maltodextrin is built of starch degradation products: linear amylose and branched amylopectin which is present in the majority. The primary building blocks of the maltodextrin chain are D-glucose units linked together by α-(1–4)- glycosidic bonds. The chemical structure of D-glucose is presented in **Fig 2.13.**

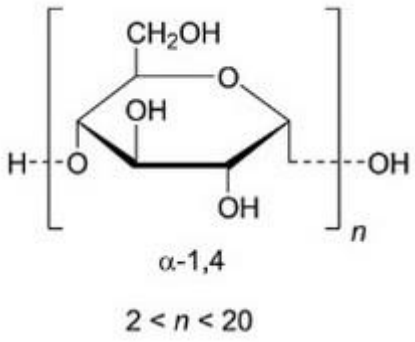

Figure 2.13 Chemical structure of primary maltodextrin chain building unit: D-glucose

Maltodextrin gel structure formation occurs during cooling. The mechanism is initiated by the formation of double helices of amylose. Helix formation is followed by aggregation and results in the creation of crystalline domains. Amylose domains interact with the outer linear chains of amylopectin which leads to the development of a hydrated, thermally reversible gel network. (Reuther, 1984) Sol to gel phase transition is a slow process, which depends mainly on the temperature, time, concentration of the maltodextrin pre-gel solution and structural uniqueness.

According to the literature, in aqueous system concentrations greater than 20 % [w/w] are required to form white gels. Structure setting is achieved within 24 hours, but to obtain final gel strength, it can take up to 4 days. (Khan, 1993)

Maltodextrins are usually added to food formulations in the liquid state where macromolecules can then hydrate and expand. Gels obtained have unique properties and are able to mimic fat/oil texture and provide fat-like mouthfeel due to strong shear thinning behaviour which is also observed when the fat is eaten. Maltodextrins are utilised in reformulated food products where an effective fat replacement is needed.

2.4. Multicomponent gel networks

Food products are mostly multicomponent physical systems. Key elements of foods which influence food structure are polysaccharides, proteins, and water (solvent, plasticizer). The behaviour of individual food components is well understood; however the performance of mixtures is more complicated and remains uncertain. Achieving an understanding of interactions between food elements in the mixture as well as the relationship between structure and properties is crucial for food structure design especially when developing replacement ingredients for reformulated food products.

The simplest multicomponent mixture contains two components. Two hydrocolloid interactions in an aqueous medium are mainly non-covalent, non-specific interactions which depend on polymer properties (molecular weight, charge, solubility) and solvent quality (pH, ionic strength). Mixing two biopolymers can result in four types of mixtures which are illustrated in **Fig 2.14.**

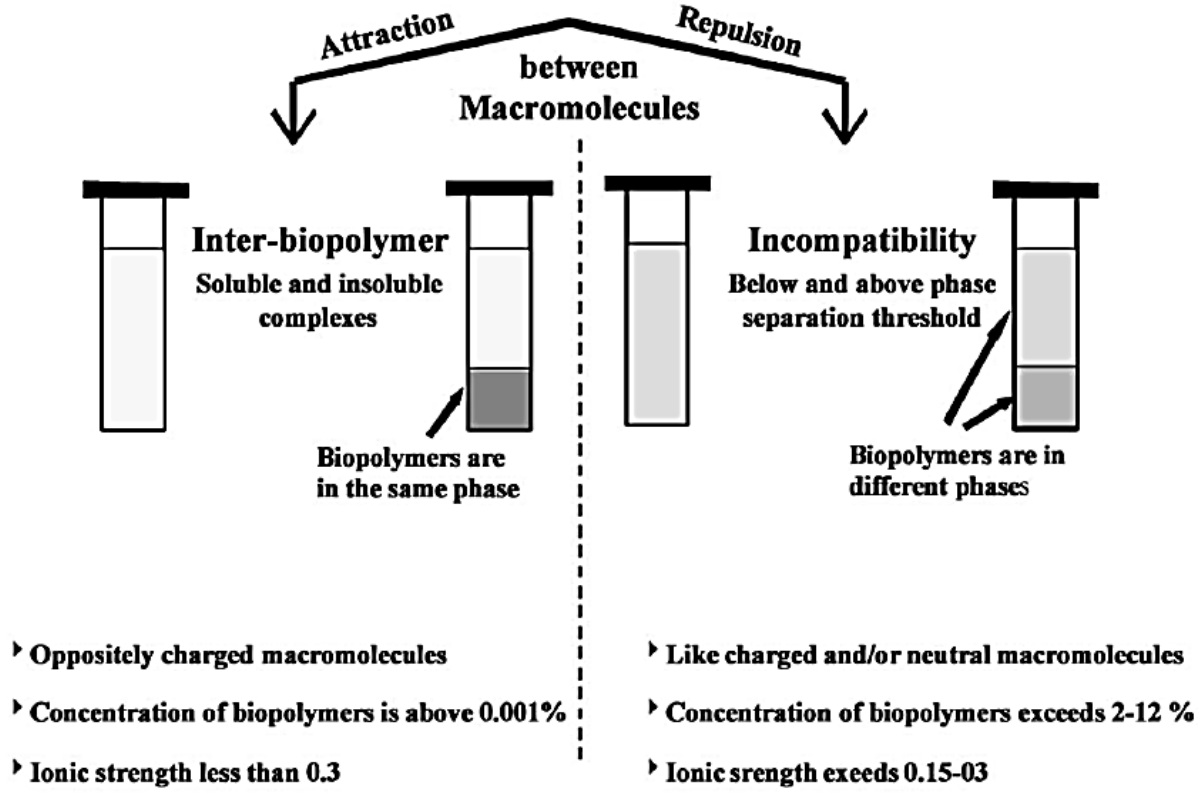

Figure 2.14 Four types of mixtures possible to obtain upon mixing two biopolymers. (Image adapted from Tolstoguzov 2008)

Upon biopolymer mixing two single phase systems (1 and 3) and two biphasic systems (2 and 4) can be formed. Interbiopolymer complexes are set up in a blend of oppositely charged biopolymers when attractive forces between different types of biopolymer exceed the attractive forces of the same kind of molecules. Complexes can be obtained when components are co-soluble (1) or insoluble (2). Interactions in interbiopolymer complexes can be reversible or irreversible as well as cooperative or noncooperative. (Tolstoguzov, 2008)

The difference between biphasic systems 2 and 4 lies in the mechanism of polymer distribution into co-existing phases. In system 2 polymers occupied the concentrated phase due to interbiopolymer complexing. In comparison, in the 4th system, polymers concentrated

in separate phases as a result of incompatibility. Polymer compatibility indicates miscibility at the molecular level. Limited thermodynamic compatibility may lead to limited cosolubility of biopolymers in system 2 and as a consequence to demixing. Another possibility is phase separation of mixed solutions in system four. All four systems structures and functional properties are significantly different. (Tolstoguzov, 2008)

When particles are present in the solution, main interactions with hydrocolloids include:

- adsorption
- charge effects (flocculation and stabilisation)
- depletion flocculation

Type and properties of gel structure formed from two biopolymers depend on components distribution between different phases and their interfacial interaction. (Tolstoguzov, 2008) Binary polymer gel networks can be divided into two groups. The first group contains gel mixtures in which only one polymer forms a network. (Morris, 1986a) The two component networks comprising a soluble polymer entrapped inside a gelling polymer is illustrated in **Fig 2.15.**

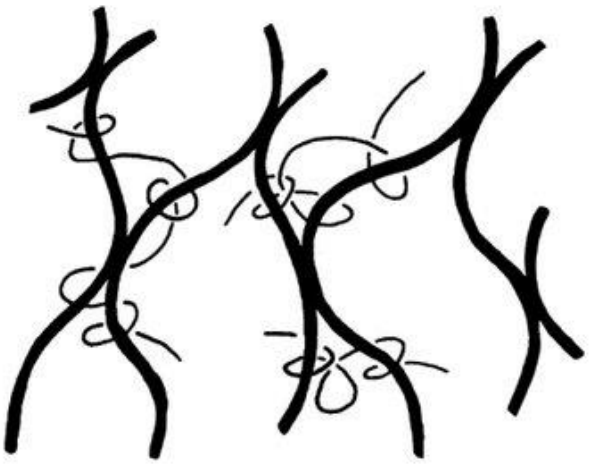

Figure 2.15 Two component network comprising a soluble polymer entrapped inside gelling polymer network (Image adapted from Brownsey 1988)

Despite the fact that the soluble polymer is not actively involved in gel network formation; it can impact on the gelation of another polymer, affect the conformational transition or swell the polymeric network. It was observed that dextran mixed with aqueous gelatin, increased the rate of helix formation and gelation. Excluded volume effect of hyaluronates entrapped in collagen resulted in network swelling and a decrease in syneresis. (Brownsey, 1988)

The second group contains gel systems in which both polymers were incorporated into the network. The type of network depends on the extent of mixing before gelation and the degree of demixing during gelation. Different types of two component gel networks formed under conditions which favour gelation: (a) coupled (b) phase-separated and (c) interpenetrating network are presented in **Fig 2.16.**

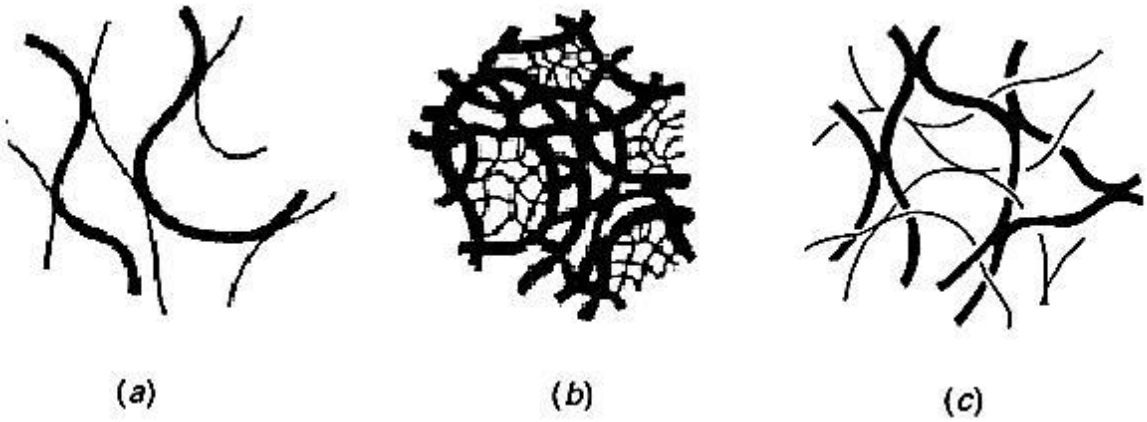

Figure 2.16 Two component gel networks formed under conditions which favour gelation: (a) coupled (b) phase-separated and (c) interpenetrating network (Image adapted from Brownsey 1988)

Coupled network formation requires favourable interactions between components. This type of network offers different mechanical and textural properties compared to those that can be obtained from those ingredients separately. In some circumstances gelation and intermolecular binding of two nongelling polymers can be promoted when the two are mixed together. Reported coupled networks include alginate esters and gelatin via chemical cross-linking or casein with κ-carraggenan. (Snoeren and Payens, 1976; Stainsby, 1980) Intermolecular binding was also discovered between algal polysaccharides (κ-carraggenan, furcellaran and agarose) and galactomannans such as tara and carob.

The phase-separated network is formed due to biopolymer incompatibility. It is the most the common gel structure formed by a mixture of two biopolymers. The structure of this type of network involves the inclusion of one polymer into the matrix of the other one. At a certain concentration phase inversion can occur. The polymer which previously formed the dispersed phase starts forming a continuous phase and entraps the second polymer. The continuous phase in a gel can be made of particles or molecules linked together, so a distinction can be

drawn between the particle and molecular gels. Molecular gels can be completely transparent; whereas particle gels are usually white or at least opaque.

An interpenetrating network is made of two biopolymer networks in which at least one is synthesized or cross-linked in the presence of the other polymer. (Brownsey, 1988) There are many preparation routes which result in various morphologies. The most common way is the preparation of a swollen gel network from one polymer and a cross-linking second biopolymer *in situ*. A condition which needs to be fulfilled for interpenetrating gel network formation is a high degree of miscibility between components. Poor miscibility results in a phase separated system. In most cases gel systems with more than one gelling agent either form phase-separated weak gels or only one of the gelling agents forms a network. (Brownsey, 1988)

Another classification of multicomponent gels distinguishes between complex mixed and filled networks. (Hill, Ledward, and Mitchell, 1998) Mixed gels are made of several interpenetrating networks. Filled gels are made when one of the components forms a continuous phase, and the other elements are present as a dispersed phase. The process of gelation reduces the excluded volume of molecules due to a decrease in their mobility. The most significant results of excluded volume which can be observed in biopolymer mixtures are synergistic or antagonistic effects. A synergistic effect is observed when the small addition of another hydrocolloid significantly increases the elastic modulus of the gel network. The increase may be a consequence of component exclusion which enhances the concentration of both biopolymers. The excluded volume effect can also impact on polymer aggregation, gelation kinetics as well as the structural and mechanical properties of the network, and it is more prominent but not reserved for helix forming biopolymers such as

agarose, gelatin or alginates. It was reported that low concentrations of dextran or agarose increased the elastic modulus of gelatin 2 - 5 times and accelerated gel network formation as an effect of component incompatibility. (Tolstoguzov, 1990; Toltoguzov, 1986) Another consequence of incompatibility is a lowering of the critical concentration required for mixed gel network formation compared to biopolymer individual gelation.

The antagonistic effect can be observed when the addition of another gelling agent results in a decrease in the elastic modulus. This is a consequence of phase separation and formation of the gel structure with dispersed liquid particles. The increase in liquid particle volume results in a decrease in the gel network elastic modulus which is related to a rise in gel network imperfection present in close proximity to liquid particles. At a certain concentration phase, inversion might occur. This results in a significant change in melting temperature and mechanical properties.

2.5. Hydrocolloid mixtures

Blends of starches and other polysaccharides have been used for many years in the food industry. (Bemiller, 2011a; Tecante, 1999; Descamps, 1986) One of the reasons for mixing starch with other polysaccharides is the need for a gelling agent replacement. This is considered when there are problems with primary agents, such as availability, price, negative publicity or public attitude towards the food additive or product reformulation. It is tough to find an equivalent gelling agent, and it is possible only if the considered agent contains the same type and number of junction zones. (de Vries, 2004b)

Another, more important reason to mix starch with other hydrocolloids is possibility to obtain new structures with superior rheological and texture-imparting properties which could be suitable for wide range of applications in food industry. (Tecante, 1999; Eidam, 1995)

A comparison of research results for carrageenan and starch mixtures are presented in **Table 2-2**

Table 2-2 Summary of research results and conclusions regarding corn starch and κ-carrageenan gels (adapted from Bemiller 2011a)

Author	Year	Starch	Hydrocolloid	Results and conclusions
Descamps et al.	1986	Modified waxy maize	κ-, ι-, λ-carrageenans	ι-carrageenan showed strong synergism, dependent on the starch type
Tye	1988	Modified corn	κ-, ι-, carrageenans	ι-carrageenan showed strong synergism, κ-carrageenan didn't
Eidam et al.	1995	Corn	κ-, ι-, carrageenans	Partial substitution sometimes resulted in lower *G´*values, and higher *G''* values than a starch alone paste at equal total concentration. Gelation was accelerated by the hydrocolloids, but final gel strength decreased.
Fanta and Christianson	1996	Corn Waxy maize	carrageenan	Properties of the composites depended both on the starch–hydrocolloid ratio and on the particular starch–hydrocolloid combination.
Tecante and Doublier	1999	Crosslinked and stabilized waxy maize	κ-carrageenan	Gels were predominantly solid-like and elastic. Granules were more swollen in the presence of hydrocolloid.
Lai et al.	1999	Modified waxy maize	κ-, ι-, carrageenans	Adding gelatinized starches to κ-carrageenan solutions increased T_{gel}, $G´$ and T_m values. Gelation of carrageenan was accelerated by adding starches

Author	Year	Starch	Hydrocolloid	Results and conclusions
Kowalski, Sikora, Tomasik, and Krystyjan	2008	Corn	κ-carrageenan	The presence of hydrocolloids reduced elasticity and viscosity of composite gels.
Chaudemanche and Budtova	2008	Corn	κ-carrageenan	AM and κ-carrageenan molecules show slight incompatibility, without phase separation in the liquid state, separate into phases upon gelation. Strong phase separation between κ-carrageenan and AP molecules in solution. Swollen granules have an effect on gel properties. Rheological responses depend on the state of the composite and its composition

In the typical biopolymer gel *G′* (elastic modulus) values are greater than *G″* (viscous modulus) along the frequency sweep test, but in starch pastes both moduli are frequency dependent. Hydrocolloids can modify the dynamic spectra of starch, and different trends can be observed. (Mandala, 2012)

The first hypothesis assumes that hydrocolloid addition leads to weaker structures, where the starch network shifts from an elastic-like to a more viscous-like one. Starch-hydrocolloid systems can be considered as biphasic. (Mandala, 2012; Tecante, 1999) In the mixtures containing swollen starch granules, the non-starch hydrocolloid occurs entirely in the continuous phase. The hydrocolloid concentration will then increase with a decrease in the accessible phase volume. This results in viscoelasticity changes in the starch. This assumption is also starch type dependent. Waxy starches contain no amylose and as a consequence short-term retrogradation does not occur. As a result, the viscoelastic properties of waxy starch pastes can't be influenced by hydrocolloid addition. (Mandala, 2012)

Another proposed hypothesis states that some hydrocolloid additions can promote associations with starches which result in increases in the elastic and the viscous modulus. (Achayuthakan, 2008; Mandala, 2004) When investigating starch-hydrocolloid systems, it is important to specify which ingredient predominates in the overall rheology.

Maltodextrin mixtures with gelatin were extensively studied by Kasapis *et al.* (1993a) and a phase separated gel network for this mixture was reported. (Kasapis 1993a) The mixed gel modulus was dependent on mixture composition. (Kasapis 1993b) An interpenetrating network formation was suggested for the gellan/maltodextrin gel by Clark *et al*. (1999). (Clark 1999) Segregative phase separation was proposed for ι-carrageenan/maltodextrin/water mixtures by Wang *et al*. (2009). (Wang 2009) ι-carrageenan was reported to have a higher water binding capacity compared to maltodextrin. The increase in potassium concentration resulted in two-phase domain enlargement. Whereas an increase in temperature increased the biopolymer compatibility. Loret *et al.* (2005) investigated agarose/maltodextrin gels and also reported phase separated gel network formation which was complicated by a polydispersity of maltodextrin molecular weight. (Loret *et al.* 2005) However, κ-carrageenan mixtures with maltodextrin have not been previously reported.

2.6. Rheology

Rheology is the science of deformation and flow. It enables food structure characterisation on the macroscopic level and classification of materials according to their consistency. Rheological behaviour depends on the internal structure of the material, outside forces which stress the material and ambient conditions (especially temperature). In general, more space between molecules enables the structure to flow easier; closely linked molecules are more resistant to flow. As a consequence ideally viscous (liquid) material flows when subjected to stress and ideally elastic (solid) material does not flow. In practice material structures are

more complicated; they are mixtures of viscous and solid elastic portions which makes their behaviour viscoelastic. Rheology describes consistency as a combination of two components, viscosity (thickness, lack of slipperiness) and elasticity (stickiness, structure). The rheological characterisation of materials involves viscosity measurements, determination of flow behaviour and material structure. (Mathisson, 2015)

To characterise the flow behaviour of the structure, it is crucial to induce stationary flow by application of a shear. The type of flow depends on the rearrangement and deformation of the particles and the breaking of bonds within a structure. The two plate model where the bottom plate is stationary and the top plate is moving was used to shear the material and to define two variables: shear stress and shear rate. They enable us to determine how much deformation is applied to the structure and how fast. The two plate model is presented in **Fig 2.17.**

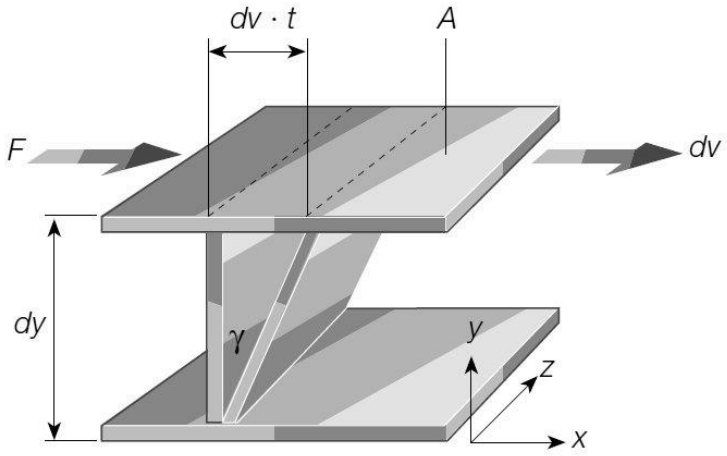

Figure 2.17 Two plates model representing shearing of the material between two parallel plates (Image adapted from Mathisson 2015)

When the top plate moves, it causes stress which is parallel to the material surface. Shear stress provides information about how much stress is applied to the material and can be

defined using **Equation 4** where σ is the shear stress, expressed as a ratio of applied force F [N] to the unit area A [m^3]:

$$\sigma = \frac{F}{A}\left[\frac{N}{m^3}\right] = [Pa] \tag{4}$$

Shear rate is determined by the velocity at which the material is stressed, and it can be calculated using **Equation 5** where $\dot{\gamma}$ is the shear rate, expressed as the ratio of velocity difference between plates to the distance between plates Δy:

$$\dot{\gamma} = \frac{\Delta V}{\Delta y}\left[\frac{m}{m \times s}\right] = \left[\frac{1}{s}\right] = [s^{-1}] \tag{5}$$

Newton's law states that the shear stress σ is equal to the shear rate $\dot{\gamma}$ multiplied by the viscosity ŋ. Newton's law is presented in **Equation 6**:

$$\sigma = \dot{\gamma} \times \text{ŋ} \tag{6}$$

Rearrangement of Newton's law provides a formula to calculate apparent viscosity. Apparent viscosity can be calculated using **Equation 7** where η is an apparent viscosity, expressed as a ratio of shear stress σ [Pa] to shear rate $\dot{\gamma}$ [s^{-1}]:

$$\text{ŋ} = \frac{\sigma}{\dot{\gamma}}\left[\frac{Pa}{\frac{1}{s}}\right] = [Pa \times s] \tag{7}$$

The process design requires a mathematical description of viscosity measurements. Examples of models used to describe flow behaviour of non-Newtonian systems include the Ostwald, Steiger-Ory, Bingham, Ellis, Herschel-Bulkley and Eyring models. However, the most extensively used in handling applications is Power law model is the model. The power law equation for Newtonian fluids is presented as **Equation 8** and for non-Newtonian shear

thinning or shear thickening fluids as **Equation 9**, where σ $[Pa]$ is the shear stress, K [Pas^n] represents a consistency, $\dot{\gamma}$ $\left[\frac{1}{s}\right]$ is the shear rate, n [-] is the flow behaviour index

$$\sigma = K \times \dot{\gamma}^n = \eta \times \dot{\gamma} \tag{8}$$

$$\sigma = K \times \dot{\gamma}^n \tag{9}$$

Values of flow behaviour index enable to recognize and describe material flow behaviour with n=1 for Newtonian flow, n<1 for shear thinning and n>1 for shear thickening flow response. Various fluids flow behaviour, expressed as shear stress/shear rate relationships are illustrated in **Fig 2.18.**

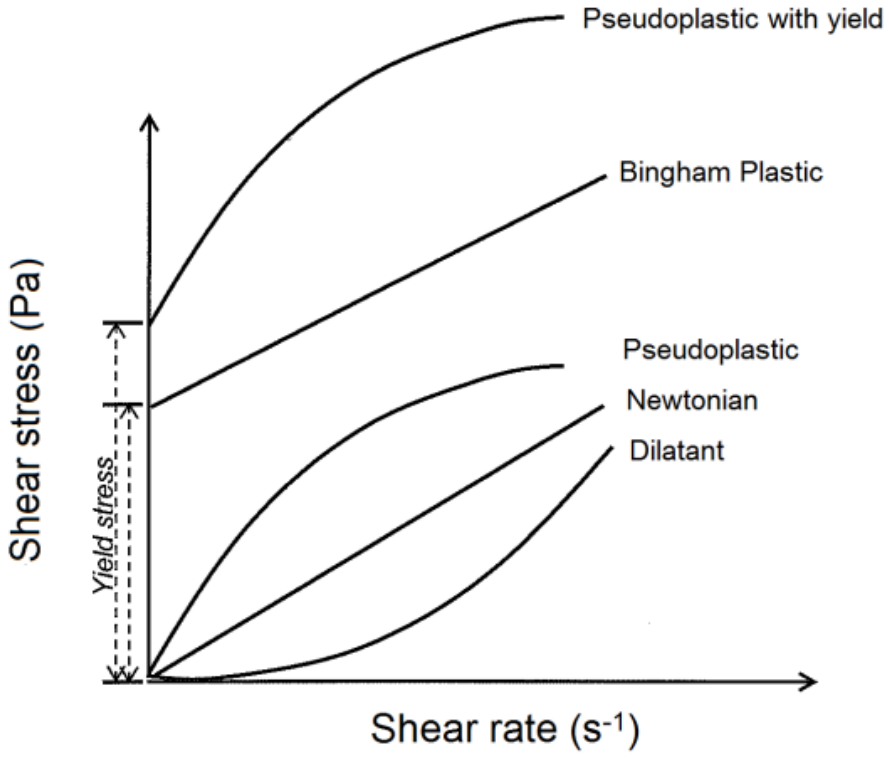

Figure 2.18 The flow behaviour of various fluids, expressed as shear stress/shear rate relationships (Image adapted from (Vieira, 2012)

At ambient conditions, the Newtonian fluid viscosity is constant and independent of the applied deformation. This behaviour is called ideal flow behaviour. Examples of Newtonian fluids are water or oil. However, the majority of fluids are non-Newtonian which means their viscosity is affected by outside forces and time even under ambient conditions. When an increase in shear rate results in an increase in material viscosity those materials show shear thickening (dilatant) behaviour. An example of a shear thickening material is starch dispersion. In comparison, when an increase in shear rate results in a decrease in material viscosity those materials show shear thinning (pseudoplastic) behaviour. Examples of shear-thinning materials (with yield) in the food industry are ketchup, mayonnaise or yoghurt. Some highly concentrated structures such as gels and dispersions have a yield point (Bingham fluid). This means that a certain force has to be exceeded to make them flow. (Earle, 1983)

Examples of shear rates for various processes, comparison of viscosities for different materials and power law constants and yield stress values for conventional food products are presented in **Fig 2.19.**

Shear rates	sedimentation	$10^{-6} - 10^{-4}$	s^{-1}	
	chewing	$10^{1} - 10^{2}$	s^{-1}	
	stirring	$10^{1} - 10^{3}$	s^{-1}	
	pumping	$10^{2} - 10^{3}$	s^{-1}	
	spraying	$10^{3} - 10^{4}$	s^{-1}	
	rubbing	$10^{4} - 10^{5}$	s^{-1}	
Viscosities	air	10^{-5}	Pas	
	water	10^{-3}	Pas	
	olive oil	10^{-1}	Pas	
	glycerol	10^{0}	Pas	
	syrup	10^{2}	Pas	
	molten polymers	10^{3}	Pas	
	molten glass	10^{12}	Pas	
	glass	10^{40}	Pas	
n and K values	fruit concentrate	n=0.7	K = 2	Pas^n
	molten chocolate	n=0.5	K = 50	Pas^n
	sour milk	n=0.3	K = 3	Pas^n
	quarg	n=0.3	K = 4	Pas^n
	apple puree	n=0.3	K = 10	Pas^n
	tomato paste	n=0.2	K = 70	Pas^n
	grease	n=0.1	K = 1000	Pas^n
Yield stress	ketchup	14	Pa	
	mustard	38	Pa	
	mayonnaise	85	Pa	

Figure 2.19 Examples of shear rates, viscosities, power law constants and yield stress values which occur in the food industry (Image adapted from Mathisson 2015)

Non-Newtonian fluids measurements are obtained using rotational rheometers which enable the application of accurate shear rates and cooling rates. Type of measurements performed using rotational rheometers includes rotational tests and oscillation tests. In rotational tests, materials are stirred or turned to define their viscosity. In oscillatory tests small, back and forward oscillation are used to determine values for the viscoelastic behaviour of materials. Comparisons of the types of measurements which can be performed using a rotational rheometer are presented in **Fig 2.20.**

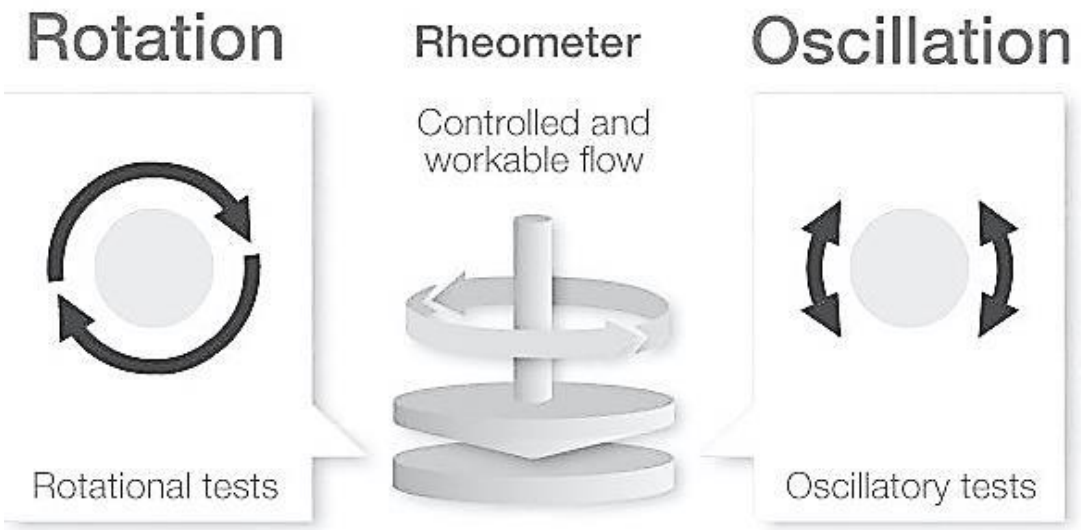

Figure 2.20 Type of measurements which can be carried out using rotational rheometer (Image adapted from Anton Paar 2009)

Measurements performed using a rotational rheometer can be divided into rotational or oscillatory tests, based on the type and direction of stress applied to the material. Appropriate geometry needs to be selected to perform measurements according to the kind of investigated material.

In this study, four-bladed vane geometry was chosen for the formation of mixed fluid gels in order to prevent wall-slip effect, which could occur as a result of an increase in viscosity during sol to gel phase transition. The single shear rate test with a temperature ramp from 85 ºC to 10 ºC was performed to form the structure. A sample hood was used to avoid heat loss and solvent evaporation.

Structure characterisation

Hooke's law states that force (F) equals spring constant (C_H) multiplied by extension (S) and it can be calculated using **Equation 10**:

$$F = C_H \times S \tag{10}$$

Rearrangement of Hooke's law enables to calculate material stiffness using **Equation 11**:

$$C_H = \frac{F}{S} \tag{11}$$

Shear deformation or shear strain can be defined using the two plate model which is presented in **Fig 2.21.**

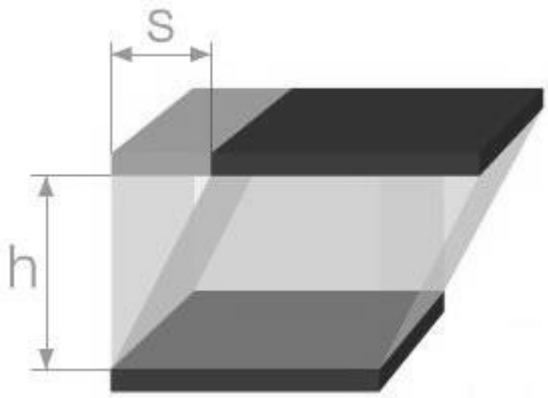

Figure 2.21 Two plate model for shear strain determination (Image adapted from Anton Paar 2009)

Equation 12 represents shear deformation or shear strain γ which is defined as a ratio of deflection path (s) to distance between plates (h) and it is dimensionless, expressed as a percentage (%)

$$\gamma = \frac{s}{h} \left[\frac{m}{m}\right] = [1]\ [\%] \tag{12}$$

Applying Hooke's law to the two plate model enables shear modulus determination, which defines material strength/stiffness and is expressed in **Equation 13,** where the shear modulus (G) equals the ratio of shear stress (σ) to shear strain (γ)

$$G = \frac{\sigma}{\gamma}\left[\frac{Pa}{1}\right] = [Pa] \tag{13}$$

The shear modulus is influenced by the internal structure of the material, external forces and by temperature and time.

In an ideal viscous liquid with a loose structure and unlinked molecules, the entire energy of applied deformation is dissipated as heat due to internal friction and it cannot be recovered just by releasing the external forces. In comparison, in an ideal elastic solid, the entire deformation energy can be stored, and the structure can fully recover its previous form when external forces are released. This is a result of tight structure and cross-linked molecules. Most materials exhibit viscoelastic behaviour. They are able to store some portion of energy and lose some energy.

Material placed on rheometer between two plates is subjected to small-amplitude periodic oscillatory shear repeated in regular cycles as presented in **Fig 2.22.**

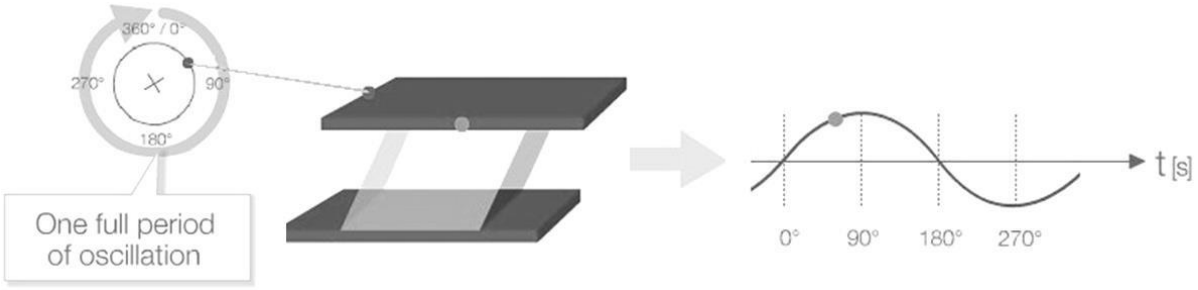

Figure 2.22 Two plate model representing one full period of small-amplitude oscillatory shear (Image adapted from Anton Paar 2009)

The measurement can be described as sinusoidal curves of deformation and material response. The two plate model representing a measurement of viscoelastic material by small-amplitude oscillatory shear is presented in **Fig 2.23.**

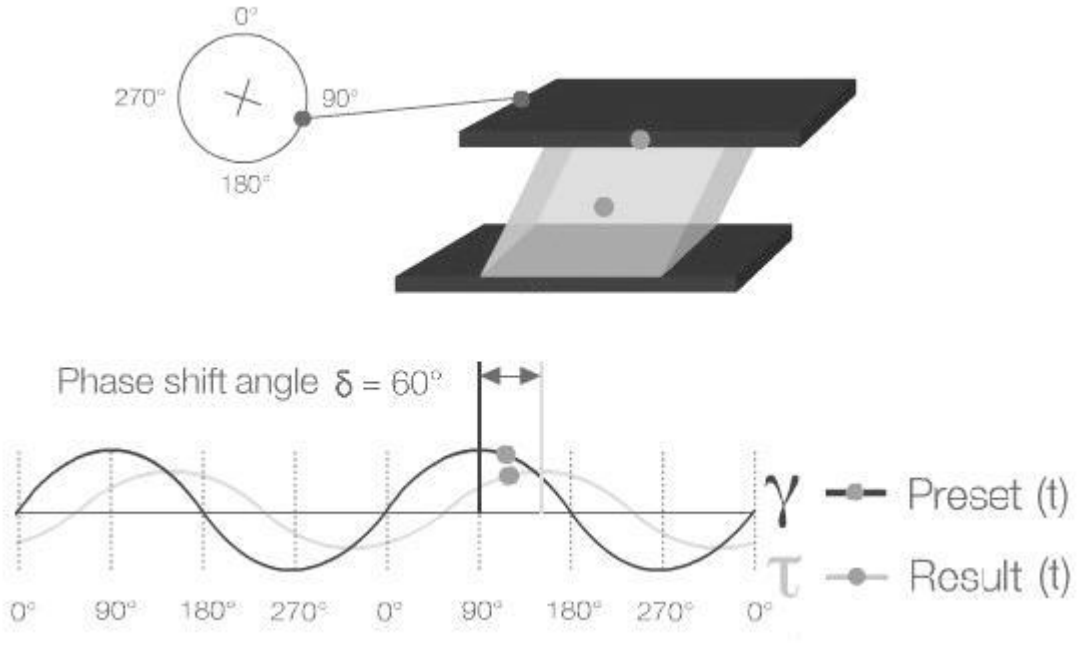

Figure 2.23 Two plate model representing measurement of viscoelastic material by small-amplitude oscillatory shear (Image adapted from Anton Paar 2009)

Phase angle shift between deformation and material response for an ideal liquid is 90 º and cannot be greater. Phase angle shift for an ideal solid is 0 º (does not exist) which means there is no delay in material response. In practice, viscoelastic materials would have a phase shift between 0 º and 90 º. Application of the model with time-dependent variables to Hooke's law enables determination of the storage modulus (*G'*) in **Equation 14** and loss modulus (*G''*) in **Equation 15:**

$$G' = \frac{\sigma_A}{\gamma_A} \cos\delta \left[\frac{Pa}{1}\right] = [Pa] \tag{14}$$

$$G'' = \frac{\sigma_A}{\gamma_A} \sin\delta \left[\frac{Pa}{1}\right] = [Pa] \quad (15)$$

Correlation between complex shear modulus (*G**), storage modulus (*G'*) and loss modulus (*G''*) is illustrated by vector diagram in **Fig 2.24.**

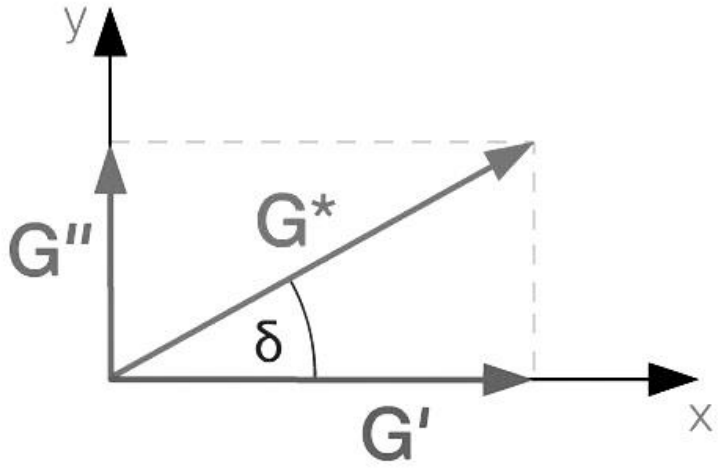

Figure 2.24 Vector diagram showing correlation between complex modulus (G^*), storage modulus (G') and loss modulus (G'') (Image adapted from Anton Paar 2009)

Loss modulus *G''* represents a viscous portion of the material (dissipated energy) and storage modulus *G'* represents the elastic portion of the material (amount of the energy which material is able to store). The correlation between the complex modulus (*G**), storage modulus (*G'*) and loss modulus (*G''*) in expressed in **Equation 16**

$$G^* = \sqrt{G'^2 + G''^2} = [Pa] \quad (16)$$

The ratio of the loss modulus to the storage modulus is defined as the loss tangent (δ) or damping factor, and its value determines viscoelastic material behaviour. (Shenoy and Saini, 1996)

Tan (δ) > 1 ($G'' > G'$) (viscoelastic liquid or 'sol')

Tan (δ) = 1 ($G' = G''$) (viscoelastic 50 % liquid and 50 % solid or sol to gel phase transition)

Tan (δ) < 1 ($G'' < G'$) (viscoelastic solid or 'gel')

Oscillatory characterisation of structures in this research was carried out at room temperature (20 ºC), using cone and plate geometry as standardised by the International Standards Organisation (ISO) and the German Institute for Standardization (DIN). The material linear viscoelastic region (LVR) was determined by the investigation of resistance to strain deformation using the amplitude sweep test. The frequency sweep test was performed within the linear viscoelastic region to investigate the behaviour of the material structure (viscous and elastic portions).

2.7. Gelation in a shear field and fluid gel structure

Red seaweed polysaccharides for many years have played an important role in the food industry as thickening, gelling and stabilizing agents. Their high water absorption capacity is responsible for their impact the material properties of food products, especially flow (viscosity) and deformation (viscoelasticity) behaviour in the presence of stress.

The ability of polysaccharides to form gel structures has been extensively investigated in the past. However, relatively recently, Sworn *et al.* (1995), discovered that the application of a shear to polysaccharides undergoing gelation results in an alternative structure - the ‘fluid gel.' Model for microstructure formation of single and mixed systems is presented in **Fig 2.25.**

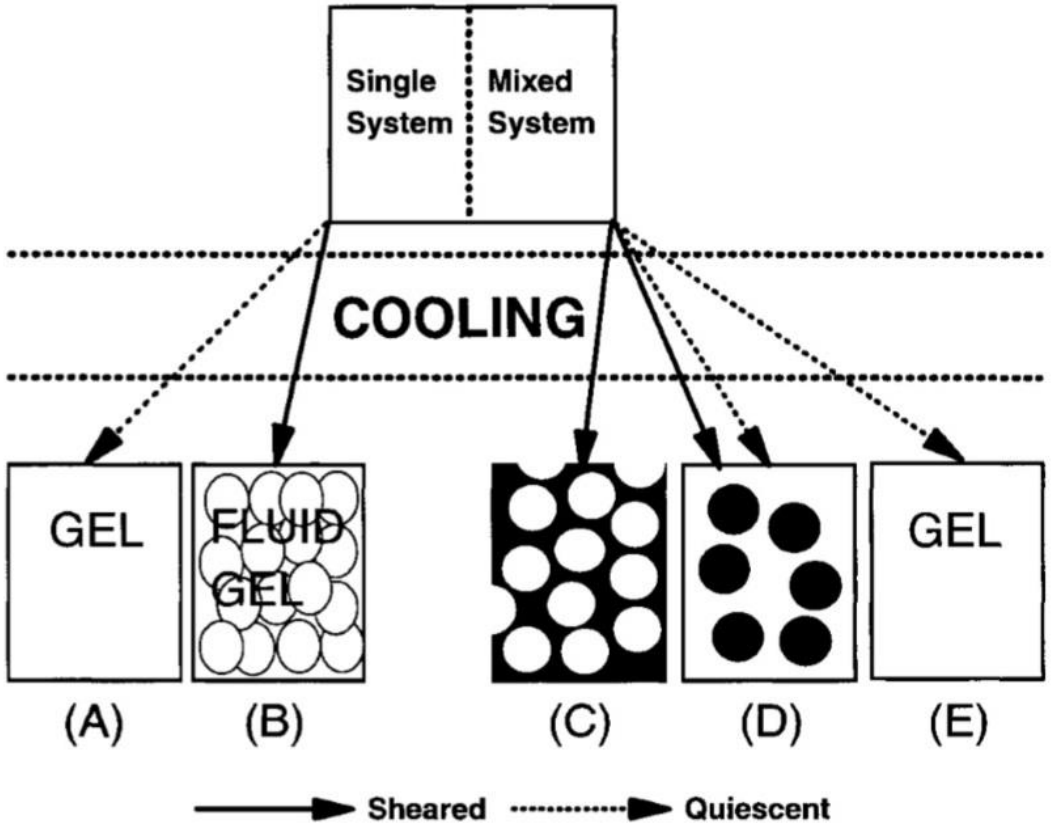

Figure 2.25 The model of single and mixed gels structure formation (Image adapted Norton 2000)

Desired microstructures in the mixed fluid gel can be achieved not only by process condition manipulation (shear or cooling rate) but also by modifying mixed system formulation and additional ingredient concentration. The concentration of components influence phase distribution within the microstructure, where one component is present as a continuous phase and other as a dispersed phase (C and D).

The main advantage of a fluid gel over a quiescently cooled gel is the possibility of obtaining various, rheologically different structures from the same starting material by manipulation of the process conditions during structure formation. Methods of fluid gel structure formation include:

1. Gelling the aqueous phase of a water-in-oil emulsion,
2. Phase separation of mixed biopolymers
3. Sheared gelation of a biopolymer solution

Only sheared gelation of a biopolymer solution will be explored further in this chapter as the most relevant to the research conducted.

A fluid gel structure formed by the sheared gelation method consists of a highly concentrated (high volume fraction) suspension of gelled particles in a non-gelled continuous medium. (Brown, 1990; Cassin, 2000; Garrec, Guthrie, and Norton, 2013) In 1999 a molecular model for the fluid gel formation was proposed by Norton and co-workers. The model was based on agarose sheared gels. (Norton, Jarvis, and Foster, 1999)

The proposed model included the following principles:

1. Formation of fluid gel particles in the shear field is possible if biopolymers gelation contains an aggregation step
2. Particles are formed by a nucleation and growth mechanism until they reach size equilibrium which is restricted by shear forces
3. Particles are ‘hairy’ before molecular ordering is completed due to unordered chains present at the particle surface
4. The molecular ordering rate is not affected, but the size of the particles or their number can be influenced
5. Particle size and volume depends on shear rate and polymer concentration
6. Particle volume fraction is related to fluid gel properties

7. A fluid gel is stable if stored away from the melting temperature which prevents de-aggregation and re-aggregation

A gradual rather than a steep increase in elastic modulus with an increase in agar concentration led to the conclusion that particles produced were highly deformable entities rather than rigid particles.

In 2002 results published by Frith *et al.* (2002) proved that during formation, fluid gels had a tendency to occupy the maximum volume fraction. They concluded that increases in the biopolymer concentration did not affect the particles' volume fraction. (Frith *et al.* 2002)

A detailed diagram illustrating molecular events during fluid gel formation and their correlation with changes in viscosity as a function of decreasing temperature was established in 2009 by Cox *et al.* (2009) and it is presented in **Fig 2.26.**

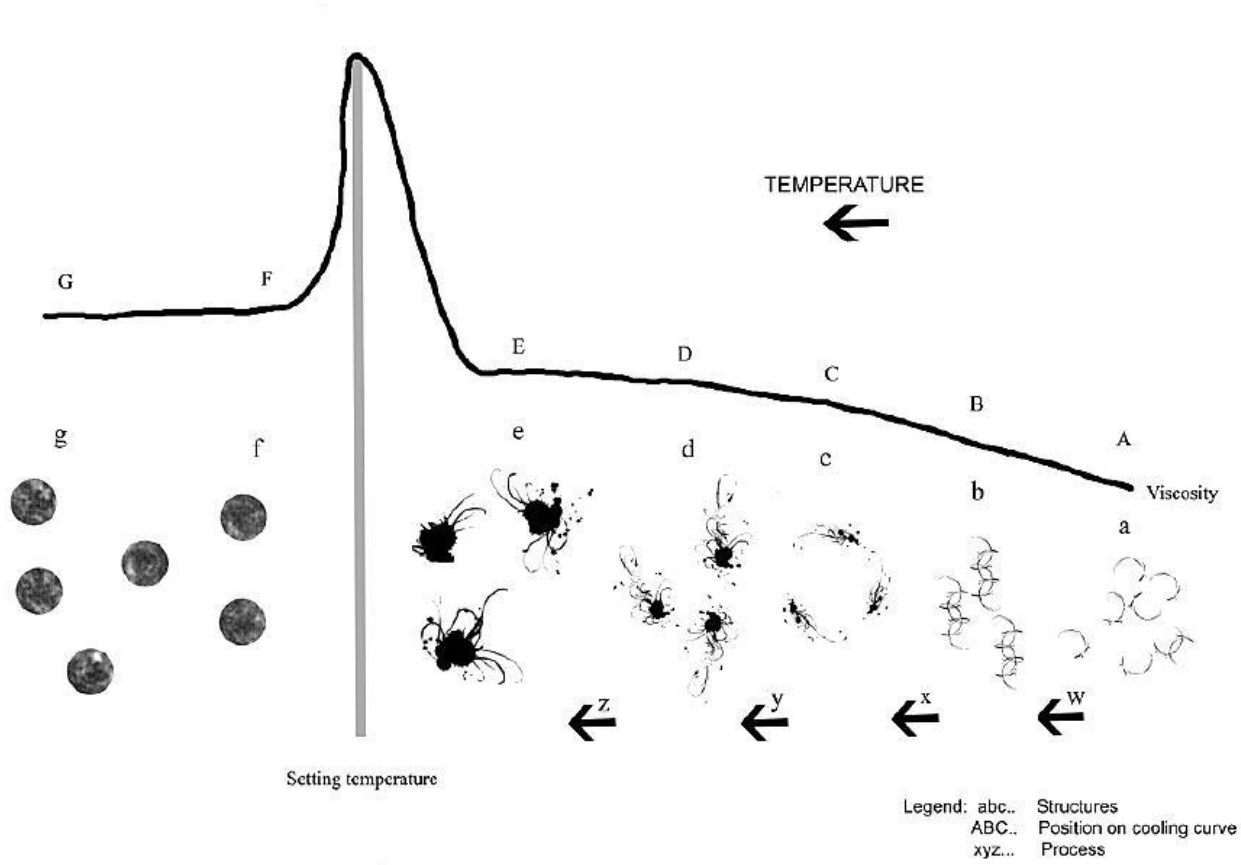

Figure 2.26 Diagram illustrating molecular events during fluid gel formation (Image adapted from Cox 2009)

According to the proposed diagram, fluid gel formation starts at the temperature above the melting point (*A*) when gel elements are in the disordered state, dispersed in a solvent (*a*). At this stage shear rate effect is not significant. Molecular ordering of the structure begins with a decrease in temperature (*w*). At this stage, individual molecules form double helixes or egg-box dimers (*b*) which results in a slight increase in viscosity (*B*). Further decrease in temperature promotes extensive aggregation (*x*). From this point the shear field starts to restrict fluid gel particles size. However, particles are still able to grow either by the addition of structures *a* or *b*, or coalescence of elements *c* (*y*) resulting in formation of structures d. Particles at this stage are 'hairy' due to the presence of unordered helixes at the surface.

Their 'hairy' nature is gradually lost during process z as an effect of an increase in ordering and aggregation rate (*E*) which occurs close to setting temperature and is related to a rapid increase in viscosity. This results in a further increase in particles size which can reach even 100s of microns. When particles grow to such large dimensions it is very likely that the shear forces will break them up. Near completion of particle formation the hydrocolloid chains became fully ordered and the surface of the particles becomes smooth (*f*) which results in a decrease in viscosity *F*. After this point a further decrease of temperature does not affect the structure of the fluid gel particles (*g*). (Cox, 2009)

The fluid gel particle size is determined by the dynamic equilibrium between two competing factors during the formation process: shear rate and cooling rate. Gel nuclei growth is mainly controlled by the cooling rate. Particle coalescence and break-up are primarily controlled by the shear rate (de Carvalho, 1997). At the high cooling rates the gelation process dominates over shear forces and as an effect, large particles are formed. At low cooling rates, the dominant factor is applied shear which results in the formation of smaller particle and a narrower size distribution (Gabriele, Spyropoulos, and Norton, 2009; Cox, 2009).

Previous research conducted by Wolf *et al.* (2000) showed that it is possible to influence the shape of the particles by manipulation of the flow stress. An increase in flow stress changed the morphology of mixed κ-carrageenan/gellan and gelatin/guar particles from ellipsoidal to cylindrical. Cylindrical particles were found to be more efficient viscosity modifiers (Wolf, 2000).

The microscopy images of the particles from the publication are presented in **Fig 2.27.**

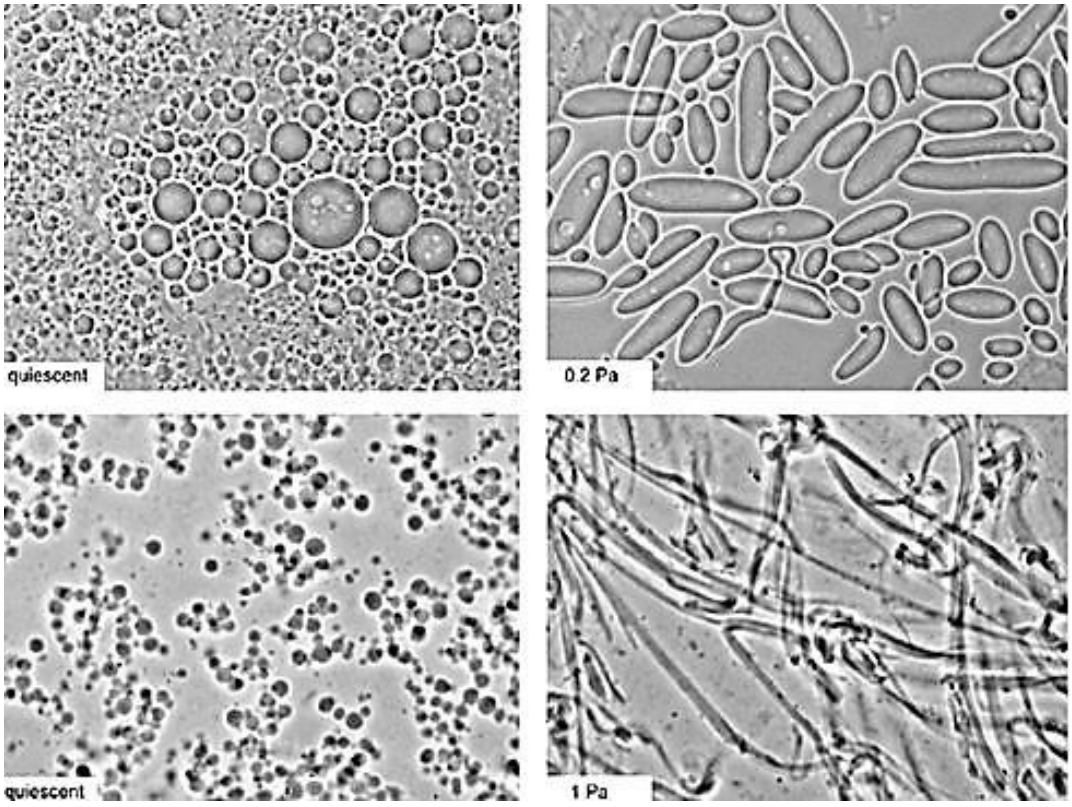

Figure 2.27 Comparison of particles of κ-carrageenan/gellan (top) and gelatin/guar (bottom) (Image adapted from Wolf 2000)

Fluid gel formation research was continued by Gabriele *et al*. (2009), who investigated the κ-carrageenan fluid gel formation process and proved that fluid gel properties are directly related to the applied shear rates and cooling rates. The κ-carrageenan fluid gel formation diagram was also proposed. The diagram illustrates the increase in viscosity during particle ordering as a function of temperature. The flow curve obtained is presented in **Fig 2.28.**

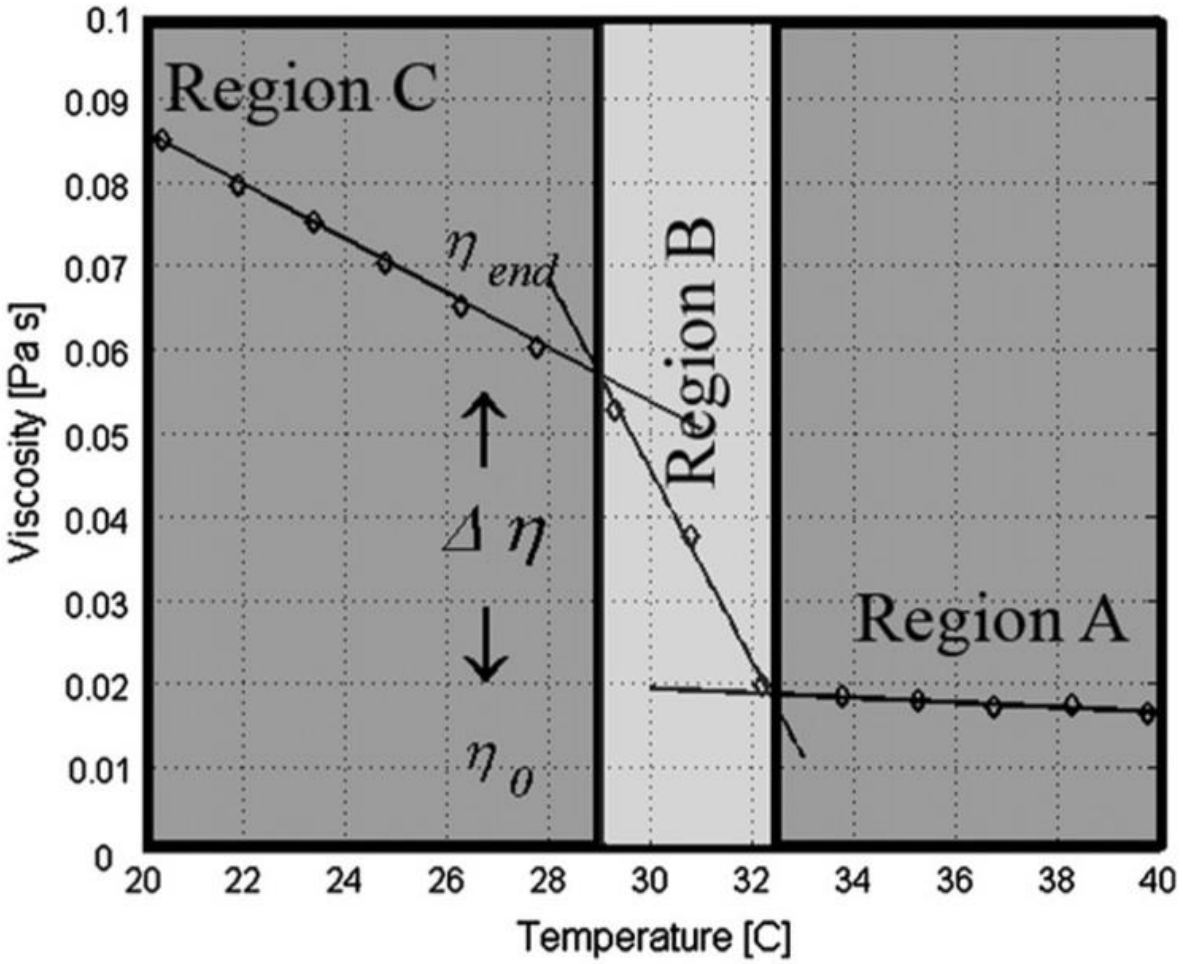

Figure 2.28 Increase in viscosity during κ-carrageenan fluid gel formation (Image adapted from Gabriele, Spyropoulos, and Norton 2009)

Three regions were identified on the fluid gel formation diagram:

1. Region A - before the onset of ordering
2. Region B - between ordering initiation and ordering completion
3. Region C - after ordering completion

In region A, κ-carrageenan was present as a low viscosity hot solution. The gradual cooling of the solution resulted in the formation of small gel nuclei which started close to the

κ-carrageenan gelation point (around 32 ºC) and caused a rapid increase in viscosity (region B). Lowering the temperature of the system further contributed to gel nuclei growth, until particle size equilibrium was reached. Reaching equilibrium indicated the end of region B. The temperature differences between ordering initiation and completion were found to be highly dependent on the investigated system and in the case of κ-carrageenan it was around 3 ºC. The process of the small gel nuclei formation was described as a consequence of a demixing phenomenon which results in the formation of polymer-rich and polymer-poor regions in the system. Researchers have proposed two hypotheses to explain the mechanism responsible for the initial stages of demixing: spinodal decomposition or nucleation and growth. (Gabriele, Spyropoulos, and Norton, 2009; Norton, 1998) Regardless of mechanism, it was established that the gel nuclei that were formed in the early stages of aggregation under shear were similar to water-in-water emulsion droplets. Therefore, the coalescence and break up of particles were expected to occur. As a result, it was concluded that the rapid increase in viscosity observed in region B was attributed to the growing number and volume of particles.

Region C was the last stage of fluid gel formation where a gradual increase in viscosity was observed. Researchers proposed two mechanisms which can lead to fluid gel particles growth after formation. 'Enrichment' from the surrounding non-gelled matrix or coalescence of particles as forced by applied shear flow. Both were taken into the account.

2.7.1. Shear rate effect

Effect of the shear rate applied to κ-carrageenan during formation process was investigated. Viscosity changes were recorded as a function of temperature and data obtained for a range of shear rates between 0.5-700 s^{-1} is presented in **Fig 2.29.**

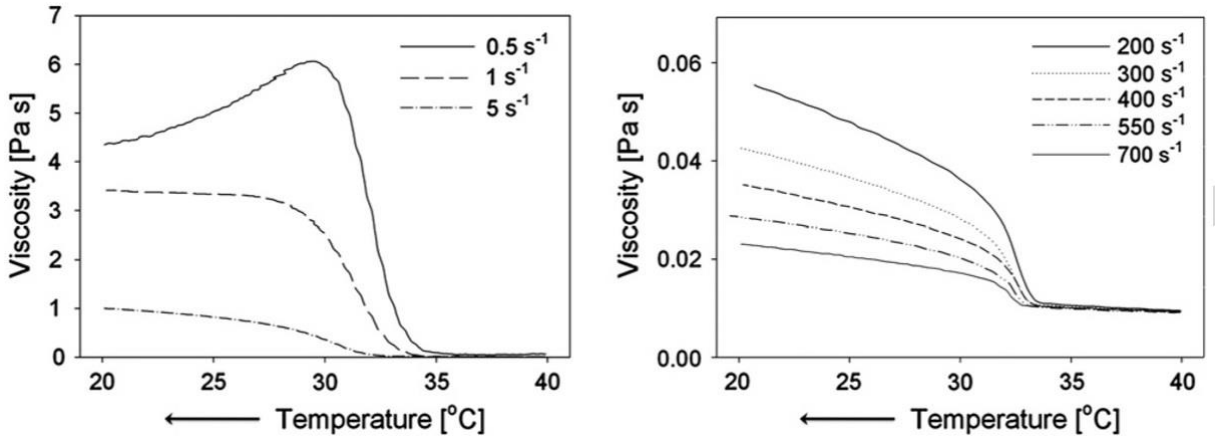

Figure 2.29 Viscosity profiles obtained during κ-carrageenan fluid gel formation under various shear rates. (Image adapted from Gabriele, Spyropoulos, and Norton 2009)

The direct relationship between particle size and applied shear rate was observed. The increase in applied shear rate resulted in a decrease in κ-carrageenan fluid gel particles size and led to decrease in structure viscosity. The rapid increase in viscosity was inversely proportional to the applied shear rates which confirmed κ-carrageenan fluid gels shear thinning behaviour. It was also noticed that below gelation temperature, fluid gel systems produced under low shear rates behaved vastly different compared to the ones produced under high shear rates. Under low shear rates, the viscosity of the system went through a maximum and then gradually decreased. This phenomenon was attributed to the faster growth of fluid gel particles and interaction between them resulting in the formation of larger aggregates which contributed to the higher increase in viscosity. Enormous fluid gel aggregates (in the range of 100s μm) were more likely to be affected by the applied shear forces and break. As a result, the system viscosity reduced in the last stage of the production. (Emanuele and Palma-Vittorelli, 1992; Gabriele, Spyropoulos, and Norton, 2009; Norton, Jarvis, and Foster, 1999)

In comparison, the increase in viscosity during fluid gel formation under higher shear rates proceeded more gradually. It was concluded that below the gelation temperature, particles

were still able to grow in size due to the ordering of the remaining chains within or on the particle surface. Another possibility was particle-particle interactions as a result of disordered charged chains present on particle surfaces which can bind to available cations and form interparticle bridges. The increase in shear rate caused the particle surface to become smoother due to cyclisation of the chains. This resulted in a decrease in final viscosity due to lack of particle-particle interactions. It was also highlighted that an increase in viscosity as a function of the reduction in temperature was in agreement with Arrhenius model.

In addition, Garrec *et al.* (2012, 2013) also concluded that fluid gel particles formed from κ-carrageenan have internal gelled network with shorter helices and smaller regions of helix aggregates in comparison to quiescently cooled gels. This was suggested to be the consequence of applying shear, which interrupted the molecular ordering process. (Garrec and Norton 2012; Garrec, Guthrie, and Norton 2013)

Average sizes obtained for κ-carrageenan fluid gels were as follows: at a shear rate 0.5 s^{-1} particles were ~ 60 μm, at 1 s^{-1} around 5 μm and at a shear rate 5 s^{-1} were observed to be at around 1 μm. κ-carrageenan fluid gel particle size followed the observations of Carvalho *et al.* (1997) who investigated gelation of gelatin gels under shear. They concluded that the size of the particles was an equilibrium obtained as a result of competition between opposing processes taking place during particle formation. The first is a process of gel nuclei growth controlled by cooling rate. At the same time coalescence and break up of particles is controlled by the applied shear field. (de Carvalho and Djabourov, 1997)

It has also been shown that κ-carrageenan fluid gel viscoelastic properties depend mainly on microstructure and interactions between particles which could be altered by formulation and

process condition modification. (Gabriele, Spyropoulos, and Norton, 2009) The increase in storage modulus (*G'*) with decrease in shear rate is due to more inter-particle interactions

2.7.2. Cooling rate effect

The effect of the cooling rate applied during κ-carrageenan fluid gel formation

(0.5-6.0 ºC min^{-1}) was also investigated by Gabriele *et al*. (2009). Viscosity of the systems was measured as a function of temperature and results obtained are presented in **Fig 2.30.**

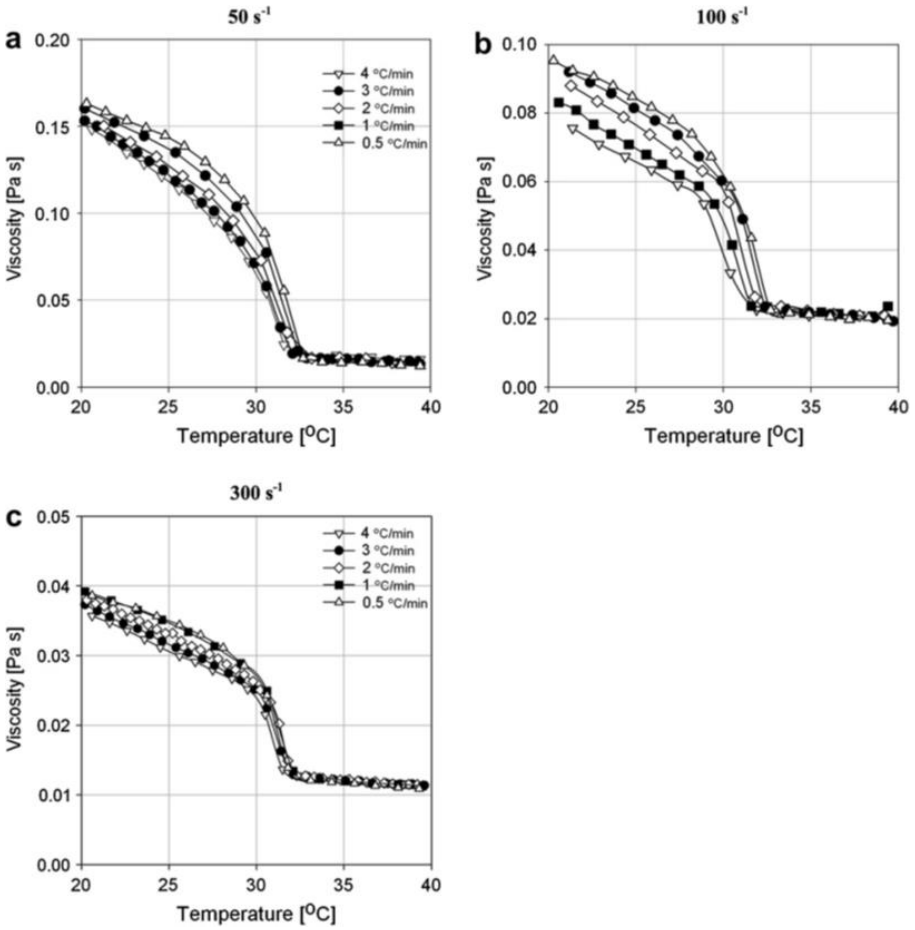

Figure 2.30 Viscosity profiles during k-carrageenan fluid gel formation under various cooling rates. (Image adapted from Gabriele, Spyropoulos, and Norton 2009)

It was observed that the reduction in applied cooling rate resulted in a slight increase in final viscosity indicating more interaction between particles and an increase in their size. This is thought to be due to the lengthening of processing time with lower cooling rates. Therefore, it is possible that more bridges are formed between particles which result in an increase in final viscosity. On the contrary, structures formed at higher cooling rates have a greater possibility for bridges to be formed post production as a consequence of shorter processing time. (Gabriele, Spyropoulos, and Norton, 2009) Further investigations revealed that particle bridging indeed developed after the formation process had finished and was more significant for structures formed at higher cooling rates. The extent of particle-particle interactions post production was found to be dependent on the cooling rate applied during the fluid gel formation process. Fluid gel particle interactions were also expected to be influenced by biopolymer and cation concentration.

Single component fluid gel formation has been described in detail in previous studies carried out by Norton *et al*. (1999), Gabriele *et al*. (2009) and Garrec *et al.* (2012,2013) (Garrec, Guthrie, and Norton, 2013; Garrec and Norton, 2012; Gabriele, Spyropoulos, and Norton, 2009; Norton, Jarvis, and Foster, 1999). Results obtained demonstrated that it is possible to design fluid gels with the desired viscosity and viscoelastic properties. They concluded that the rheological properties of fluid gel depend greatly on aggregation rate (junction zones density) within particle as well as particle size and interaction between them. Aggregation rate can be affected by biopolymer and ion concentration. Particle size and interactions can be controlled by shear and cooling rate applied during the structure formation process.

Nevertheless, it is very difficult to precisely mimic fat using a fluid gel based only on one hydrocolloid. As has been shown for hydrocolloid systems, mixtures give greater control of texture and in use performance. Introducing another component and making complex fluid gel

system can lead to structures with greater control over texture (viscosity and viscoelasticity) which can be more closely matched with desired application at reduced cost.

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Chapter 3. κ-CARRAGEENAN AND PREGELATINIZED CROSS-LINKED WAXY MAIZE STARCH SHEARED GEL STRUCTURES

3.1. Overview

The aim of this chapter is to demonstrate a greater understanding of correlations between blended formulation, formation process conditions, structure and rheological properties of κ-carrageenan and pregelatinized cross-linked waxy maize starch gel mixtures produced in a shear field. Results and discussions included in this chapter have been published within:

Gladkowska-Balewicz, I., Norton, I. T., & Hamilton, I. E. 2014, Food Hydrocolloids 42, 355-361, 'Effect of process conditions, and component concentrations on the viscosity of kappa-carrageenan and pregelatinized cross-linked waxy maize starch mixed fluid gels'.

3.2. Abstract

The formation and characterisation of κ-carrageenan and pregelatinised cross linked waxy maize starch mixed fluid gels on a Kinexus Pro rotational rheometer using vane geometry has been described. The viscosity of mixed fluid gels containing between 0.5 to 4 % [w/w] starch was investigated and compared to fluid gels of κ-carrageenan only. The dependence of the fluid gels initial and final viscosity on the application of shear rates between 1 - 600 s^{-1}, and cooling rate between 0.5-3 °C applied during hydrocolloid gelation was studied, along with the effect of altering the concentration of its components. The presence of pregelatinised cross linked waxy maize starch during fluid gel formation has been shown to decrease the onset gelling temperature by approximately 2 °C when compared to the κ-carrageenan only fluid gel. The apparent 26 fold increase in the final fluid gels viscosity as a result of producing a 2 % starch mixed fluid gels has the potential for composite fluid gels of this kind to be used as a more efficient thickening agent in fat replacement products than their kappa-carrageen only counterparts.

3.3. Introduction

The food industry has a challenging task of constantly improving existing food products to satisfy consumer's needs and expectations. (Consultation, 2000; Funami, 2011; Miller & Groziak, 1996) Consumers require their foods to be healthier but remain tasty, as such food technologists and engineers have a growing interest in designing new food microstructures that can be used to reduce fats and sugars whilst, maintaining the same oral response as the potentially less healthy alternative. (Aguilera, 2005; Aryana & Haque, 2001; Douaire & Norton, 2013; Gidley, 2013; Kavas, Oysun, Kinik, & Uysal, 2004; Koutsopoulos, Koutsimanis, & Bloukas, 2008; McMahon, Alleyne, Fife, & Oberg, 1996; J. E. Norton & Norton, 2010; Passos & Ribeiro, 2010; Prindiville, Marshall, & Heymann, 2000; Totosaus, Alfaro-Rodriguez, & Perez-Chabela, 2004) The understanding and engineering of food microstructures is important when tailoring many properties such as flavour, smoothness, creaminess, as well as textural and sensual traits. (Aguilera, 2005; Funami, 2011; I. T. Norton, Frith, & Ablett, 2006) Previous research has indicated that sensation such as creaminess is associated with the perceptions of a foods thickness, smoothness and slipperiness, and has shown correlation with properties such as viscosity, particle size, shape and hardness. (Garrec & Norton, 2013; Guinard & Mazzucchelli, 1996; Kokini & Plutchok, 1987; Richardson, Morris, Ross-Murphy, Taylor, & Dea, 1989; Tyle, 1993)

It has been reported that mixed hydrocolloid systems can deliver greater control of texture in foodstuffs compared to single hydrocolloid systems, when used as thickening agents or fat replacers, (Eidam, Kulicke, Kuhn, & Stute, 1995; Tecante & Doublier, 1999) a property that has led to blends of the κ-carrageenan and starch being adopted in the food industry as a texture enhancer for several dairy products. (Matignon, *et al.*, 2014; de Vries, 2004; Descamps, Langevin, & Combs, 1986)

Although the characterisation of interactions of starch-carrageenan hydrocolloid composites has been attempted by several authors (BeMiller, 2011; Lai, Huang, & Lii, 1999; Tecante, et al., 1999; Verbeken, Thas, & Dewettinck, 2004) however their finding have not been conclusive.

Fluid gels made from polysaccharides that are known to form gels in the presence of water such as κ-carrageenan have been reported in the literature as a potential fat replacer for emulsion based semi solid foods such as mayonnaise and margarine, custards, gravies and pourable dressings. (Garrec & Norton, 2012; Sworn, Sanderson, & Gibson, 1995) These fluid gels are produced by a technique of applying shear to the biopolymer whist it undergoes gelation, resulting in a structured fluid that contains gel particles suspended in a carrier liquid. (Norton, Jarvis, & Foster, 1999) Research in the area of κ-carrageenan fluid gel production has shown that by controlling the process conditions, and ingredient concentrations a wide range of textures can be produced from a single fluid gel formulation. (Gabriele, Spyropoulos, & Norton, 2009; Garrec, Guthrie, & Norton, 2013; Norton, 2006)

Formation and characterisation of mixed fluid gels systems have been briefly described before. (Norton *et al.,* 2006) In this study, the formation of κ-carrageenan and cross-linked waxy maize starch mixed fluid gels produced at various hydrocolloid and starch concentrations (between 0.1-2 % and 0.5-4 % respectively) is described. The effect of the presence of starch on the initial and final viscosity along with changes in gelling temperature has been examined. Precisely controlled shear rates between 1-600 s^{-1} and cooling rates between 0.5-3 °C min^{-1}, were applied to the gelling systems via a Kinexus Pro rotational rheometer using a vane geometry, to investigate and measure process effects on the resultant mixed fluid gel viscosity. Finally, the effect of the hydrocolloid and starch concentrations on

the dynamic viscosity was measured in order to gain a greater understanding of the mechanisms governing the mixed fluid gels microstructure formation.

3.4. Experimental

3.4.1. Materials

κ-carrageenan powder and HiForm® instant pregelatinized cross-linked waxy maize starch (CLWM) powder were kindly provided by Cargill. Potassium Chloride BioXtra, ≥99.0% salt, and 0.01 M iodine solution were purchased from Sigma-Aldrich. All materials were used without further purification.

3.4.2. Sample preparation

3.4.2.1 Preparation of κ-carrageenan solutions

The dry κ-carrageenan powder was weighed and added to distilled water heated to 85 ºC (50 ºC above expected phase transition temperature) under constant agitation and allowed to dissolve. Solutions were kept isothermal and stirred for a further 30 minutes before experimentation to ensure complete hydration.

3.4.2.2 Preparation of CLWM-starch solutions with potassium chloride addition

Potassium chloride (KCl) (0.1-0.6 g l^{-1}) was dissolved in hot distilled water (85 ºC) and continually stirred until complete dissolution. The dissolved KCl was mixed with the required amount of pre-gelatinised CLWM-starch and stirred until fully dissolved. Solutions were kept isothermal at 85 ºC and stirred for a further 30 minutes before testing to ensure full hydration.

3.4.2.3 Mixed fluid gel preparation

The concentrations of κ-carrageenan and pregelatinized CLWM starch used in the mixtures varied between 0.1-2.0 % and 0.5-4.0 % of the overall mass of the pre-gel solution respectively. The addition of 0.1 % [w/w] KCl was chosen as the most suitable to allow mixed fluid gel structure formation.

Solutions of pre-gel mixtures were prepared using the same method as single ingredient solutions. Components were first dissolved separately to ensure complete uninterrupted hydration. After full hydration required amount of hot pregelatinized CLWM starch solution was added to a solution of hot κ-carrageenan so as to achieve the desired concentrations [w/w/w]. Mixtures were kept at a constant temperature of 85 ºC whilst stirring for a further 30 minutes to ensure homogeneous mixing prior to performing the experiment. The vessel was covered so as to avoid evaporation of the water at elevated temperatures.

3.5. Methods

3.5.1. Differential scanning calorimetry measurements

A Seteram μDSC3 evo Dynamic Scanning Calorimeter (DSC) was used to detect and measure the temperature at which thermal phase transitions occur. The transitions in question are changes from liquid to gel state during cooling (sol to gel transition) and from gel to liquid state during heating (gel to sol transition). Screw-top 'closed batch cells' were used for the analysis. 0.5-0.6 g of sample was added to the sample cell and an equal mass of distilled water added to the reference cell. Gels were tested by placing the samples in the instrument and allowing the temperature to stabilise at 20 ºC before taking measurements. Samples were then heated to 85 ºC and cooled from 85 ºC to 5 ºC in three cycles at a rate of 1 ºC min^{-1}. 10 min isothermal holds were applied in between each step to ensure temperature equilibrium.

Results obtained were calculated from the average of three heating cycles (for the gel to sol transition) and three cooling cycles (for sol to gel transition).

3.5.2. Rheometer configuration

Mixed fluid gel formation and characterization of rheological properties were investigated using a Kinexus pro rotational rheometer with vane geometry (internal cup diameter 27 mm, the vane rotor 25 mm) calibrated with pure water (at 20 ºC). An evaporation control hood was fitted for the duration of the experiment. The geometry was preheated to 85 ºC and allowed adequate time for the temperature to stabilize. Fluid gel formation was observed as the temperature of the solution was gradually reduced via a peltier controlled cylinder cartridge whilst the appropriate shear rate was simultaneously applied.

3.2.1 Fluid gel production and characterisation

38 ml of heated pre-gel solutions were poured into the vane cup and given five minutes to reach an equilibrium temperature. Mixtures were cooled from 85 ºC to 10 ºC under precisely controlled shear rates of 0.5-600 s^{-1} and cooling rates of 0.5-3 ºC min^{-1}. Any increase in viscosity due to changes in mixture resistance to flow as a result of fluid gel structure formation were monitored and recorded at two-second intervals by the instrument. A rapid increase in viscosity during the ordering and aggregation of the helices was determined by the point of intersection of tangents fitted to the viscosity profiles. Tangents were fitted to the three regions observed in the flow curves (prior, during, and post aggregation). Values of viscosity at the intersection of the tangents were taken as an on-set and end-set of the ordering transition. The viscosity increase was calculated as the difference between the values of these two points. Values were compared for each of the mixed fluid gel systems.

3.6. Results and discussion

3.6.1. Identification of the gelling and melting transition temperatures by differential scanning calorimetry

Measurements of the melting and gelling points of the 0.5 % [w/w] κ-carrageenan and 0.5 % κ-carrageenan, 2 % pregelatinized CLWM starch [w/w/w] mixed fluid gels solutions were recorded by differential scanning calorimetry. The results are shown in **Fig 3.1.**

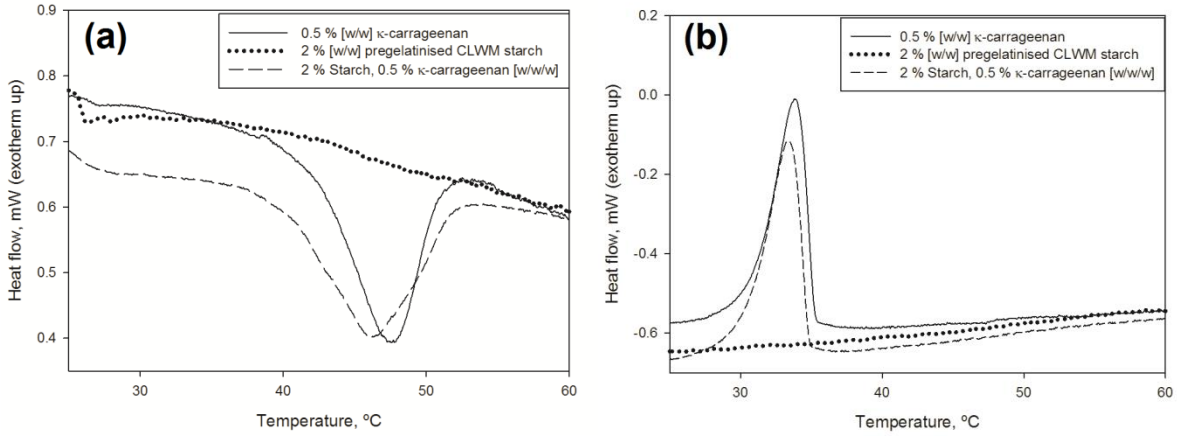

Figure 3.1 Thermograms representing heat effects during a) melting (heating) and (b) gelling (cooling) phase transitions as a function of temperature for 2 % [w/w] pregelatinized CLWM starch, 0.5 % [w/w] κ-carrageenan and 0.5 % κ-carrageenan, 2 % pregelatinized CLWM starch [w/w/w] mixed gels obtained on μDSC at a heating and cooling rate of 1 ºC min^{-1}

The investigation of the melting curves of mixed fluid gels recorded a peak temperature of 45.8 ± 0.1 °C (ΔH = 32.8 ± 2.4 j g $^{-1}$). When 0.5 % [w/w] κ-carrageenan was measured, an increase of 2 °C in the peak temperature was found (47.61 ± 0.31 °C, ΔH = 30.0 ± 4.0 j g $^{-1}$) compared to gels which contained CLWM-starch. Measurements of ΔH for both the mixed and κ-carrageenan only fluid gels showed good agreement, indicating that the formation of both fluid gel types was dominated by the gelling of κ-carrageenan even in the presence of

CLWM- starch. Thermograms of the solutions of 2 % [w/w] pregelatinized CLWM starch as expected showed that in the temperature range investigated there was no evidence of a thermal event.

Thermograms produced by the cooling solutions, showed similar trends to the curves obtained when monitoring the melting transition of the gels. As in the heating experiments the mixed fluid gel systems exhibited the lowest gelling temperature, with an onset recorded at 34.17 ± 0.18 °C peaking at 32 ± 0.03 °C (ΔH = -36.8 ± 3.0 j g $^{-1}$). The equivalent κ-carrageenan only systems, showed a higher onset and peak temperature of the transition 35 ± 0.67, 34 ± 0.09 °C respectively, ΔH = -33.2 ± 2.8 j g $^{-1}$). Again around a 2 °C difference in the gelling temperature was recorded between the mixed fluid gel and the κ-carrageenan counter-part.

The results showed a similar trend to those reported by Garrec *et al.* (2012) who described lower values of ΔH when fluid gels of κ-carrageen only were produced and compared to gels formed under quiescent conditions. The discrepancies in the values were attributed to a decline in the number of helical domains formed upon fluid gel production. The ΔH measurements of the mixed fluid gels systems recorded an increase of 8.5 % over κ-carrageen only fluid gels, suggesting that the inclusion of CLMW-starch increased the number of aggregated domains within the sample.

3.6.2. Effects of the addition of pregelatinized cross-linked waxy maize starch on the mixture viscosity and gelation temperature during fluid gel structure formation.

The changes in the viscosity as a function of temperature of the mixed fluid gels and its components, formed under a constant shear rate 100 s^{-1} and cooling rate 3 ºC min^{-1} is shown in **Fig 3.2**. 0.1 % [w/w] KCl solutions were added to the pre-gel in order to promote the

κ-carrageenan gelation of the mixed fluid gels, κ-carrageenan only. For comparison the equivilent mass of KCl solution was also added to the 2% pregelatinised CLWM starch solution.

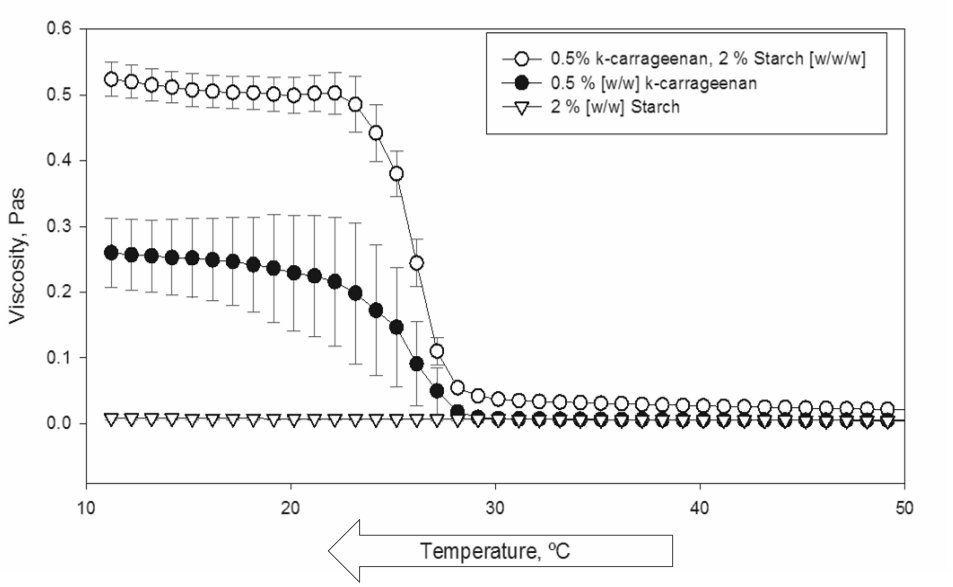

Figure 3.2 Comparison of viscosity changes as a function of temperature as samples were cooled at a rate of 3 ºC min^{-1} whilst subjected to a constant shear at the rate of 100 s^{-1}. The samples were 2 % [w/w] pregelatinized CLWM starch only, 0.5 % [w/w] κ-carrageenan only and 0.5 % κ-carrageenan with 2 % pregelatinized CLWM starch [w/w/w].

As can be seen, viscosity measurements of the solutions containing κ-carrageenan showed that as the temperature decreased there was a marked increase in viscosity. This trend was far less evident when starch with potassium chloride solutions were measured in the absence of κ- carrageenan, where a moderate increased in viscosity was recorded during the experiment (0.0028 Pa s at 85 ºC to 0.008 Pa s at 15 ºC). Solutions of 0.5 % κ-carrageenan with 0.1 % KCl exhibited a significant fortyfold increase in viscosity from 0.0040 Pas at 85ºC to 0.1629 Pas at 15 ºC as a consequence of the gelation of κ-carrageenan. The change in viscosity occurred at an onset temperature of 30 ºC (around 5 °C lower than the onset recorded by

the DSC). The variation in gelling temperatures recorded by the different techniques due to the requirement for significant ordering of the κ-carrageenan to take place before viscosity could build. The mixed fluid gel system showed an increase from an initial viscosity of 0.0097 to a final viscosity of 0.2485 Pa s. The onsets of gelation observed at 28 ºC, 2 ºC lower than that of the single component fluid gel showed a similar difference to that observed by DSC. The addition of the starch was also shown to increase the initial viscosity of the pre-gel solution. This increase is thought to be related to the starch excluded volume effect, (Chaudemanche & Budtova, 2008; Mandala & Bayas, 2004, Tecante, 1999) where the presence of the swollen starch granules in the system causes a reduction of the accessible water that can be structured by κ-carrageenan. As a result the κ-carrageenan concentration increases which facilitates an increase in the ordering, aggregation rates, and final viscosity.

The absence of an abrupt increase in viscosity in the solutions where κ-carrageenan was not present indicated the addition of CLWM-starch to the pre-gel solution was not directly responsible for the viscosity increases recorded for mixed fluid gels. The initial and final viscosity increase observed when pregelatinized CLWM-starch was present is believed to be a result of the presence of the swollen starch granules in the system causing a starch excluded volume effect. A 26 fold increase in the final viscosity of the mixed fluid gels were recorded when compared to fluids gels made from (0.5 %) κ-carrageenan alone. This significant increase has an advantage of using κ-carrageenan with pregelatinized CLWM starch as a fluid gel mixture instead of separate ingredients as a fat replacer with enhanced thickening capabilities.

3.6.3. Effect of the shear rate applied during production process on mixed fluid gel structure formation

A comparison of the change in viscosity as a function of temperature of κ-carrageenan only fluid gels and mixed fluid gels formed under a range of the shear rates between 0.5-600 s^{-1} at a constant cooling rate of 3 ºC min^{-1} is shown in **Fig 3.3.**

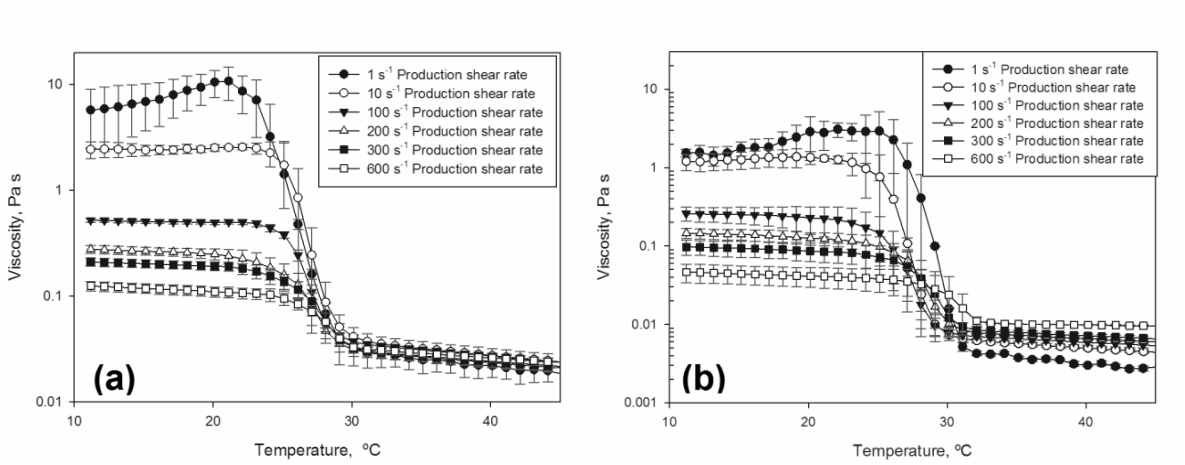

Figure 3.3 The Effect of shear rates (1.0-600 s^{-1}) applied during production process on the viscosity of 0.5 % κ-carrageenan mixed with 2 % pregelatinized CLWM starch [w/w/w] (a) and 0.5 % [w/w] κ-carrageenan only fluid gels (b). Systems formed at a constant cooling rate of 3 ºC min^{-1}.

It can be seen, when directly compared that the initial and final viscosity of the mixed fluid gels systems were about an order of magnitude greater than κ-carrageenan only fluid gels, produced under the same shear rate. Both the κ-carrageenan only and pregelatinised CLWM starch, κ- carrageenan mixed fluid gels systems showed an increase in the dynamic viscosity observed at the gelling temperature (28, 30 ºC for single and mixed fluid gels respectively) regardless of the applied shear rate.

The reduction in the final viscosity exhibited by the single and mixed fluid gel systems became more pronounced at higher shear rates, probably due to the applied shear field increasingly limiting large network ordering, and facilitating the production of concentrated suspended gel particles. In mixed systems the increased shear rates was also shown to facilitate even distribution of starch granules throughout the gel reducing large areas of aggregation in the final fluid gel.

The investigation highlighted that under the current process condition the conformational transition of biopolymers such as κ-carrageenan occur over the same time interval as fluid gel formation, it is therefore possible to strongly affect the viscosity properties of the final product by modifying the production shear rate. Prevention of the agglomeration of starch granules by increase in applied shear rates during production was shown to reduce the final viscosity of the mixed fluid gels. In general, each of the fluid gel systems showed an inversely proportional relationship of the final viscosity to the applied production shear rate.

3.6.4. Effect of applied cooling rate during production process on mixed fluid gel structure formation

The consequence of altering the cooling rate applied during the formation process to the mixed fluid gels, produced under constant shear rate 200 s^{-1} was investigated and changes in the viscosity were compared with the κ-carrageenan only fluid gels systems formed under the same process conditions. Changes in the viscosity of the structures were recorded as a function of temperature and results for the κ-carrageenan/starch fluid gels are presented in **Fig 3.4**.

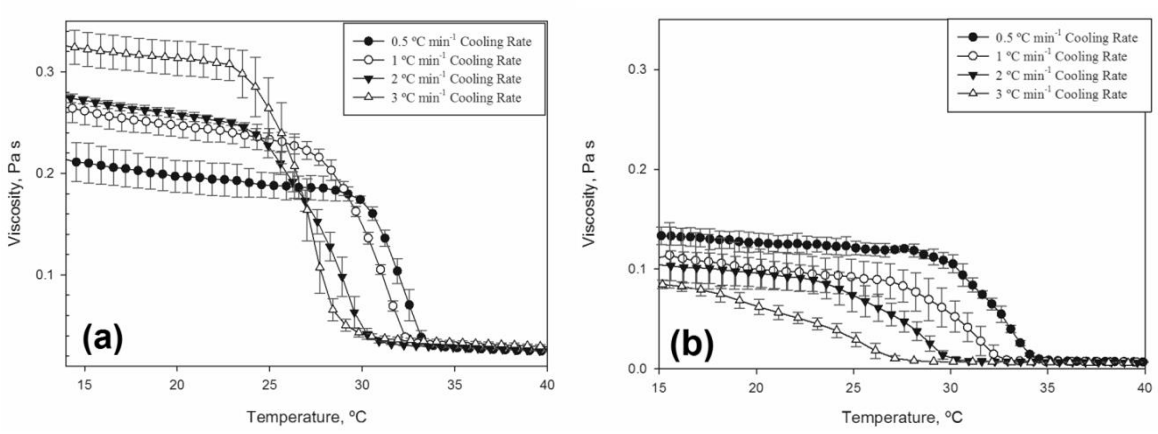

Figure 3.4 Effect of applied cooling rate on the viscosity of the fluid gels formed from (a) 0.5% κ-carrageenan and 2% [w/w/w] pregelatinized CLWM starch (b) 0.5% [w/w] κ-carrageenan. Structures formed under a constant shear rate of 200 s^{-1}.

From the behaviour shown in **Fig 3.4**, it was observed that as the cooling rate increased the onset gelation temperature decreased. Mixed fluid gels produced at cooling rates of 0.5 ºC min^{-1} showed gelation occurred at 34 ºC, whereas mixed fluid gels cooled at 3.0 ºC min^{-1} exhibited an onset gelation temperature at 28 ºC. The discrepancy is probably due to the speed of significant ordering occurring slower than incremental changes in the measured temperature when higher cooling rates were used.

Viscosity measurements during mixed fluid gel formation showed that the final viscosity increased with increases in the cooling rate. Most significantly when mixed fluid gels were produced at a cooling rates of 3 ºC min^{-1}. It is believed this response is the result of rapid gelation leading to immobilisation of dispersed starch granules throughout the fluid gel. When the cooling rate was decreased, a reduction in final viscosity was recorded, as phase separation can occur faster than the gelation can take place. The resultant viscosities of the mixed fluid gels formed at low cooling rates tended towards that of a κ-carrageenan only fluid gel system further suggesting separation of the κ-carrageenan and starch phases may have

occurred. An opposing trend was observed when the viscosities of κ-carrageenan only fluid gels were measured. Where the fluid gels showed an inverse relationship between cooling rate and final viscosity. It is believed that the slower cooling rates allowed for longer times for the gel network to equilibrate and thus produce networks with a greater probability of bridged particles. (Gabriele, et al., 2009; Nunes, Raymundo, & Sousa, 2006)

The applied cooling rate utilised during fluid gel production altered the final viscosity of both mixed and singular component fluid gels. Higher cooling rate (3 °C min^{-1}) were shown to be suitable to produce dispersed mixed 0.5% κ-carrageenan and 2% [w/w/w] pregelatinised cross linked waxy maize starch fluid gels by facilitating structuring before significant agglomeration could take place. The same cooling rate was also shown to give a reduction in the final viscosity of κ-carrageenan only fluid gels, where viscosity was governed by allowing sufficient time for the dynamic equilibria of the gel network formation to be reached rather than limiting phase separation.

3.6.5. Effect of κ-carrageenan and pregelatinised cross linked waxy maize starch concentration on the initial and final viscosity of mixed fluid gels

3.6.5.1 Changes in viscosity as a result of κ-carrageenan concentration

Concentrations of κ-carrageenan were altered between 0.1-2 % [w/w] (2 % [w/w] CLWM-starch) to measure changes in gelling temperature along with initial and final viscosities (**Fig 3.5.**), the increase in measured viscosity as a result of mixed fluid gel structure ordering is shown in **Fig 3.6.**

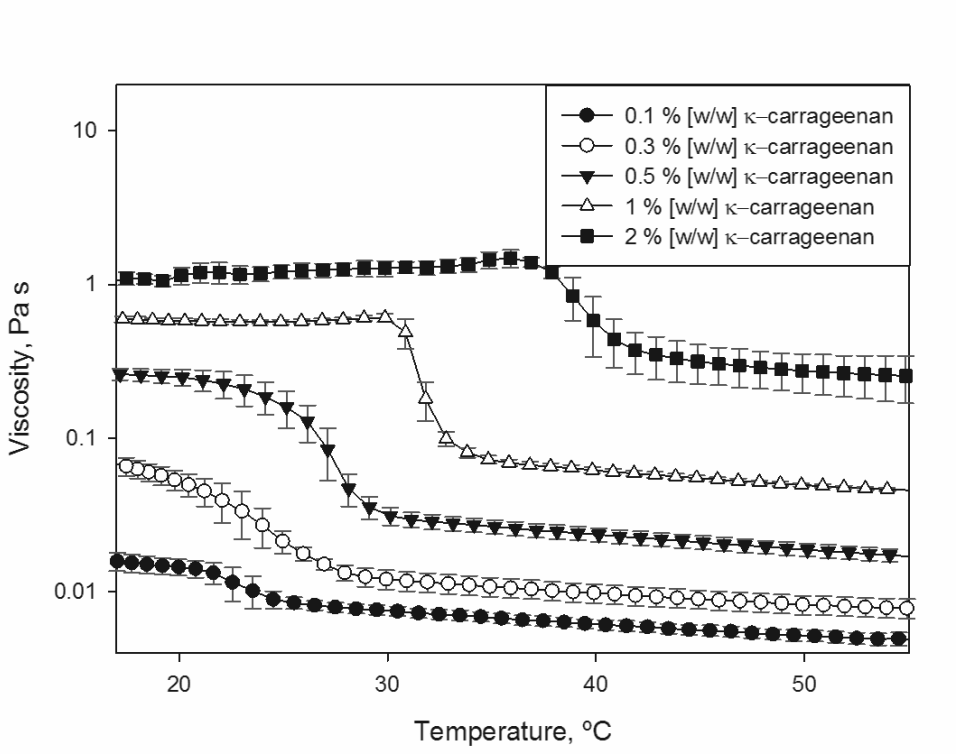

Figure 3.5 Changes in the viscosity of mixed κ-carrageenan and 2 % [w/w/w] pregelatinized cross-linked waxy maize starch fluid gels as an effect of an increase in κ-carrageenan concentration in the mixture. Fluid gels formed at a shear rate of 200 s^{-1} and a cooling rate of 3 ºC min^{-1}

As κ-carrageenan concentrations increased it was observed that both the initial and final viscosity also increased as a result of dispersed fluid gel particles occupying larger portions of the total volume available. The investigation highlighted a large increase in the onset temperature of the gelling transition. When κ-carrageenan concentrations were low (0.1 % [w/w]) gelation occurred at 25 °C increasing to 42 °C when the concentration was increased to 2 % [w/w].

A linear correlation (R^2=0.9952) was observed between the increase in gelling temperature and increase in κ-carrageenan concentration.

The gelling temperature as a function of the κ-carrageenan concentration for the mixed fluid gel systems (2 % [w/w] CLWM-starch) can be expressed using **Equation 1**, where x is the κ-carrageenan concentration % (w/w) in the pre gel solution.

$$T_{\text{gelling}} = 8.7054x_{\kappa-Carrageenan} + 24.53 \quad (1)$$

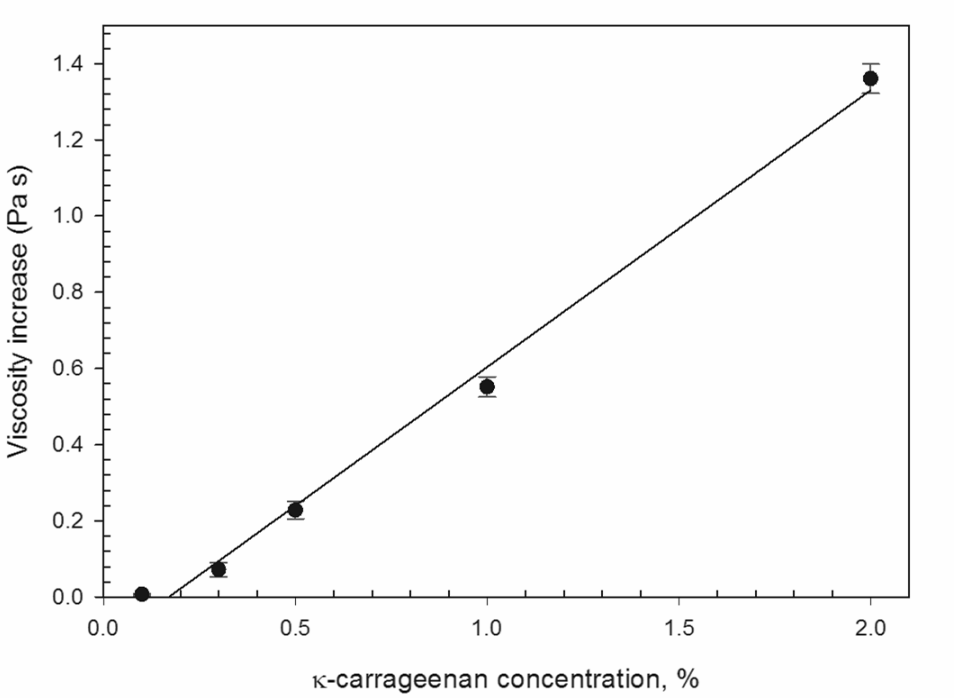

Figure 3.6 Viscosity increase during gel structure ordering and aggregation (Δη) for mixed fluid gels containing a constant amount of starch 2.0 % [w/w] and κ-carrageenan in concentrations ranging between 0.1-2.0 % [w/w], formed under shear rate 200 s^{-1} and cooling rate 3 ºC min^{-1}

At various κ-carrageenan concentrations it was evident that as with the change in transition temperature the gain in viscosity upon gelation and κ-carrageenan concentration was directly proportional (between 0.1–2 % κ-carrageenan, R^2 = 0.9938). The change in viscosity can be expressed using **Equation 2**, where again x is the % (w/w) κ-carrageenan concentration in the pre-gel solution.

$$\Delta\eta_{\text{mixed fluid gel}} = 0.7270x_{\kappa\text{-Carrageenan}} - 0.1233 \quad (2)$$

3.6.5.2 *Changes in viscosity as a result of pregelatinized cross-linked waxy maize starch concentration*

The effect of starch concentrations between 0.5-4.0 % [w/w] (0.5 % [w/w] κ-carrageenan) on the viscosity of mixed fluid gel systems during their formation is shown in **Fig 3.7**, the measured increase in viscosity as a result of gelation is shown in **Fig 3.8**.

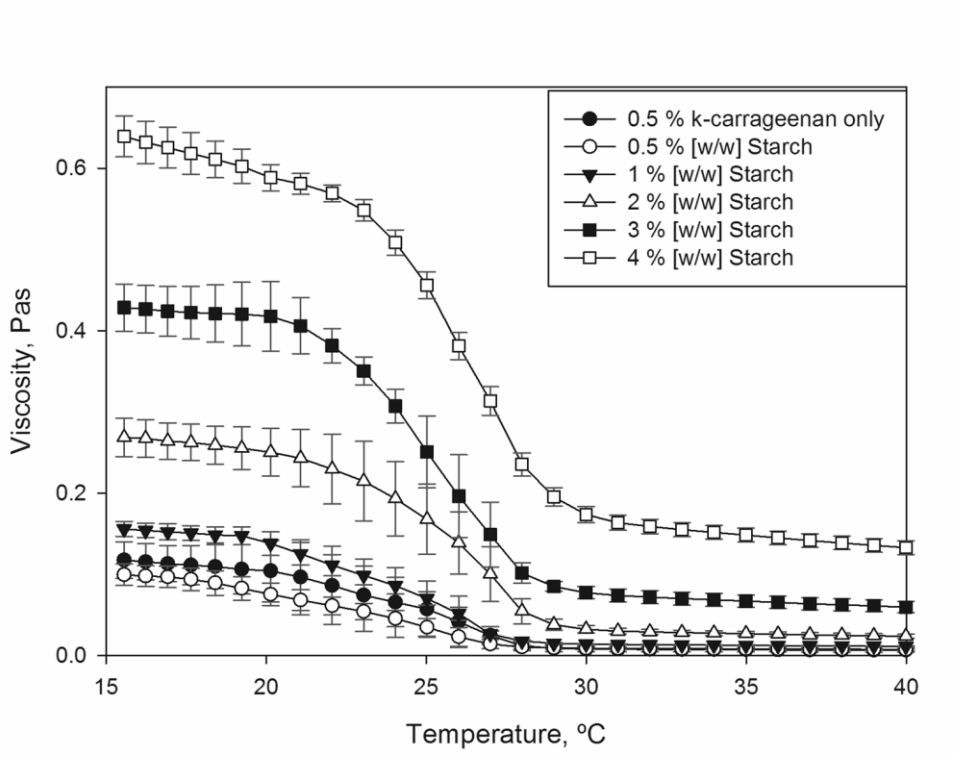

Figure 3.7 Viscosity changes during mixed fluid gel structure formation. Mixtures contain a constant amount of 0.5 % κ-carrageenan and a range of starch concentrations between 0.5 % - 4.0 % [w/w]. Structures were formed at a shear rate of 200 s^{-1} and a cooling rate of 3 ºC min^{-1}

It was evident from the results that with the increase in pregelatinized cross-linked waxy maize starch concentration an increase in initial and final viscosity was observed. Comparison of the initial and final viscosity showed, that when the starch concentration was below 2 % [w/w] there was no significant difference to the results of κ-carrageenan only fluid gels.

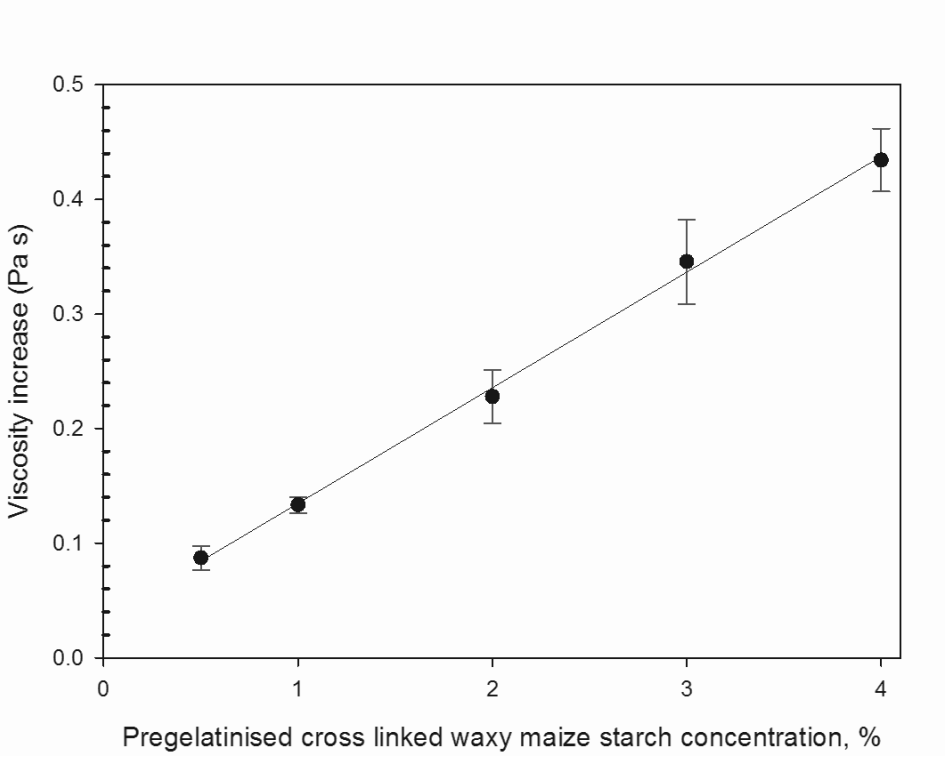

Figure 3.8 Viscosity increase during sol to gel transition under shear (Δη) for mixed fluid gels containing 0.5% κ-carrageenan and starch concentrations between 0.5 %-4.0 % [w/w]. Structures were formed under shear rate 200 s^{-1} and cooling rate 3 $^{o}C\ min^{-1}$

As in the investigation of hydrocolloid concentration, a linear relationship (R^2 = 0.9981) also was found to exist between the viscosity developed during gelation and the percentage starch concentration of the solution (between 0.5-4.0 %). The increase in viscosity during mixed fluid gel formation as a result of pregelatinized CLWM starch addition could be expressed

using **Equation 3** taking x as the % [w/w] pregelatinized CLWM starch concentration in the pre gel solution.

$$\Delta\eta_{\text{mixed fluid gel}} = 0.1009x_{\text{Starch}} + 0.0338 \quad (3)$$

In the concentration range investigated a direct relationship between the increase in gelling temperature and the increase in pregelatinized CLWM starch concentration was also evident (R^2 = 0.9790). Gelling temperatures of the mixed fluid gel systems, associated with altering the starch concentration can be calculated using **Equation 4**, where x is a % [w/w] pregelatinized CLWM starch concentration in the pre gel solution.

$$T_{\text{gelling}} = 0.5317x_{\text{Starch}} + 27.7634 \quad (4)$$

With fairly low concentrations of pregelatinized CLWM starch in pre-gel solution it was shown that it is possible to obtain κ-carrageenan mixed fluid gels with various final viscosities.

Conclusions

The investigation has shown that κ-carrageenan, pregelatinised cross linked waxy maize starch mixed fluid gels can be produced using a Kinexus Pro rotational rheometer using vane geometry. It has been shown that viscosity properties of κ-carrageenan and pregelatinised cross- linked waxy maize starch mixed fluid gels microstructures depend strongly on the formation process conditions, and components concentration.

Thermal analysis of mixed fluid gel systems showed the addition of CLWM-starch resulted in a 2 °C decrease in the gelling temperature when compared to κ-carrageenan only fluid gels. Rapid viscosity increases during mixed fluid gel production was shown to be inversely

proportional to the applied shear rates but proportional to the applied cooling rates. The investigation has shown that the presence of as little as 2 % pregelatinized CLWM-starch during κ-carrageenan fluid gel production resulted in a 26 fold increase in the final viscosity compared to κ-carrageenan only fluid gels made under the same conditions. Between concentrations of 0.5-4 % [w/w], it was shown that the addition of starch to the fluid gel showed a linear relationship with the increase in viscosity, without significant change in gelling temperature of the mixed fluid gel. Finally, the viscosity increase as a result of ordering was found to be proportional to the κ-carrageenan concentration in the presence of 2 % CLWM-starch.

Additional data not included in the publication:

Introduction:

κ-carrageenan and CLWM starch mixed fluid gels were shown to be effective viscosity enhancers. However to be used as a fat replacers and replicate sensation of creaminess it is also necessary for their particle size to be below 10 μm. The additional experiments were carried out to investigate the effect of shear on mixed fluid gel aggregates size, shape and distribution. The data obtained was fitted into rheological Power law model to compare the consistency and flow index of single and mixed κ-carrageenan fluid gel.

3.6.6. Determination of κ-carrageenan/starch aggregate sizes using optical microscopy

An optical microscope (Leica DMRBE, Leica Microsystems Imaging Solution LTD) equipped with a camera (3CCD, Colour Vision Camera Module) was used to obtain microstructure images and measure the size of κ-carrageenan/starch aggregates, since it is difficult to make precise measurements via light scattering techniques due to the similar refractive indexes of the fluid gel aggregates and water. Due to the large size of the aggregates

images of the size, shape and distribution of the samples were obtained in macro scale, using camera only. The dimensions of the aggregates in the micrographs and macrographs were measured in pixels and converted into millimetres using public software ImageJ. In order to facilitate the measurement process, final gel samples were diluted in 0.1% [w/w] KCl solution (by a factor of 3:1) which made it possible to observe individual aggregates more clearly and therefore obtain a better estimate of their sizes. Furthermore, we were able to increase the visual contrast in the micrographs and macrographs, by adding 200 ppm of iodine dye to each sample. Iodine dye reacts with starch granules and stains them brown.

The formation of a κ-carrageenan/starch mixed fluid gel structure under shear along with associated increase in viscosity presented as a function of temperature is illustrated in **Fig 3.9**.

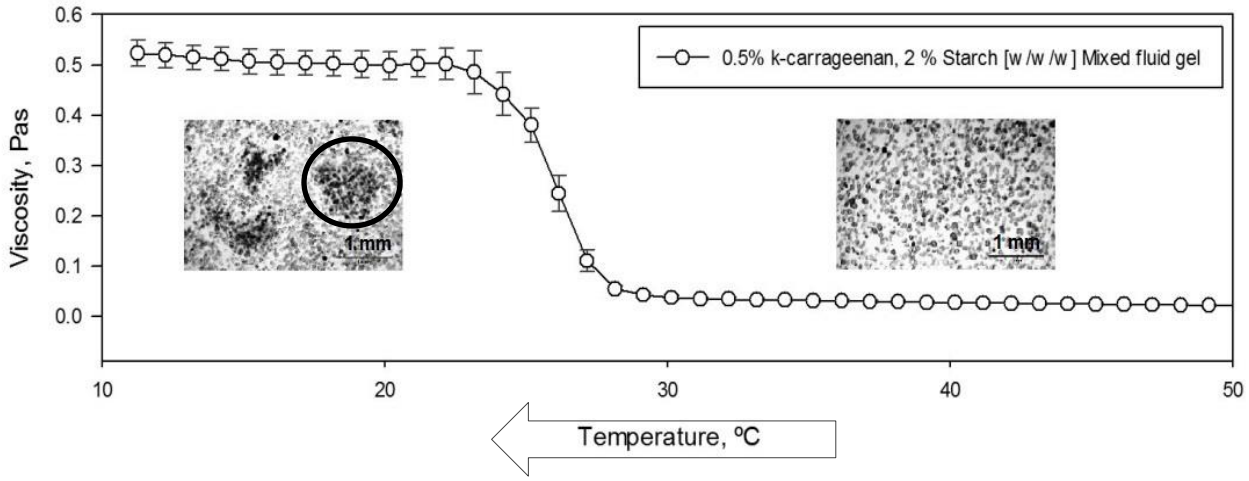

Figure 3.9 The formation of 0.5 % κ-carrageenan and 2 % pregelatinized cross-linked waxy maize starch mixed fluid gel structure. Results present changes in viscosity as a function of temperature. κ-carrageenan/starch aggregate is circled in black.

The micrograph on the right shows that in hot mixed solution, above gelation point, swollen starch granules were spread evenly and their size was around 30 μm. Close to the gelation

point of κ-carrageenan, (around 30 ºC) the viscosity of the mixture rapidly increased as the mixed fluid gel structure formed. The structure is illustrated on the micrograph on the left. The size of the κ-carrageenan/starch aggregates in the fluid gel structures produced at the shear rate of 600 s^{-1} was around 1 mm. Not all of the starch granules were incorporated into the κ- carrageenan/starch aggregates. Some remained in the continuous medium. The mixed fluid gel structure contained irregular aggregates built from starch granules linked by κ-carrageenan particles (example circled in black). Continuous phase was formed of free starch granules and remaining water.

Macro scale images of mixed fluid gel structures illustrating the decrease in κ-carrageenan/starch aggregate size as a result of an increase in shear rate applied during the formation process are presented in **Fig 3.10.** An image of κ-carrageenan/starch pre-gel hot solution was added as a comparison. Fluid gel structures were diluted 1:3 and iodine dye was used to stain starch granules and obtain better contrast between dispersed and continuous phase in the structures.

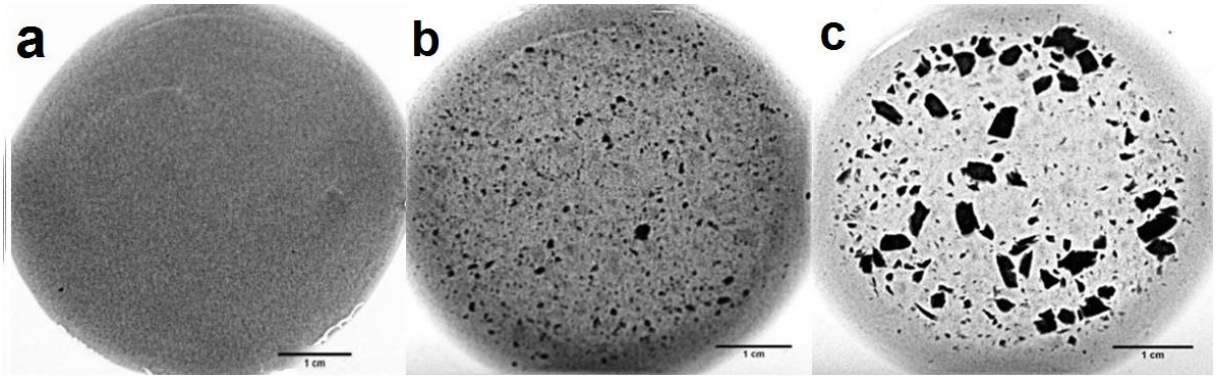

Figure 3.10 Macro scale images of 0.5 % κ-carrageenan/2 % starch pre- gel hot solution (a), mixed fluid gel structure (diluted 1:3) formed under shear rate 600 s^{-1} (b) and 0.5 s^{-1}(c)

It can be observed that above the gelation temperature, starch granules and κ-carrageenan molecules are spread evenly and form homogenous pre-gel solution (a). It is known that addition of the starch granules to a κ-carrageenan contributes to the 'excluded volume effect'. (Chaudemanche & Budtova, 2008; Mandala & Bayas, 2004; Tecante, *et al.*, 1999) This means that the swollen starch granules occupy a certain amount of water and exclude κ- carrageenan molecules from this occupied solvent volume. As a result biopolymer effective concentration increases, mobility, and the distance between molecules decrease, which enhance intermolecular and intramolecular interactions. Upon cooling the pre-gel solution, close to the gelation temperature, κ-carrageenan particles start to order and interact with each other. Application of the shear to the mixed pre-gel solution induces flow which increases the rate of interactions between particles with similar size due to creation of flow lines. (Walkenstrom, 2002) However, the aggregation of κ-carrageenan particles increases the possibility of interactions with starch granules, resulting in formation of κ-carrageenan/starch aggregates. The size of the aggregates is dictated by the rate of the shear applied during the formation process. At a low shear rate of 0.5 s^{-1} (c), κ-carrageenan particle aggregate to the greater extent and more starch granules can be incorporated into the aggregate structure with size of around 5 mm on average. The lighter colour of the continuous phase indicates that majority of starch granules have been immobilized into κ-carrageenan/starch aggregates. Increasing the shear rate up to 600 s^{-1} (b) resulted in the reduction of the κ- carrageenan particle aggregation, therefore interactions between κ-carrageenan and starch granules were less possible and size of the κ-carrageenan/starch aggregates and their decreased to around 1 mm. The dark colour of the continuous phase indicated that a lot of starch granules remained in the continous phase and were not included in the aggregate structure, partially explaining the smaller aggregate size. In brief, increase in shear rate during production results in decrease in κ-carrageenan

particle-particle interactions, increasing amounts of free starch granules in the continous phase and decreasing size of the κ-carrageenan/starch aggregates.

The increase in aggregates size is also responsible for significant increase in mixed fluid gel viscosity. The measurements of rapid viscosity increase ($\Delta\eta$) that occurred during particles ordering and aggregation in the single and mixed fluid gel structure were plotted as a function of applied shear and fitted into power law rheological model to observe the impact of shear rate on κ-carrageenan/starch aggregates size in comparison to κ-carrageenan only structures. The results obtained are presented as double logarithmic plot in **Fig 3.11.**

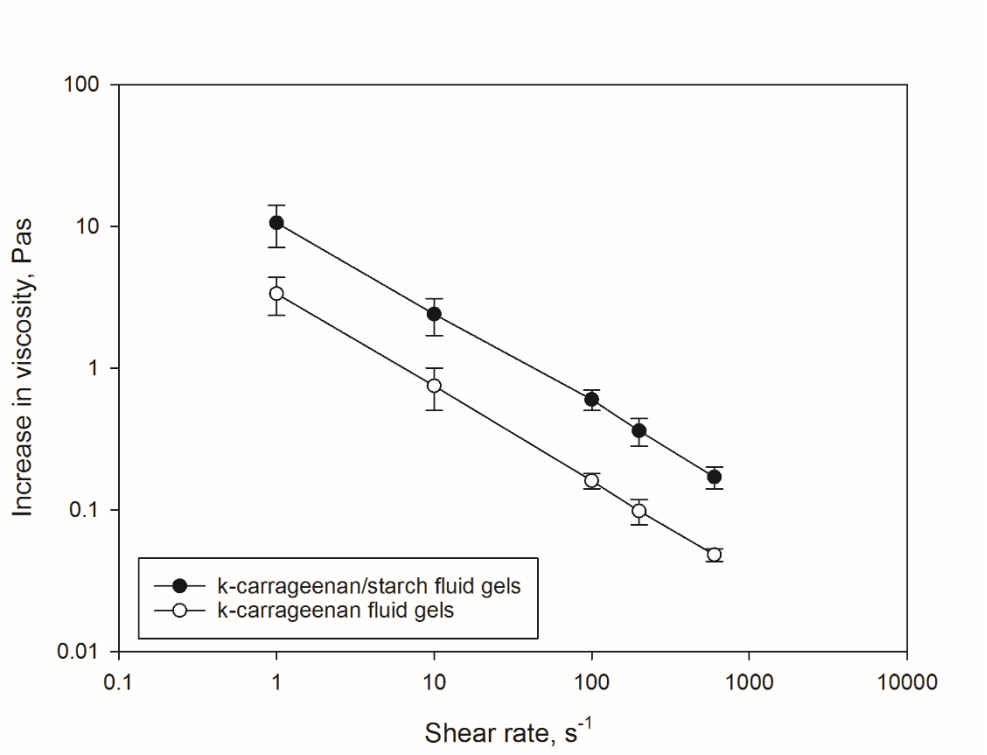

Figure 3.11 Increase in viscosity during fluid gel structure formation process as a function of applied shear rate

The rate of shearing had a significant impact on the size of aggregate. Reduction in the viscosity was observed in both fluid gel types as a result of increasing shear rate. As in

previous experiments, viscosity was greater in a fluid gel containing starch, due to the formation of larger κ-carrageenan/starch aggregates. Differences in viscosity increase (aggregates size) between κ- carrageenan and κ-carrageenan/starch structures were a proportional across entire shear rate range. To establish a detailed comparison of the rheological behaviour of the structures, data was fitted into the power law rheological model presented in **Equation 5** which has previously been used to describe the inversely proportional relationship between increase in viscosity from ordering initiation to completion ($\Delta\eta$) and the shear rate ($\dot{\gamma}$) applied during production process for agarose and κ-carrageenan gels produced in shear field. (Emanuele and Palma-Vittorelli 1992; Gabriele, Spyropoulos, and Norton 2009)

$$\Delta\eta = B(\dot{\gamma})^{C} \quad (5)$$

Power law coefficients *B* representing material consistency and flow index *c* obtained for 0.5 % [w/w] κ-carrageenan and 0.5 % κ-carrageenan \ 2 % starch [w/w] fluid gel structures are presented in **Table 3.1**

Table 3-1 Power law coefficients B and c calculated from increase in viscosity obtained for fluid gels produced under various shear rates

Parameters	***B***	***c***	R^2
0.5 % κ-carrageenan	3.34	- 0.67	0.995
0.5 % κ-carrageenan/ 2 % starch	10.65	- 0.65	0.997

The coefficient *B* value for the κ-carrageenan/starch fluid gel was three times greater than that of the single κ-carrageenan, thus confirming that the 2 % [w/w] starch contributed to the increase in fluid gel structure consistency by causing an increase in aggregate size. Similar flow index values for both structures indicated comparable shear thinning behaviour. This behaviour is common in suspensions containing non-attracting, large molecules as their

orientation, determined by laminar flow reduces resistance to the shear. Flow index values are also similar to the exponent previously obtained by Gabriele *et al.* for 0.5 % [w/w] κ-carrageenan only fluid gel where exponent c = -0.69.

The impact of cooling rate on fluid gel structures formation was also investigated. The measurements of increase in viscosity ($\Delta\eta$) occurring during aggregation of both types of fluid gel structures were fitted into the power law rheological model (**Equation 5**) where (γ) is the cooling rate, B is consistency coefficient and c is the power law index. (Emanuele and Palma-Vittorelli 1992; Gabriele, Spyropoulos, and Norton 2009)

Power law coefficients B and c calculated from the increase in viscosity occurring as fluid gel structures are formed under cooling rates 0.5-3 ºC min^{-1} are presented in **Table 3-2.**

Table 3-2 Power law coefficients B and c calculated from increase in viscosity obtained for fluid gel structures produced under various cooling rates 0.5 -3 ºC min^{-1}

Parameters	***B***	***c***	***R*^{2}**
0.5 % κ-carrageenan	0.10	-0.37	0.954
0.5 % κ-carrageenan/ 2 % starch	0.22	0.28	0.996

The B coefficient value for the κ-carrageenan/starch mixed fluid gel was double that of the κ-carrageenan only fluid gel, indicating that the addition of starch resulted in a thickened structure. The power law index c of the mixture showed that an increase in applied cooling rate led to an increase in final structure viscosity as a result of the formation of larger aggregates. In comparison, the κ-carrageenan fluid gel power law index revealed that in the absence of starch the opposite relationship was observed. The fluid gel aggregates size decreased with increase in cooling rate. Slower cooling rates allowed for longer processing time and as a result for extended gel nuclei growth. The particles had a greater probability of

interaction and increasing the size of the aggregates. (Nunes, Raymundo, and Sousa 2006; Gabriele, Spyropoulos, and Norton 2009)

In comparison, in the fluid gel structures containing starch, the greatest viscosity increase was observed at the highest of the investigated cooling rates, 3 ºC min^{-1}. It is believed that this is the result of rapid κ-carrageenan gelation leading to immobilisation of a vast number of starch granules within the κ-carrageenan/starch fluid gel aggregates. When the cooling rate was decreased, a reduction in structures viscosity was recorded. The decrease in cooling rate increased processing time and resulted in prolonged exposure to shear which could lead to a decrease in the size of the κ-carrageenan/starch aggregates. Extended production process could also lead to separation of the κ-carrageenan and starch phases prior to κ-carrageenan gelation with fewer starch granules being incorporated into aggregate structures. The effect of cooling rate on the fluid gel structure formation was not as substantial compared to the impact of shear rate as the differences in increase in viscosity of the structures formed at various cooling rates were not significant.

The κ-carrageenan/starch fluid gels are effective viscosity modifiers. However, the size of the aggregates is too large to provide fat-like mouthfeel (creaminess). Therefore lower molecular weight components than pregelatinized cross linked waxy maize starch needs to be considered in order to obtain mixed fluid gels structures with fat-like texture and mouthfeel.

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Chapter 4. κ-CARRAGEENAN AND MALTODEXTRIN PASELLI SA2 SHEARED GEL STRUCTURES

4.1. Overview

The aim of this chapter is to gain a better understanding of the relationship between mixture composition, formation process parameters, gel structure and rheological properties of κ-carrageenan and maltodextrin Paselli SA2 gel mixtures produced under shear. Results and discussions included in this chapter are in preparation for publication in a peer reviewed journal:

Gladkowska-Balewicz, I., Hamilton, I. E., I.T. Norton 2016, 'Relationship between formulation, process parameters, structure and rheological properties of κ-carrageenan/maltodextrin Paselli SA2 fluid gel mixtures produced under shear'.

4.2. Abstract

The formation of κ-carrageenan/maltodextrin Paselli SA2 structures under precisely controlled shear rates between 1-600 s^{-1} and cooling rates between 0.5-3.0 ºC min^{-1} and their characterisation were investigated in order to gain a greater understanding of the mechanisms governing mixed fluid gel structure formation. Specifically, the impact of process parameters and component concentrations on the increase in viscosity and on the gelation onset temperature during fluid gel aggregate formation was studied. The mixed fluid gel structure had a significantly higher melting temperature than the single κ-carrageenan fluid gel indicating that maltodextrin addition had an impact on κ-carrageenan helix aggregation resulting in a more thermally stable structure. Confocal microscopy images of the structures revealed an increasing phase separation between components with increasing concentration of maltodextrin in the system. Segregative interactions lead to significant increase in κ-carrageenan/maltodextrin aggregate size and fluid gel viscosity. The increase in viscosity during fluid gel aggregates formation along with onset gelation temperatures were observed to

be influenced by the applied process conditions (shear and cooling rate) and with concentrations of maltodextrin above 8 % [w/w]. In addition, rheological models for the rate of mixed κ-carragenan/maltodextrin fluid gel structures aggregation are presented. Post-production characterisation of the mixed fluid gel structures revealed that adding maltodextrin extended the linear viscoelastic region of the structure as well as improved the strength and fluid gel resistance to strain deformation. The importance of the time post-production for continuous phase viscosity development was evident from observations of the slower ordering rate of maltodextrin.

4.3. Introduction

Hydrocolloids are widely used by food manufacturers as thickening and gelling agents due to their high water binding capacity at very low concentrations. (Saha, 2010) Application of shear during red algal polysaccharides resulted in the formation of the structures containing gel particles and non-gelling medium. (Norton, 1999; Norton, 2000; Norton, 2006; Gabriele, 2009) These structures poses bulk rheological behaviour similar to emulsions used in soft solid foods, therefore fluid gel structures could be utilized as a potential fat replacer in low-calorie product formulations to mimic the texture and mouthfeel of fat particles. (Hippleheuser, Landberg, and Turnak, 1995; Brennan and Tudorica, 2008; Zoulias, Oreopoulou, and Tzia, 2002; Sanchez, Klopfenstein, and Walker, 1995) However, using only one polysaccharide as a fat replacer has proven to be insufficient if the intention is to mimic closely fat-like behaviour. (Kruse *et al.* 1994; Augustine 1996)

The process of mixing biopolymers whilst controlling gelation conditions is believed to be the one way in which superior, mixed fluid gel structures can be designed with the potential to cover a wider range of fat functions in food products compared to currently employed fat

replacers. (Le Reverend *et al.* 2010) Starch and its derivatives can significantly impact on creaminess sensation due to their ability to form a gel structure with disc-shaped particulates, which under shear in the mouth give fat droplets perception.

Sheared gel mixtures formed from κ-carrageenan and pregelatinized cross-linked waxy maize starch have shown that introducing another ingredient in the system can significantly influence gel particle morphology and, as a result, favourably alter the final gel rheological properties, leading to more efficient viscosity modifiers, with potential application in reformulated soft solid food products. (Gladkowska-Balewicz *et al.*, 2014) However, one of the consequences of using starch is formation of too large aggregates to duplicate creaminess sensation as well as unwanted starchy aftertaste. These difficulties can be eliminated by replacing starch with lower molecular weight maltodextrin Paselli SA2, which provides a neutral taste. (Altschul, 1993)

Maltodextrin Paselli SA2 (SA2) is a low dextrose equivalent (*DE* < 3) product of potato starch enzymatic hydrolysis. Gels obtained from SA2 are used as fat replacers owing to their ability to mimic important fat properties, such as smooth, even texture and fat-like mouthfeel. (Altschul, 1993)

Detailed understanding of the rheological performances of mixed fluid gel structures is crucial in predicting their flow behaviour during production and identifying factors which can impact on fluid gel structure formation. Those parameters can be appropriately manipulated to improve product properties, such as texture and mouthfeel. (Ding *et al.* 2005; Norton and Frith, 2001; Stokes, Wolf, and Frith, 2001; Wolf and Frith, 2003; Wolf, Frith, and Norton, 1998)

In this research sheared gel structures of κ-carrageenan mixed with maltodextrin Paselli SA2 were investigated to gain an understanding of the relationship between mixture composition, formation process parameters, mixed fluid gel structure, and rheological properties.

4.4. Experimental

4.4.1. Materials

Samples of κ-carrageenan were kindly provided by Cargill. Maltodextrin Paselli SA2 was obtained from Avebe (The Netherlands). Potassium Chloride BioXtra, ≥99.0 % salt was purchased from Sigma-Aldrich. All materials were used without any further purification.

4.4.1.1 Sample preparation

4.4.1.1.1 Preparation of κ-carrageenan solutions

κ-carrageenan powder 0.3-0.5 % [w/w] was added to hot distilled water at 90 °C (~60 °C) above expected phase transition temperature) under constant agitation and allowed to hydrate fully. 0.05-0.1 % [w/w] potassium chloride (KCl) by mass was added to the homogenous κ-carrageenan solution. Solutions were kept isothermal and stirred for a further 30 minutes prior to the experiment to ensure complete hydration.

4.4.1.1.2 Preparation of Maltodextrin Paselli SA2 solutions

White maltodextrin Paselli SA2 powder 2-20 % [w/w] was added to distilled water at ambient temperature (20 ºC). Mixtures were then heated and continuously stirred until completely transparent (fully dissolved). Solutions were kept isothermal at 90 ºC and stirred for a further 30 minutes before mixing with the κ-carrageenan solution.

4.4.1.1.3 Mixed pre-gel solution preparation

The concentrations of κ-carrageenan and maltodextrin Paselli SA2 in the pre-gel solutions varied between 0.1-1.0 % [w/w] and 2.0-20.0 % [w/w] of the overall mass of the mixture respectively. Potassium chloride addition was kept constant at 0.05 % or 0.1 % by mass of the overall solution.

Mixed pre-gel solutions were prepared using the same method as single component solutions. The appropriate portions of hot SA2 and κ-carrageenan solutions were mixed to achieve the desired concentration of the final mixed fluid gel [w/w]. Mixtures were kept isothermal at 90 °C whilst stirring for a further 30 minutes to ensure homogeneous mixing prior to performing the experiment. The vessel was covered so as to avoid evaporation of the water at elevated temperatures.

4.4.2. Methods

4.4.2.1 Differential scanning calorimetry measurements

A Seteram μDSC3 evo Dynamic Scanning Calorimeter (DSC) was used to detect the temperature at which the components underwent a thermal transition as well as any potential thermally driven interactions between them. Screw-top 'closed batch cells' were used for the analysis, where 0.5-0.6 g of the sample was added to the sample cell and an equal mass of distilled water added to the reference cell. Samples were tested by placing the cells in the instrument and allowing the temperature to stabilise initially at 20 °C before the examination. Samples were then heated from 20 °C to 95 °C at a rate of 1 °C min^{-1}. The samples were then cooled to 5 °C at the same rate of 1 °C min^{-1}. 10 minute isothermal holds were applied

between each step. Reported results were calculated from an average of three heating cycles and three cooling cycles.

4.4.2.2 Rheometer measurements

Single and mixed fluid gel formation and post-production characterisation of rheological behaviour were investigated using a Kinexus pro rotational rheometer equipped with a vane geometry (internal cup diameter 27 mm, the vane rotor 25 mm) calibrated with pure water (at 20 °C). Prior to the experiment and before the sample was placed in the equipment, the geometry was heated to 85 °C and allowed adequate time for the temperature to stabilise. An evaporation control hood was fitted for the duration of the experiment. Fluid gels were formed by controlled cooling of pre-gel solutions via a peltier controlled cylinder cartridge whilst the appropriate shear rate was applied.

4.4.2.3 Fluid gel structures formation

Heated pre-gel solutions were poured into the vane cup and heated to 85 °C. The temperature between sample and geometry was equilibrated over a 5 minutes interval. Single component solutions and mixtures were cooled from 85 °C to 5 °C using precisely controlled cooling rates 0.5 - 3 °C min^{-1} and under constant stirring under shear rates 1.0-600 s^{-1}. Changes in the viscosity were monitored at two second intervals. Stages of mixed κ-carrageenan fluid gel structure formation are illustrated in **Fig 4.1.**

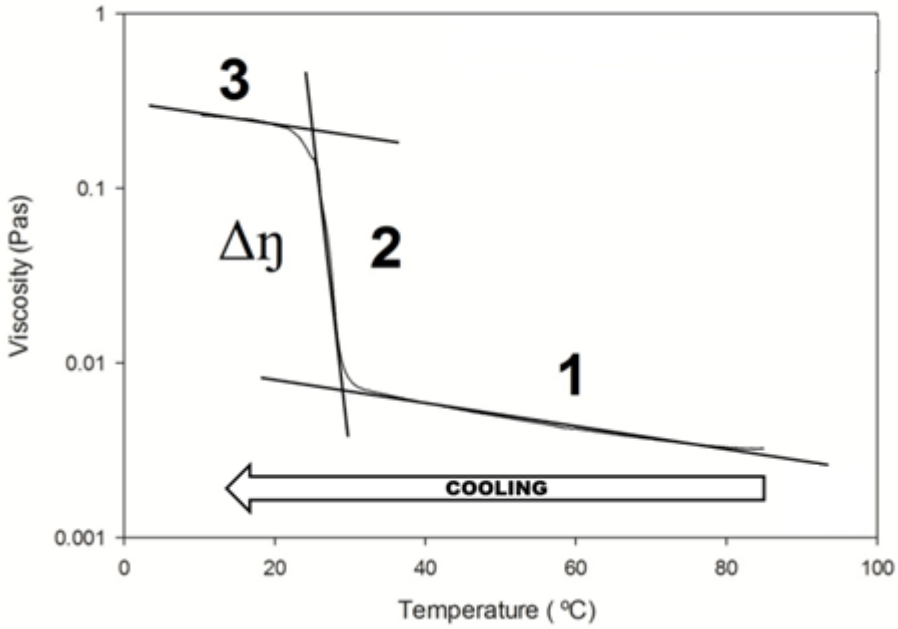

Figure 4.1 the κ-carrageenan/maltodextrin mixed fluid gel structure formation in a shear field.

The increase in viscosity was determined by the points of intersection of tangents fitted to the viscosity curve. Tangents were fitted to the three regions observed during structure formation (1-prior, 2-during, and 3-post aggregation). Values of viscosity at the intersection of the tangents were taken as an on-set and end-set of the structure formation. The increase in viscosity was calculated as the difference between the values of these two points. Values were compared for each of the mixed fluid gel systems to allow comparison of differences in aggregate size as a consequence of component composition in the mixture and production process conditions.

4.4.2.4 Fluid gel structures characterisation

4.4.2.4.1 Linear viscoelastic region and structure elasticity

Sample characterization tests were performed immediately after mixed gel production using a cone and plate 4/40 geometry. An amplitude sweep test was used to determine the stability of the mixed fluid gels and their resistance to strain deformation. Their linear viscoelastic region was determined in strain range between 0.1 to 100 % and at a constant frequency (1 Hz). The point at which the storage modulus (*G'*) changed by more than 10 % from linearity indicated the end of the linear viscoelastic region. The strength of the fluid gel microstructure was also evaluated. Values of the storage modulus (*G'*) were obtained using the frequency sweep test within the samples' linear viscoelastic region.

4.4.2.4.2 Fluid gel structure images

Images of the κ-carrageenan fluid gel structure with varying maltodextrin Paselli SA2 content were obtained using confocal laser scanning microscopy to investigate the type of structures formed under shear . Hot distilled water was dyed with the fluorescent Nile blue. 0.5 % [w/w] κ-carrageenan powder was fully dissolved in hot water containing 1 % [w/w] Nile blue. 0.1 % [w/w] potassium chloride salt was added afterwards. κ- carrageenan pre-gel solution was cooled on the rheometer whilst undergoing shearing. Post production, gel particles were added to hot 5 %, 15 % and 25 % [w/w] maltodextrin solutions and stirred for 15 minutes to obtain homogenous mixtures. The fluid gel structure was observed via confocal microscope after 48h.

4.5. Results and discussion

4.5.1. Identification of the gelling and melting phase transition by differential scanning calorimetry

Differential scanning calorimetry was used to analyse κ-carrageenan phase transitions in the presence and absence of maltodextrin Paselli SA2. Analyses were carried out by successive heating and cooling cycles at 1 ºC min^{-1}. The exothermic (left column) and endothermic (right column) phase transitions along with their onset temperatures for the 0.5 % [w/w] κ-carrageenan separately and mixed with 2 % (a), 8 % (b), 15 % (c), 20 % (d) [w/w] SA2 are illustrated as a function of temperature. The thermographs obtained are presented in **Fig. 4.2.**

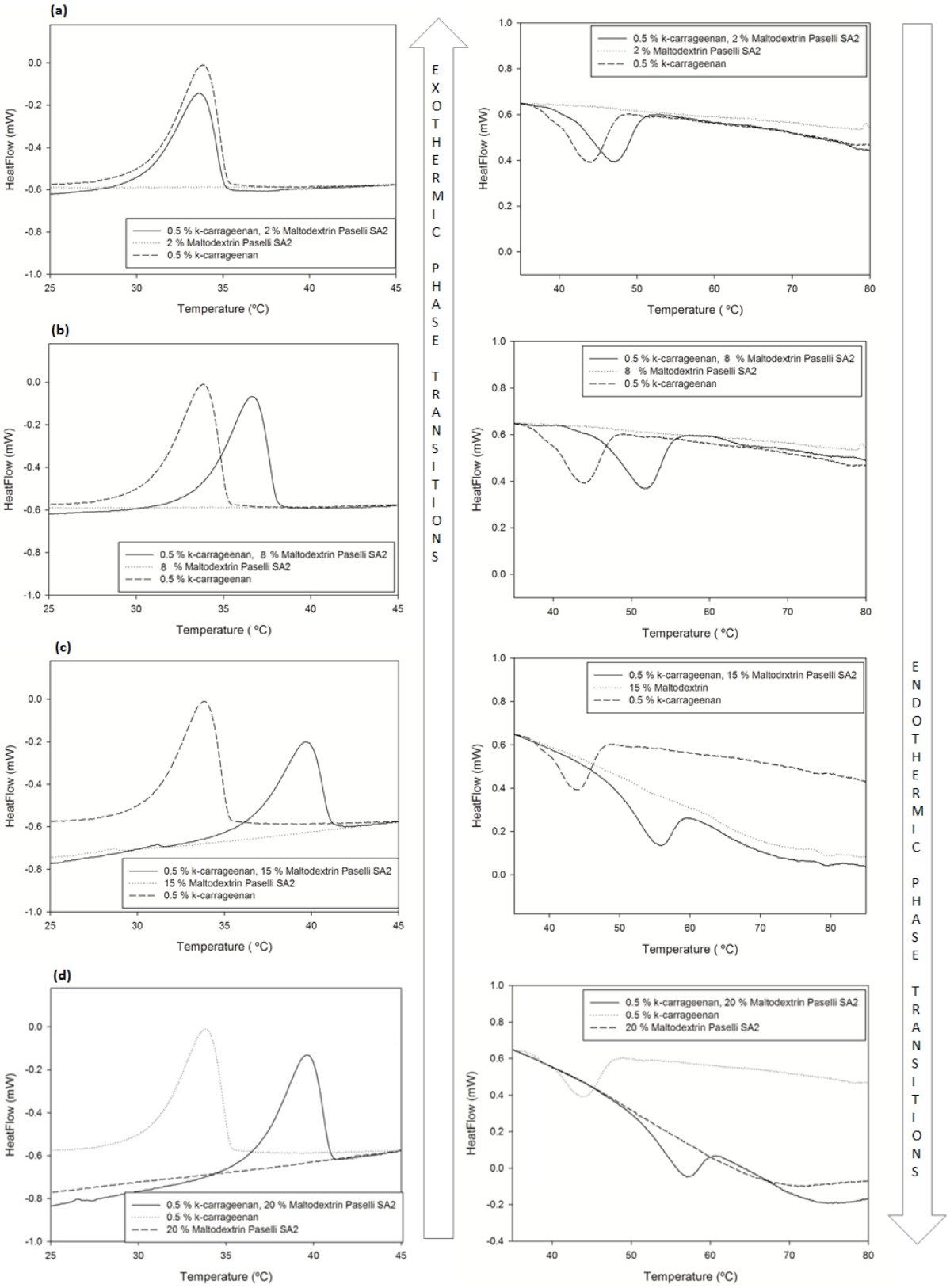

Figure 4.2 Thermographs illustrating exothermic (left column) and endothermic (right column) phase transitions for individual 0.5 % [w/w] κ-carrageenan gel and mixtures with 2 % (a), 8 % (b), 15 % (c) and 20 % (d) SA2 obtained at a rates of 1 ºC min^{-1}.

Only one exothermic peak was detected during cooling of κ-carrageenan single component gels and gels mixed with maltodextrin. No peak, in this concentration and temperature range, was observed during cooling of maltodextrin Paselli SA2 only. Furthermore, when the concentration of maltodextrin was raised to 15 % [w/w] of maltodextrin and above, the peak followed the maltodextrin only baseline, suggesting phase inversion had occurred, and the maltodextrin was dominating in the continuous phase of the mixture. The peak observed in the κ- carrageenan/maltodextrin sample during the sol-to-gel phase transition, was associated with κ-carrageenan coil-to-helix ordering and aggregation (gelation). κ-carrageenan gel structure formation in the absence of maltodextrin started at around 35 ºC. Sol-to-gel phase transition for κ-carrageenan/maltodextrin structure revealed no change in onset temperature with addition of up to 8 % [w/w] of maltodextrin. However, adding 8 to 20 % [w/w] Paselli SA2 resulted in the onset gelation temperature of the mixed gel increasing gradually from 35 ºC to 42.0 ºC (± 0.1). The gelation peak maximum increased from 32.7 ºC (ΔH = - 36.0 ± 2.0 j g $^{-1}$) to 39.6 ºC (ΔH = - 32.0 ± 2.0 j g $^{-1}$). These results indicate that maltodextrin had an impact on κ-carrageenan helix aggregation. The presence of an additional co-solute in the system increased the overall solids concentration and decreased the amount of water available for κ-carrageenan. As a result, the distance between κ-carrageenan molecules also decreased, accelerating helix ordering and allowing aggregation to occur at a higher temperature.

One endothermic peak for κ-carrageenan helix-to-coil transition was observed at the onset temperature of 38 ºC. In the case of κ-carrageenan/maltodextrin mixtures, a second endothermic peak appeared at higher maltodextrin concentrations, which is above 15 % [w/w]. The appearance of two peaks indicates the formation of phase separated gel structure. Additionally, phase inversion was observed as the peak baseline switched from following the κ-carrageenan curve to the maltodextrin only one, indicating the formation of a continuous

maltodextrin structure. The differences in thermal behaviour between κ-carrageenan and maltodextrin are attributed to the dissimilar chemical structure and molecular weight. κ-carrageenan is a high molecular weight, linear polysaccharide with a conformational transition timescale in the order of seconds to minutes which appears as a tall but narrow peak on the thermograph. Maltodextrin Paselli SA2 exhibits a slower gelation mechanism in the hours timescale, due to its lower molecular weight and because it is constructed mainly of highly branched amylopectin chains from which only short chains take part in gel network development. (Kalichevsky, Orford, and Ring 1990; Ring et al. 1987) As a consequence, the phase transition appears on thermographs as a shallow but broad peak.

Further increases in the maltodextrin content up to 20 % [w/w] resulted in an increase in the onset melting temperature, up to 50 ºC. The first melting peak, attributed to κ-carrageenan, reached its maximum at 56.1 ºC ($\Delta H = 28 \pm 2.0$ j g $^{-1}$). This temperature is 11.9 ºC higher than that recorded during the melting of κ-carrageenan only gels. The second gel-to-sol phase transition peak, attributed to the melting of maltodextrin, was observed at 66.2 ºC. The significant increase in the melting temperature of κ-carrageenan in the mixed gel indicated that the addition of maltodextrin had an impact on κ-carrageenan helix aggregation, and more thermally stable structures were formed as a result of phase inversion.

4.5.2. The effect of maltodextrin Paselli SA2 addition on the viscosity and gelation temperature of κ-carrageenan during structure formation in the shear field.

To investigate the effect of maltodextrin addition on the formation of mixed fluid gel, solutions of κ-carrageenan and maltodextrin were cooled individually and as a mixture at a cooling rate of 3 ºC min^{-1} while being subjected to a shear rate of 100 s $^{-1}$. The viscosity and

onset gelation temperatures during fluid gel formation were monitored as a function of temperature. The flow curves obtained are shown in **Fig. 4.3.**

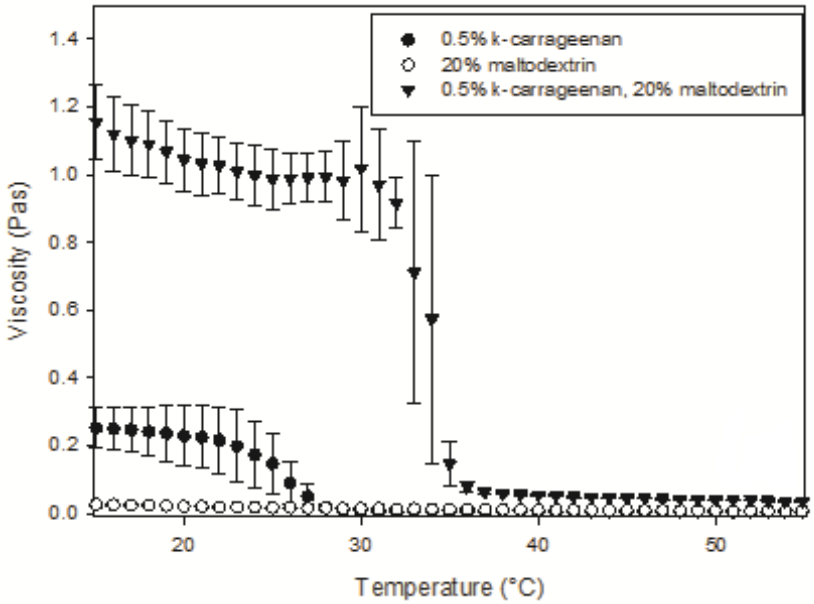

Figure 4.3 The comparison of viscosity changes during 20 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] κ-carrageenan fluid gel structure formation individually and as a mixture. Changes in viscosity are presented as a function of temperature. Solutions were subjected to a shear rate of 100 s^{-1} and a cooling rate of 3 ^{o}C min^{-1}.

Comparison of flow curves revealed that the addition of 20 % [w/w] maltodextrin Paselli SA2 to 0.5 % [w/w] κ-carrageenan resulted in a shift in gelation onset temperature from around 30 ºC to around 35 ºC. The elevated mixture gelation temperature is due to the excluded volume effect which determines space occupancy and, in the presence of an additional co-solute (maltodextrin), caused a localised increase in κ-carrageenan concentration. The addition of maltodextrin also resulted in a significant increase in viscosity during mixed fluid gel structure formation. At the end of the production process, mixed fluid gel structure viscosity was fifty times higher than maltodextrin only viscosity and five times higher than κ-carrageenan only fluid gel structure viscosity. The rapid increase in viscosity which occurs

close to the mixed fluid gel gelation temperature is explained by the formation and growth of κ-carargeenan small gel nuclei followed by mixed aggregates creation. Small gel nuclei formation is believed to be a result of the demixing process. This process is not fully understood yet. However, it has been suggested that it may be initiated by the spinodal decomposition or nucleation and growth mechanism. Fluid gel particles start to form in the early stages of κ- carrageenan helix aggregation, and this process is defined by the dynamic equilibrium between biopolymer interactions and applied shear forces. The behaviour of the resultant structure is similar to the water in water emulsion and because of that particle agglomeration and/or break up is expected. The growth of fluid gel particles is believed to occur either via enrichment from the surrounding non-gelling medium or particle coalescence restricted by applied shear flow. Consequently, the rapid increase in viscosity observed during mixed κ- carrageenan/maltodextrin structure formation (between 35 ºC and 30 ºC) is directly related to aggregate number, size and volume fraction. (Gabriele, Spyropoulos, and Norton 2009) The interference of ungelling maltodextrin on the association of gelling κ-carrageenan resulted in an increase in aggregation rate and larger size of formed entities. These types of segregative interactions occur due to components incompatibility when neutral polysaccharide is mixed with a helix-forming polysaccharide, and modification of gelation rate could be observed. (Morris and Wilde 1997)

When the maltodextrin was subjected to formation under the same conditions, no rapid increase in viscosity was recorded at any point in the production process, which indicated that no sol- to- gel phase transition had occurred and at the end of formation process maltodextrin remained in the sol state with a viscosity of 0.026 Pas. These results are in agreement with the findings obtained using differential scanning calorimetry suggesting that maltodextrin conformational changes occur over a different timescale, to those of κ-carrageenan.

Therefore, during formation of mixed fluid gel aggregates only κ-carrageenan gelation occurs and their properties are dominated by κ-carrageenan. However, significant difference in aggregation rate between single and mixed κ-carrageenan structure suggest that maltodextrin is immobilised in the κ-carrageenan aggregates and at the end of the formation process structure consist of κ- carrageenan/maltodextrin aggregates suspended in non-gelling medium. Additional experiments confirmed that some maltodextrin chains, not incorporated into the aggregates with κ-carrageenan are dispersed in the non-gelling medium. The proposed model for the κ-carrageenan/maltodextrin mixed fluid gel structure is presented in **Fig 4.4**.

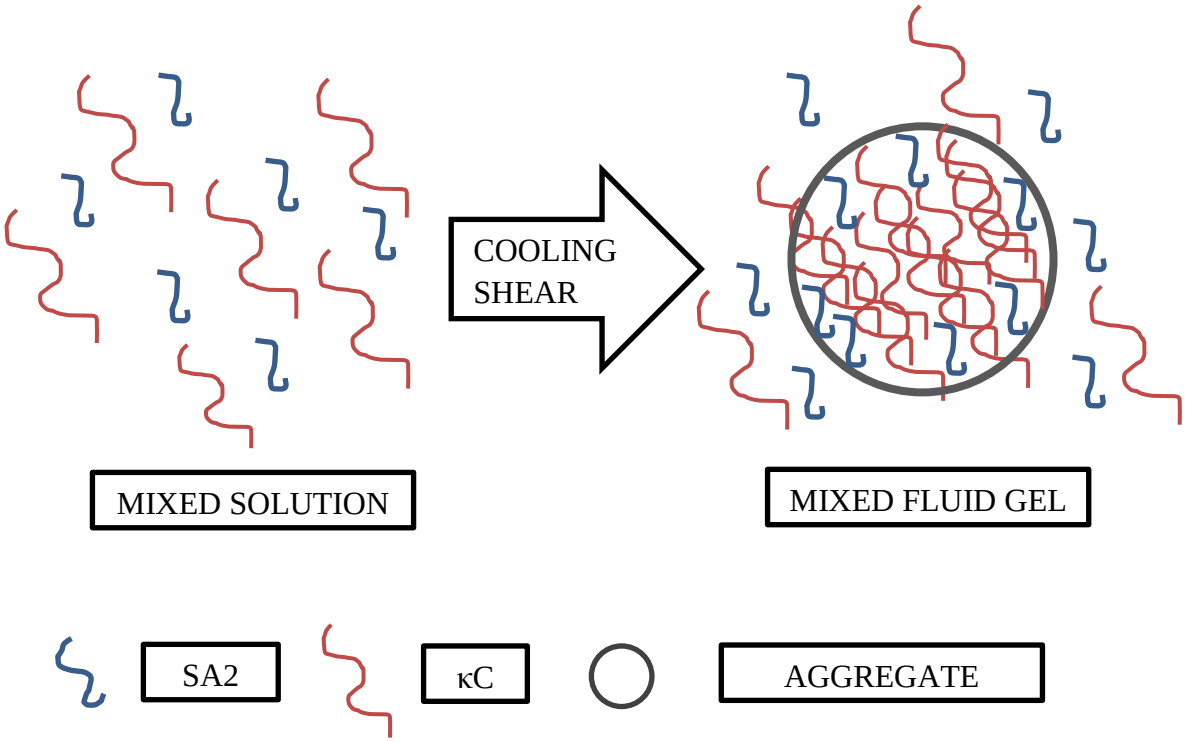

Figure 4.4 The model of the κ-carrageenan/maltodextrin fluid gel structure

4.5.3. Sheared gel structure characterisation

4.5.3.1 Linear viscoelastic region and storage and loss moduli

The length of the linear viscoelastic region (LVR) of maltodextrin and κ-carrageenan fluid gel structures, formed separately and as a mixture were determined in order to investigate the effect of maltodextrin on the structure resistance to strain deformation and hence, structure stability. Results obtained are presented in **Fig 4.5.**

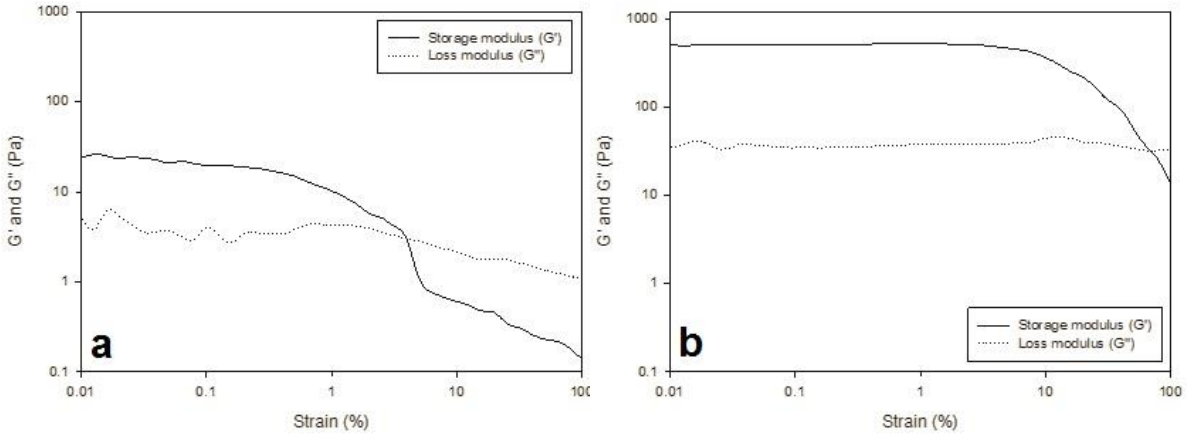

Figure 4.5 Linear viscoelastic region of 0.5 % κ-carrageenan only fluid gel (a) and mixed with 20 % maltodextrin Paselli SA2 (b)

The storage (*G'*) and loss (*G"*) moduli were measured within the linear viscoelastic region and loss tangent (δ) values were calculated in order to observe the effect of maltodextrin on structure strength and viscoelastic behaviour. Structures were investigated 24 hours post formation process, and the results are presented in **Table 4-1**.

Table 4-1 Viscoelastic properties of 0.5 % κ-carrageenan, 20 % maltodextrin Paselli SA2 [w/w/w] sheared gels formed separately and as a mixture

Sample	LVR length (%)	Cross point (%)	G' (Pa)	G'' (Pa)	Tangent δ
0.5 % κ-carrageenan	0.1	4	12	1.4	0.12
20 % Paselli SA2	2.0	53	166	10	0.065
0.5 % κ-carrageenan / 20 % Paselli SA2	5.0	79	470	52	0.11

It was observed that addition of maltodextrin Paselli SA2 to 0.5 % κ-carrageenan extended the length of the linear viscoelastic region, which suggested improved resistance to strain deformation and consequently, increased structure stability. Separately, structures were able to resist deformation up to 2 % strain. However, upon mixing the two components together, resistance to strain deformation increased up to 5 %. A significant development of storage modulus (G') in the mixed κ-carrageenana/maltodextrin structure was observed. The elasticity of mixed structures was 470 Pa, compared to 12 Pa for the κ-carrageenan only fluid gels. The increase in structure strength is solely as a result of the additional co-solute present in the system and incorporation of maltodextrin into the κ-carrageenan aggregates. However, non-gelling maltodextrin partially remained in the continuous medium and no significant change in the loss to storage moduli ratio suggested no shift in the viscoelastic behaviour of the mixed fluid gel structure towards more solid- or more liquid-like.

4.5.4. Effect of component concentrations on κ-carrageenan/maltodextrin structure formation under shear

4.5.4.1 Maltodextrin Paselli SA 2

Various concentrations of maltodextrin Paselli SA2 from 2 % to 20 % [w/w] were mixed with 0.5 % [w/w] κ-carrageenan to observe the effect on the viscosity and onset gelation temperature during gel structure formation under shear. Changes in the mixtures' viscosity were monitored during the production process, and the results obtained are presented as a function of temperature in **Fig 4.6. a**. The increase in viscosity as a function of maltodextrin concentration in the mixture is shown in **Fig 4.6. b**

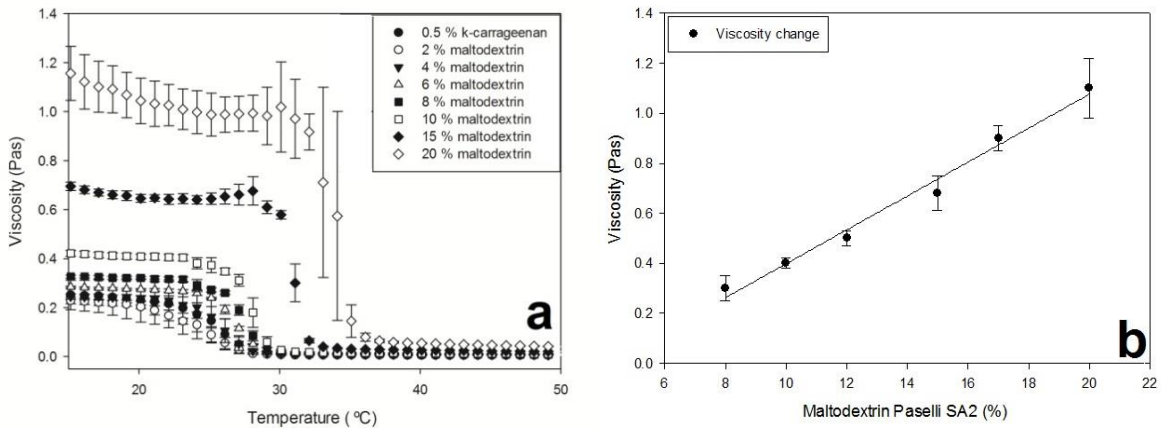

Figure 4.6 The comparison of viscosity for 0.5 % [w/w] κ-carrageenan mixed with 2-20 % [w/w] maltodextrin Paselli SA2 as a function of temperature. Solutions were subjected to a constant shear rate of 100 s^{-1} and a cooling rate of 3 $^{o}C\ min^{-1}$.

In the concentration range, 0-8 % [w/w] maltodextrin no significant increase in viscosity was observed in mixed fluid gels compared to the κ-carrageenan only system. However, in the concentration range 8-20 % [w/w] maltodextrin, a direct relationship between the increase in

viscosity during gel structure formation and increase in maltodextrin concentration was evident (R^2 = 0.9812).

The increase in viscosity during κ-carrageenan/maltodextrin mixed sheared gel formation as a result of maltodextrin addition can be expressed using **Equation 7** where x is the concentration of maltodextrin in the pre-gel solution expressed as a percentage by weight. This equation is valid for mixtures with maltodextrin content of 8 % [w/w] and above.

$$\Delta\eta_{\text{mixed fluid gel}} = -0.2803x_{\text{Maltodextrin}} + 0.0678 \quad (7)$$

4.5.4.2 Confocal laser scanning microscopy images

Confocal laser scanning microscopy was used to obtain images of κ-carrageenan/maltodextrin shear gel microstructures. 0.5 % [w/w] κ-carrageenan was mixed with 5 % (a), 15 % (b) and 25 % (c) of maltodextrin to observe the effect of maltodextrin concentration effect on κ-carrageenan gel network formation under shear. The images obtained are presented in **Fig 4.7.**

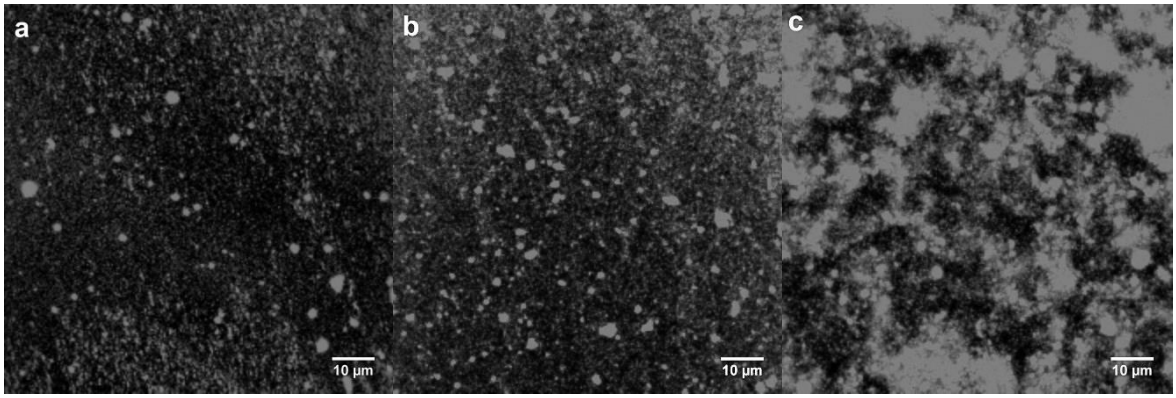

Figure 4.7 Confocal laser scanning microscopy images of mixed fluid gel structures formed from 0.5 % κ-carrageenan with (a) 5 %, (b) 15 % and (c) 25 % maltodextrin Paselli SA2 48h after production. The white colour corresponds to κ-carrageenan, and the black corresponds to maltodextrin.

The white areas on the confocal images represent κ-carrageenan selectively stained with Nile blue, and the black areas correspond to maltodextrin. It was observed that when maltodextrin concentration was as little as 5 % [w/w] the resulting fluid gel structure appears to be equally divided between two components with κ- carrageenan and maltodextrin aggregates dispersed in the non-gelling medium. Increasing the maltodextrin concentration to 15 % [w/w] resulted in more maltodextrin domains in the system and, as a consequence of the excluded volume effect; the localised aggregation of κ-carrageenan particles can be seen. A subsequent increase in maltodextrin concentration to 25 % [w/w] promoted further thermodynamic incompatibility between the components and a significant increase in κ-carrageenan particles aggregation. As a result of the segregative interaction, a heterogeneous, phase-separated structure was formed.

4.5.4.3 Changes in gelation onset temperature

When various concentrations of maltodextrin Paselli SA2 were mixed with 0.5 % [w/w] κ-carrageenan, an increase in onset gelation temperatures was observed. Gelation temperatures

were recorded during a production as a function of the maltodextrin concentration in the mixture, and the results obtained are illustrated in **Fig**. **4.8.**

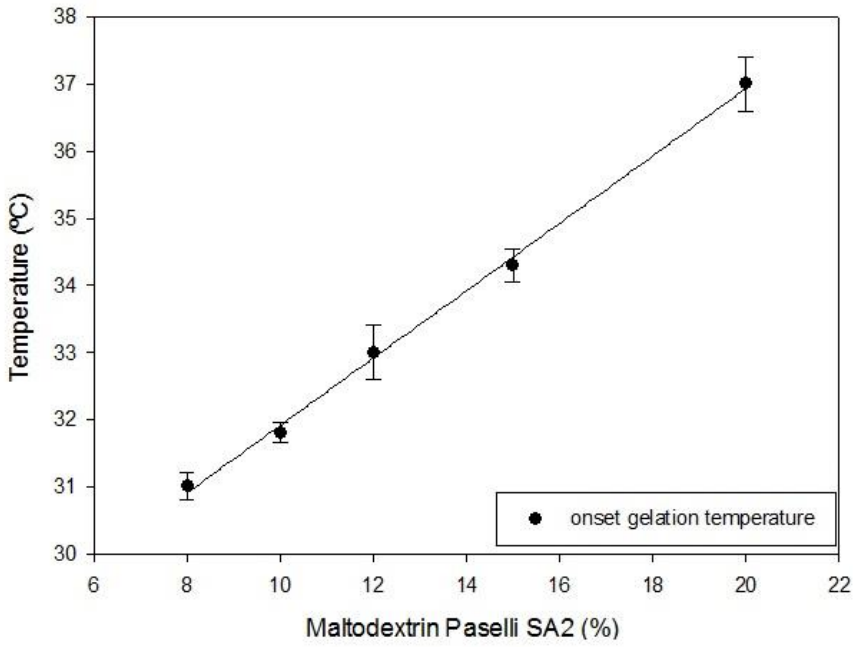

Figure 4.8 The onset gelation temperatures of 0.5% [w/w] κ-carrageenan mixtures with maltodextrin, presented as a function of maltodextrin Paselli SA2 concentration in the formulation.

The individual 0.5% [w/w] κ-carrageenan structure onset temperature was recorded at around 30 ºC and this temperature was not changed with the addition of up to 8 % [w/w] maltodextrin. In the concentration range, 8-20 % [w/w] a linear relationship between the increase in gelation temperature and increase in maltodextrin concentration in the mixture was observed (R^2 = 0.9979).

The gelation temperatures of the κ-carrageenan/maltodextrin sheared gel systems, associated with increasing maltodextrin Paselli concentration can be calculated using **Equation 8**, where

x is the maltodextrin Paselli SA2 concentration in the pre-gel solution, expressed as a percentage by weight [w/w].

$$T_{gelling} = 26.8905 + 0.5023x_{maltodextrin} \quad (8)$$

At maltodextrin concentrations of 8 % [w/w] and above, the number of maltodextrin domains increased. This enabled segregative interactions between the maltodextrin and κ-carrageenan and a localised increase in concentration which enabled κ-carrageenan to form fluid gel structures faster and at higher temperatures.

4.5.4.4 κ-carrageenan

To investigate the effect of κ-carrageenan concentration on the κ-carrageenan/maltodextrin structure formation under shear, 8 % [w/w] maltodextrin Paselli SA2 was mixed with a range of κ-carrageenan concentrations from 0.5 % to 2 % [w/w]. The viscosity during the production process was monitored as a function of temperature, and the results are presented in **Fig 4.9. a**. The increase in viscosity during gel structure formation as a function of κ-carrageenan concentration in the mixture is presented in **Fig 4.9. b**.

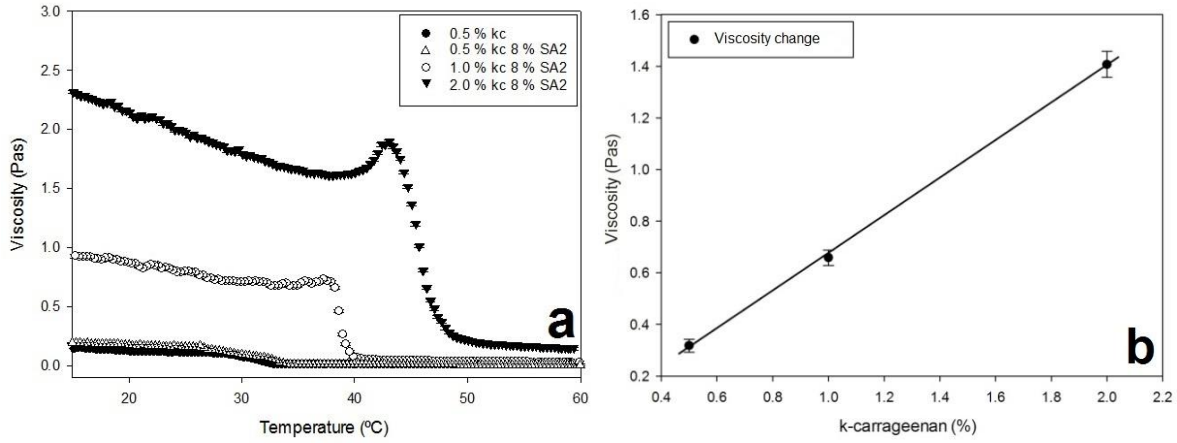

Figure 4.9 Increase in viscosity during mixed fluid gel structure formation for κ-carrageenan/maltodextrin systems as a function of temperature (a) and κ-carrageenan concentration (b)

In the κ-carrageenan concentration range from 0.5-2.0 %, a linear relationship between the increase in viscosity and the increase in κ-carrageenan concentration was evident (R^2 = 0.9995). The increase in viscosity during mixed sheared gel formation as a result of increasing κ-carrageenan concentration could be expressed using **Equation 9** where x is a κ-carrageenan concentration in the pre-gel solution expressed in percentage by weight.

$$\Delta\eta_{\text{mixed fluid gel}} = 0.7296x_{\text{k-carrageenan}} - 0.057 \quad (9)$$

4.5.4.5 Changes in gelation onset temperature

Onset gelation temperatures recorded during κ-carrageenan/maltodextrin gel structure formation as a function of the κ-carrageenan concentration are presented in **Fig 4.10.**

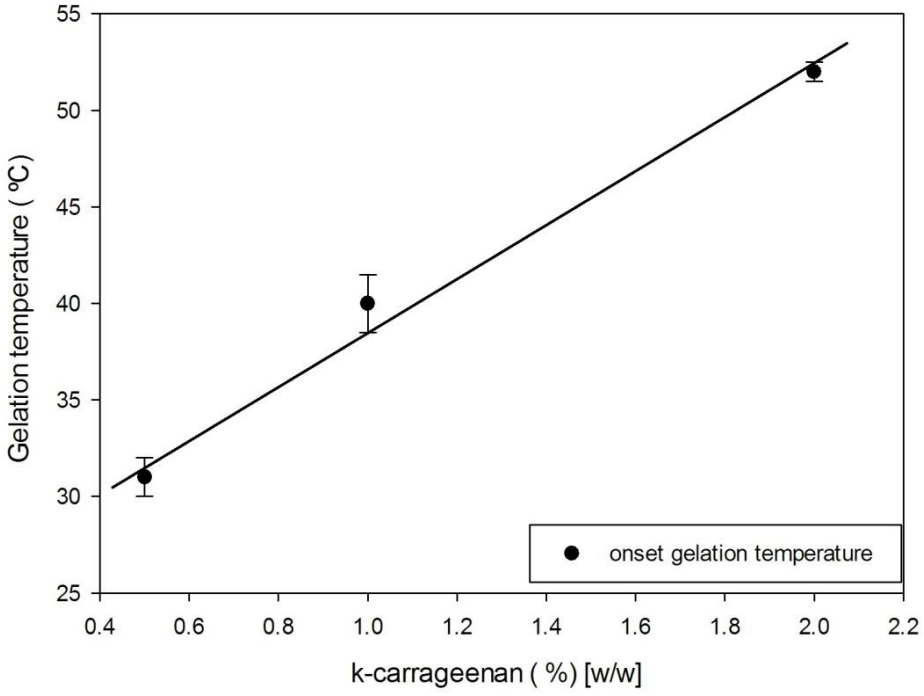

Figure 4.10 Increase in gelation temperature as a function of κ -carrageenan concentration in the formulation. Mixtures containing 8 % [w/w] maltodextrin Paselli SA2

The onset temperature of 0.5 % [w/w] κ-carrageenan fluid gel structure was recorded at around 30 ºC. It was observed that increased κ-carrageenan concentration in a mixture with 8 % [w/w] maltodextrin resulted in increased onset gelation temperature. In the κ-carrageenan concentration range from 0.5-2.0 % [w/w] a linear relationship between the increase in gelation temperature and the increase in κ-carrageenan concentration in the mixture with 8 % [w/w] Paselli SA2 was observed (R^2 = 0.9884)

Onset gelation temperatures for κ-carrageenan/maltodextrin sheared gel mixtures, associated with the increase in κ-carrageenan concentration can be calculated using **Equation 10**, where x is the concentration of κ-carrageenan expressed as a percentage by weight [w/w] in the pre-gel solution.

$$T_{gelling} = 25 + 13.714x_{k-carrageenan} \quad (10)$$

4.5.5. The effect of applied shear rate on mixed κ-carrageenan/maltodextrin structure formation

To investigate the effect of shear rate on κ-carrageenan/maltodextrin structure formation, a broad range of shear rates from 1-600 s^{-1} was applied to the mixtures of 0.5 % κ-carrageenan with 20 % maltodextrin Paselli SA2 [w/w/w]. The viscosity of the structures was recorded as a function of temperature. The obtained flow curves are illustrated in **Fig 4.11.**

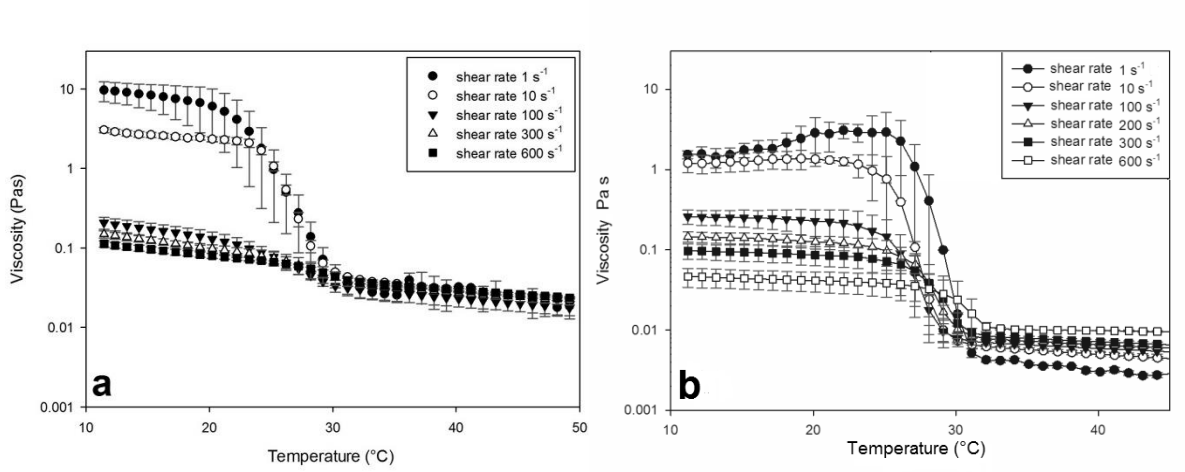

Figure 4.11 Effect of the applied shear rate on the viscosity of mixed 0.5 % [w/w] κ-carrageenan, 20 % [w/w], Paselli SA2 (a) and single 0.5 % [w/w] κ-carrageenan (b) fluid gel. Structures formed with a cooling rate of 3 ºC min^{-1}

The application of low shear rates (1 s^{-1}) the association forces dominated over the shear forces and as a result, viscosity of mixed fluid gel structure rose to around 10 Pas compare to 1 Pas for κ-carrageenan fluid gel only. This increase indicated the formation of relatively large κ- carrageenan/maltodextrin aggregates. In comparison, when the shear rate was

increased to 10 s^{-1}, there was evidently competition between the two opposing forces and as a consequence, structure viscosity dropped to around 3 Pas for mixed fluid gel structure, owing to a reduction in the size of fluid gel aggregates. The greater increase in shear rates to 100 s^{-1}, further decreased the size of the κ-carrageenan/maltodextrin aggregates, and fluid gel viscosity was reduced to around 0.1 Pas. At higher shear rates, above 100 s^{-1}, the viscosity differences between structures were minor (0.1 Pas). Overall, it was observed that an increase in applied shear rate encouraged the formation of smaller κ-carrageenan/maltodextrin aggregates and resulted in a decrease in structure viscosity. Investigations of the shear thinning behaviour of mixed biopolymer systems showed agreement with κ-carrageenan fluid gels and mixed κ-carrageenan/starch fluid gel structures. (Gladkowska-Balewicz, Norton, and Hamilton 2014)

For detailed structure comparisons, data obtained was fitted into the power law rheological model presented in **Equation 11.** The model describes the relationship between the increase in viscosity from structure ordering initiation to completion (Δŋ) and shear rate ($\dot{\gamma}$) applied during production process. (Emanuele and Palma-Vittorelli 1992; Gabriele, Spyropoulos, and Norton 2009)

$$\Delta ŋ = B(\dot{\gamma})^C \tag{11}$$

Viscosity increase recorded during mixed κ-carrageenan/maltodextrin fluid gel structure formation were compared to single κ-carrageenan fluid gel structures produced under the same condition. The results obtained are presented in **Fig 4.12.**

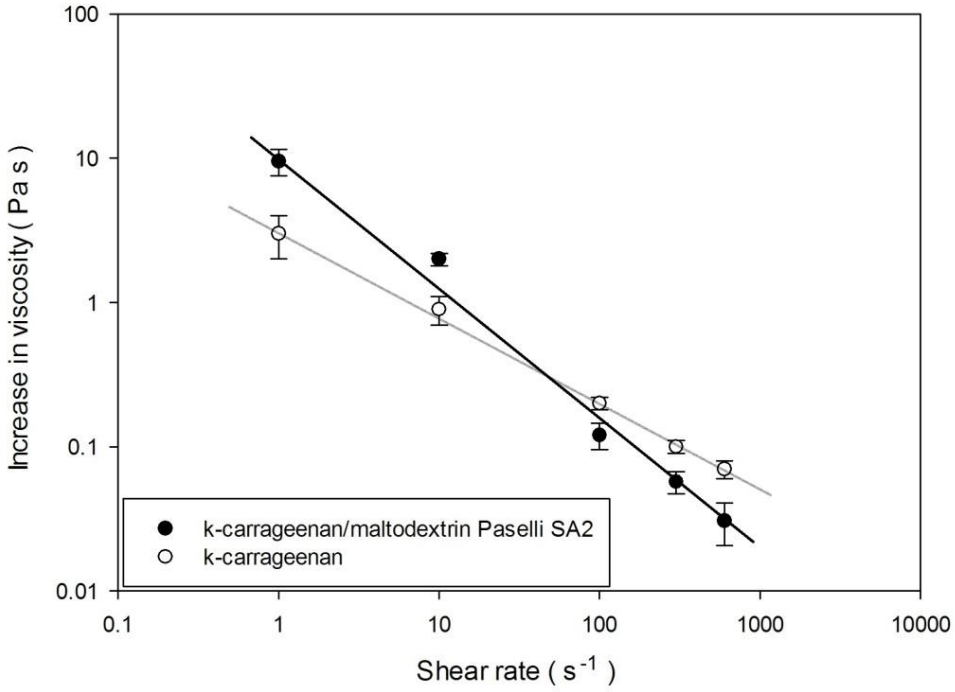

Figure 4.12 Increase in viscosity during 0.5 % [w/w] κ-carrageenan and 0.5 % κ-carrageenan\ 20 % maltodextrin [w/w/w] fluid gel structure formation as a function of applied shear rate

The power law statistical correlation coefficients *B* (flow consistency index) and *c* (flow index) calculated from the increase in viscosity during 0.5 % [w/w] κ-carrageenan and 0.5 % κ-carrageenan\ 20 % maltodextrin [w/w/w] fluid gel structure formation under shear are presented in **Table 4-2.**

Table 4-2 Power law coefficients B and c calculated from increase in viscosity during κ-carrageenan and κ –carrageenan/maltodextrin structure formation under various shear rates

Parameters	***B***	***c***	R^2
0.5 % κ-carrageenan	3.34	- 0.67	0.995
0.5 % κ-carrageenan/ 20 % maltodextrin Paselli SA2	10.58	- 0.92	0.987

The flow consistency coefficient *B* value was more than two times higher for the κ-carrageenan/maltodextrin mixture indicating that the addition of maltodextrin significantly increased structure consistency compared to a κ-carrageenan only fluid gel structure formed without maltodextrin. This is a consequence of maltodextrin incorporation into aggregates with κ-carrageenan which resulted in an increase in their size and number compared to those observed in individual κ-carrageenan fluid gel structures. However, at higher shear rates, above 100 s^{-1}, the growth in viscosity during mixed fluid gel structure formation was lower than for κ-carrageenan only systems. This indicated a relative decrease in the size of κ-carrageenan/maltodextrin aggregates. Flow index *c* for the mixed κ-carrageenan/maltodextrin structure was also lower compared to κ-carrageenan only systems, showing greater effect of shear on mixed structure formation. The association forces in the mixed κ-carrageenan/maltodextrin structure were weaker than those in the κ-carrageenan fluid gel structure as mixed fluid gels were more significantly affected by higher shear rates, resulting in smaller aggregate. This is a consequence of a greater alignment of molecules in the mixture under the higher shear rates and easier flow which results in a decrease in mixed structure viscosity. Similar behaviour was observed for individual κ-carrageenan fluid gel structures. Gabriele *et al.* previously reported that a flow index *c* for 0.5 % κ-carrageenan was -0.69 and decreased with increasing polymer concentration. (Gabriele, 2009)

4.5.6. Effect of the cooling rate applied during the formation process on viscosity of κ-carrageenan/maltodextrin Paselli SA 2 structures produced under shear

Another process parameter that can influence fluid gel structure formation and led to the fluid gel structure with different rheological properties is the cooling rate. (Gabriele, 2009; Gladkowska-Balewicz, 2014)

To investigate the effect of cooling rate on κ-carrageenan/maltodextrin fluid gel structure formation, the range of cooling rates from 0.5 ºC min^{-1} to 3 ºC min^{-1} was applied to the hot pre-gel solutions of 0.5 % [w/w] κ-carrageenan mixed with 20 % [w/w] SA2. The increase in viscosity during structure formation was recorded as a function of temperature and results obtained are presented in **Fig 4.13. (a)** κ-carrageenan structures formed under the same conditions are added as a comparison **(b)**.

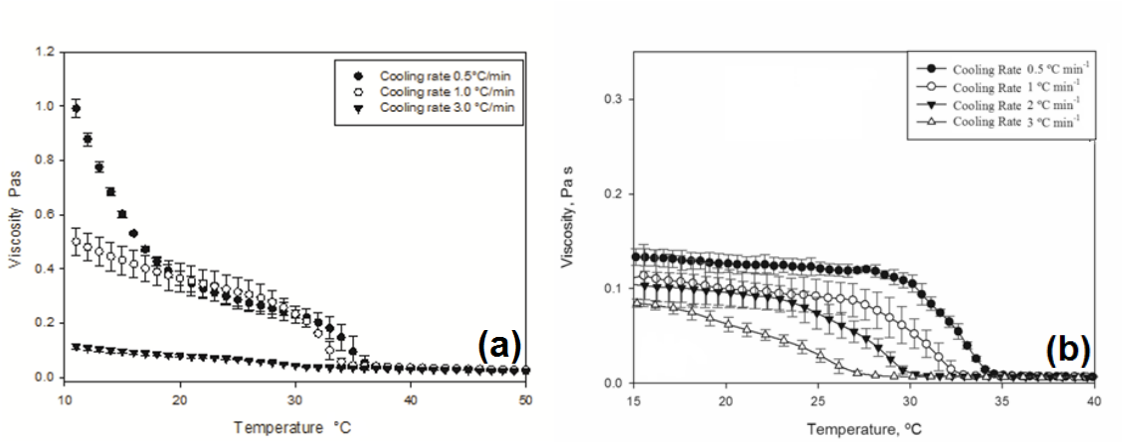

Figure 4.13 The effect of applied cooling rate on the increase in viscosity during mixed 0.5 % [w/w] κ-carrageenan, 20 % [w/w] SA2 (a) and single 0.5 % [w/w] κ-carrageenan (b) fluid gel structure formation. Solutions subjected to a constant shear rate of 100 s^{-1}

Cooling rates applied to the gelling biopolymer structures are directly related to the duration of the production process. As a consequence, higher cooling rates resulted in less time allocated for fluid gel structure development. The investigation revealed that application of the fastest cooling rate, 3 ºC min^{-1} to the κ-carrageenan/maltodextrin mixture resulted in the lowest increase in viscosity, around 0.1 Pas. This indicated that the structure containing the smallest aggregates was formed under these process conditions. Decreasing the cooling rate to 1 ºC min^{-1} prolonged the formation process and enabled the formation of the structure with greater aggregate size. The flow curve obtained under the slowest cooling rate of 0.5 ºC min^{-1}

showed that a rapid increase in viscosity occurred twice. The first increase in viscosity observed between 38 and 32 ºC was related to the ordering and aggregation of κ-carrageenan helices and the formation of κ-carrageenan/maltodextrin aggregates. The second increase in viscosity observed below 20 ºC was assigned to the development of the maltodextrin structure development in the remaining, continuous medium. These results revealed differences in component structure formation kinetics. Lower temperature and longer processing times were needed for the SA2 structure to be formed compared to κ-carrageenan. As a result, greater differences could be observed in the viscosity of fluid gel structures formed at various cooling rates. The partial overlap of the flow curves during structure formation at the cooling rates of 0.5 ºC min^{-1} and 1.0 ºC min^{-1} suggests that the second increase in viscosity observed is attributed to the formation of maltodextrin structures in the continuous medium. This increase would be expected to appear in the flow curves representing higher cooling rate, if the amount of time allowed for the fluid gel structure formation were increased.

The size of the fluid gel aggregates is the result of the competition between shear forces and structure formation forces which are driven by cooling rate. For a detailed comparison of individual κ-carrageenan structures and those mixed with starch and maltodextrin, data obtained was fitted into the power law rheological model presented in **Equation 12** which describes the correlation between the increase in viscosity (Δŋ) and cooling rate (γ) applied during the production process for structures produced in a shear field.

$$\Delta\eta = B(\gamma)^C \qquad (12)$$

Results are plotted as a function of cooling rate and presented in **Fig 4.14.**

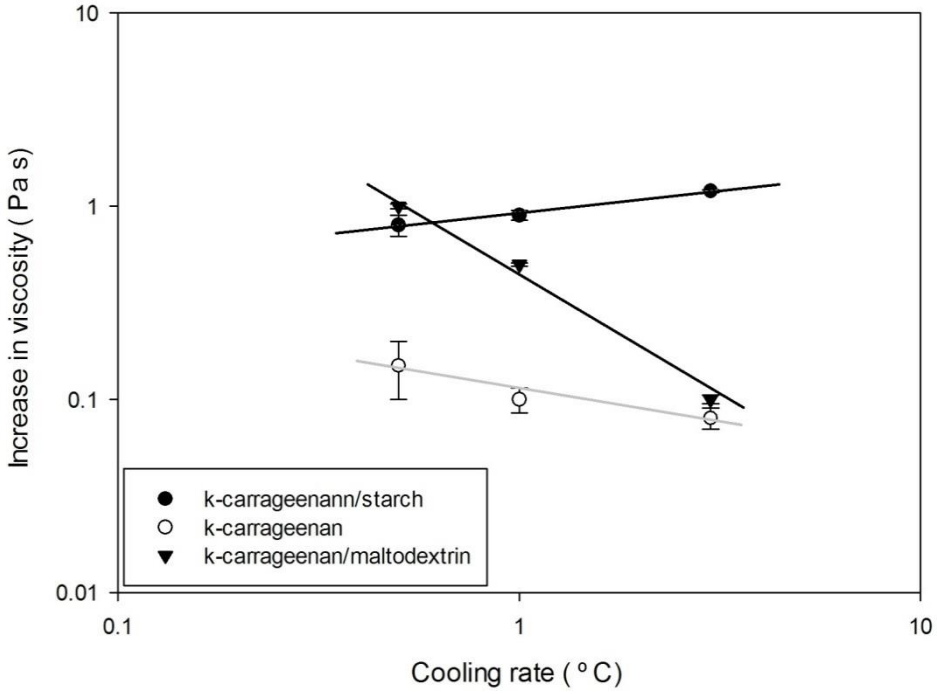

Figure 4.14 Comparison of fluid gel structures of κ-carrageenan, κ-carrageenan/starch, and κ-carrageenan/maltodextrin. The increase in viscosity is presented as a function of cooling rates between 0.5 ºC to 3 ºC min^{-1}. Structures subjected to shear rate of 100 s^{-1}

Flow consistency coefficient *B* and flow index *c* were calculated from the increase in viscosity obtained during κ-carrageenan/maltodextrin fluid gel structure formation under cooling rates 0.5-3 ºC min^{-1}. The results are compared to κ-carrageenan fluid gels and κ-carrageenan/starch mixed fluid gel structures and are presented in **Table 4-3**

Table 4-3 Power law coefficients B and c calculated from increase in viscosity obtained for fluid gel structures produced under the range of cooling rates between 0.5 - 3 ºC min^{-1}and constant shear rate of 100 s^{-1}

Parameters	***B***	***c***	***R*^{2}**
0.5 % κ-carrageenan	0.10	- 0.37	0.954
0.5 % κ-carrageenan/ 20 % Paselli SA2	0.44	-1.30	0.990
0.5 % κ-carrageenan/ 2 % starch	0.94	0.28	0.975

The values of coefficient *B* indicated that the consistency of the κ-carrageenan/maltodextrin structure was four times higher than the κ-carrageenan only structure, but only half that of the κ-carrageenan/starch structure. These differences are attributed to the variation in fluid gel aggregates size which impact on structure viscosity. As expected, the addition of high molecular weight starch significantly increased the aggregate size and greatly influenced mixed fluid gel structure viscosity. In comparison, the addition of maltodextrin, with an intermediate molecular weight (intermediate between starch and sugar) resulted in smaller aggregates.

It was observed that the κ-carrageenan fluid gel aggregates size decreased with increased cooling rates. When κ-carrageenan was mixed with pregelatinized cross-linked waxy maize starch, the opposite relationship was noted as aggregate size increased with increasing cooling rate. This is due to more starch granules incorporated into the aggregates (less phase separated structure) as a result of shorted processing time and more rapid gelation of κ-carrageenan. However differences in viscosity between structures formed using various cooling rates are not significant. In comparison, it was observed that viscosity of κ-carrageenan/maltodextrin structure could be greatly manipulated by cooling rate applied during formation process as indicated by flow index *c* value of -1.3. Application of faster cooling rate of 3 ^{o}C min^{-1} resulted in the structure with behaviour similar to single κ-carrageenan fluid gel system which suggested that only gelation of κ-carrageenan and occurred and process was too fast for maltodextrin ordering and aggregation. Processing using slower cooling rate of 0.5 ^{o}C min^{-1} resulted in the structure with viscosity similar to κ-carrageenan/starch fluid gel structure which suggested that aggregation of both, κ-carrageenan and maltodextrin took place.

4.5.7. κ-carrageenan/maltodextrin structure development post-formation process

Owing to the slower ordering rate of maltodextrin, investigation of κ-carrageenan/maltodextrin structure development post-formation was required. Therefore, κ-carrageenan/maltodextrin structures were formed using the slowest cooling rate of 0.5 ºC min^{-1} and a shear rate of 100 s^{-1}. The structure formation process finished at a temperature of 5 ºC. After production, structures were kept *in situ* at the same process conditions, a constant temperature of 5 ºC and shear 100 s^{-1}, for a further 360 minutes. The viscosity development post-formation was recorded as a function of time. The point, at which the maximum viscosity value was reached, followed by a plateau region, marked the completion of structure formation. Individual κ-carrageenan and maltodextrin structures were formed under the same process conditions, and results obtained are compared in **Fig 4.15.**

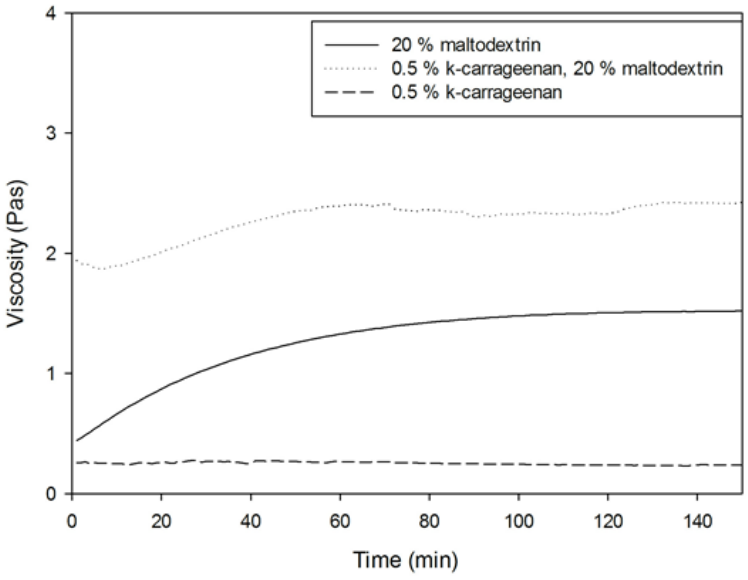

Figure 4.15 Structure viscosity development post production process for 0.5 % [w/w] κ-carrageenan, 20% [w/w] Paselli SA2 separately and as a mixture presented as a function of time. Structures subjected to a constant shear rate of 100 s^{-1}at the temperature of 5 ºC

The measurements of the viscosity at the temperature of 5 ºC, post production, showed no change for the single κ-carrageenan fluid gel structure. The κ-carrageenan viscosity remained constant during the entire investigation time. These findings indicated that κ-carrageenan fluid gel structure formation was complete at the end of the fluid gel production process. In comparison, gradual viscosity development post production was observed for the maltodextrin Paselli SA2 structures. The maltodextrin viscosity reached its maximum after an additional 90 minutes under shear. At this point, the viscosity reached a plateau, and no further increase was observed. The performance of the mixed κ-carrageenan/maltodextrin structure was similar to maltodextrin system. This indicated that after formation of κ-carrageenan/maltodextrin aggregates, part of the maltodextrin remained in the continuous medium. Mixed structure development reached a plateau after around 60 minutes post production. The time required for maltodextrin structure formation in a mixtures with κ-carrageenan was one-third shorter, compared to the maltodextrin only structure. The shortening of the maltodextrin formation time in the mixture is a consequence of less solvent being available when the two components are mixed together. Maltodextrin, which was not incorporated in the fluid gel aggregates, remained with not structured water in the continuous phase of the structure. The development of the viscosity of the continuous phase depends on the maltodextrin and water ratio. If the maltodextrin concentration in the continuous medium is below critical for gelation, the κ- carrageenan/maltodextrin structure will remain post-production as a fluid gel due to the liquid continuous phase. However, if the concentration of maltodextrin is above critical, the structures formed of κ-carrageenan/maltodextrin aggregates trapped in the maltodextrin solid gel network will be formed.

Conclusions

This research has shown that κ-carrageenan/maltodextrin structures produced under shear contained κ-carrageenan/maltodextrin aggregates dispersed in non-gelled water. The maltodextrin which was not incorporated in the aggregates during fluid gel structure formation remained in the continuous phase. The type of structure formed depended strongly on the processing time available, owing to the slower ordering rate of maltodextrin. Confocal microscopy images of the mixed fluid gel structures revealed that thermodynamic incompatibility between components increased with increasing maltodextrin concentration in the system. Maltodextrin concentrations higher than 8 % [w/w] led to segregative interactions which resulted in increased κ-carrageenan gelation temperatures and viscosity during fluid gel aggregate formation (increase in aggregates size). A significant increase in melting temperatures was also observed when maltodextrin was added at concentrations above 8 % [w/w]. This confirmed the positive impact of maltodextrin on κ-carrageenan helix aggregation and a more thermally stable gel structure formation. Differential scanning calorimetry results also revealed that at 15 % [w/w] of maltodextrin in the formulation, a phase inversion to a maltodextrin continous system occurred. An extended linear viscoelastic region indicated an increased resistance to strain deformation and structure stability in the presence of maltodextrin. Investigation of the process conditions revealed that an increase in the shear rate and cooling rate applied during the formation process decreased the final structure viscosity owing to reduced size of the aggregates.

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Chapter 5. RED ALGAL POLYSACCHARIDES AND MALTODEXTRIN SHEARED GEL STRUCTURES

5.1. Overview

This chapter aims to further understand the effect of adding maltodextrin Paselli SA2 to red algal polysaccharide of different charge density in terms of rate of ordering and aggregation and onset gelation temperature during fluid gel structure formation under shear. The viscoelastic properties of the resulting sheared gel structures were also characterised 48 hours post-production. The optical microscopy images were obtained to investigate the compatibility of the sheared gel components, the strength of the structure, interactions between material particles and stability post-formation. The results and discussion included in this chapter are in preparation for publication in the peer reviewed journal:

Gladkowska-Balewicz, I., Hamilton, I. E., Norton, I. T., 2016, 'Rheological studies of the fluid gel structure formation in mixtures of different charge density red algal polysaccharide and maltodextrin Paselli SA2'.

5.2. Abstract

This research focuses on mixed fluid gel structures formation and characterisation. The changes in viscosity, gelation onset temperature and viscoelastic properties of charged (ι-carrageenan, κ-carrageenan, furcellaran) and uncharged (agarose) polysaccharide structures produced under shear in the presence of maltodextrin Paselli SA2 are monitored. The results obtained suggested that mixed fluid gel structure formation and interactions between particles are affected greatly by red algal polysaccharide charge density, specific cation addition and neutral co-solute concentration. The addition of neutral maltodextrin (Paselli SA2) had impact on component compatibility and aggregates formation. The resultant structure viscosity and elasticity was influenced by aggregate size, number and volume fraction. It has been shown that increase in viscosity during mixed fluid gel structure formation depend strongly on the

formation process conditions; particularly shear rate and polysaccharide charge density. If mixture gelation was observed, the mixed fluid gel structure viscosity and elasticity decreased with a decrease in red algal polysaccharide charge density.

5.3. Introduction

Consumer food choices are mainly dictated by taste and texture. Therefore, in the process of product reformulation, it is crucial to replace ingredients such as fat with low-calorie structures which have similar impact on those features. Polysaccharides extracted from red algae seaweed have been extensively employed in the food sector owing to their ability to significantly increase the viscosity of aqueous solutions and form gels at low concentrations between 0.5 to 2.0 %. (Usov, 2011; Coviello *et al.* 2007; de Vries, 2004a) Red algal polysaccharides are long chains of repeating disaccharide units linked by glycosidic bonds. Some of these disaccharide units may carry a negative charge if either of the sugars has a sulfate group attached to it. The number and position of the ester sulfate groups on the sugars in the polysaccharide chain affect the rate of helix ordering and aggregation since they cause repulsion between helices. It has been shown that gel structure elasticity increases as the charge density of the polysaccharide decreases. (Morris and Wilde, 1997) The charge of red algal polysaccharides investigated in this study decrease in the following order:

ι-carrageenan (2 SO_3^-) > κ-carrageenan (1 SO_3^-) > furcellaran (0.5 SO_3^-) > agarose (0 SO_3^-)

It has been previously reported that some ions have the abbility to reduce the electrostatic repulsion between polysaccharide chains, promoting their aggregation and increase gel elasticity. The cations with the most significant effect are called specific ions (Rinaudo, 1993). It has been shown that potassium is specific for κ-carrageenan and calcium for ι-carrageenan. (Norton *et al.* 1978; Morris, Rees, and Robinson, 1980; Norton *et al.* 1983)

Application of shear forces during biopolymer gelation is also known to affect the emerging structure. Instead of a three-dimensional, strong gel network with significant resistance to mechanical deformation, the resultant structure is made up of concentrated gel particles suspended in a non-gelling medium. The change in the microstructure entails modification of the response to mechanical deformation, which moves towards the 'weak gel' structure. Sheared gel is capable of sustaining small strains or stresses, however at large enough deformation structure flows, hence the name, fluid gel. (Altmann, 2004) The advantage of the fluid gel over the quiescently cooled gel is that by varying the processing conditions, especially shear rate, the same starting material can provide a range of structures with differing rheological properties due to the various particle number, size, shape and volume which leads to the manipulation of the particle-particle interactions. In this way, the fluid gel structure rheology can be controlled and modified to achieve the desired features of the food product, such as texture, taste, performance during production, stability during storage, release of flavours and active ingredients. (Norton and Norton, 2010; Aguilera, 2005)

Research results obtained by Norton *et al.* (2010) have shown that bulk rheological behaviour of full-fat mayonnaise can be successfully mimicked by an agar fluid gel structure. This indicates that the fluid gel structures have the potential to be used as a fat replacer in soft solid food products with gel particles exhibiting the rheological behaviour of oil droplets. (de Carvalho, 1997; Norton, Jarvis, and Foster, 1999; Norton, Frith, and Ablett, 2006; Gabriele, Spyropoulos, and Norton, 2009; Garrec and Norton, 2012)

The formation of fluid gel structures based on one polysaccharide has been widely studied and is well understood. However, water based fluid gel structure composed of a single polysaccharide does not sufficiently match the texture and mouthfeel provided by fat based

emulsions. Investigations of κ-carrageenan sheared mixtures with pregelatinized cross-linked waxy maize starch (Gladkowska-Balewicz, Norton, and Hamilton, 2014) and maltodextrin Paselli SA2 (Gladkowska-Balewicz, *et al.*, 2016 in preparation) have shown that the addition of the second, neurtal polysaccharide can significantly increase fluid gel structure viscosity, elasticity and resistance to strain deformation due to the formation of mixed aggregates and an increase in continuous phase viscosity. However, work still needs to be done to fully understand the mechanisms involved in mixed fluid gel structure formation.

In this study, the formation and viscoelasticity of mixed fluid gel structure from ι-carrageenan, furcellaran and agarose mixed with maltodextrin Paselli SA was investigated and κ-carrageenan/maltodextrin mixture was added as a comparison. The aim was to investigate whether mixed fluid gel structure can be obtained from mixtures containing other red algal polysaccharides and neutral co-solute, to understand how different charge density impact on mixed fluid gel structure formation and viscoelastic properties.

5.4. Materials and methods

5.4.1. Materials

Food-grade κ-carrageenan was kindly provided by Cargill (Vilvoorde, Belgium). Furcellaran was kindly provided by Est-Agar (Kärla, Estonia). Maltodextrin Paselli SA2 was obtained from Avebe (Veendam, The Netherlands). ι-carrageenan, agarose, potassium chloride salt BioXtra, ≥ 99.0 %, and calcium chloride salt, were purchased from Sigma-Aldrich (UK). All materials were used without further purification.

5.4.2. Methods

5.4.2.1 Pre-gel solution preparation

5.4.2.1.1 Red algal polysaccharides solutions

The 0.5 % [w/w] of polysaccharide powder was weighed and added to hot distilled water at 90 °C (~60 °C above the expected phase transition temperature) under constant agitation and allowed to hydrate fully. The 0.1 % [w/w] of KCl was added to the κ-carrageenan and furcellaran and 0.15 % [w/w] of $CaCl_2$ was added to the ι-carrageenan post-dissolution to avoid the negative impact of salt of polysaccharides hydration. Solutions were kept isothermal at 85 ºC and stirred for a further 30 min prior to the experiment to ensure homogenous blends.

5.4.2.1.2 Maltodextrin Paselli SA2 solutions

Due to the high concentrations of maltodextrin Paselli SA2 needed for the experiments weighed powders were added to distilled water at the ambient temperature of 20 ºC. Afterwards, the solution was heated on the hotplate up to 85 ºC whilst being continuously stirred until complete dissolution.

5.4.2.1.3 Mixed solution preparation

The concentrations of red algal polysaccharide and maltodextrin Paselli SA2 in the pre-gel solutions varied between 0.5-12.0 % of the overall mass of the mixture. Potassium chloride salt at 0.1 % by mass of the overall solution was added to κ-carrageenan and furcellaran as potassium is a specific ion which promotes helix ordering and aggregation (gelation).

Calcium chloride at 0.15 % by mass of the overall mixture was added to ι- carrageenan as calcium is a selective ion, promoting gel structure formation in this type of carrageenan.

Pre-gel solutions were prepared using the same method as the single component solutions. The appropriate mass of hot maltodextrin Paselli SA2 solution mixed with KCl or $CaCl_2$ was added to the heated ι-carrageenan, κ-carrageenan, furcellaran or agarose solution to achieve the desired concentration of the final mixed fluid gel [w/w]. Mixtures were kept at 90 °C whilst stirring for a further 30 min to ensure homogenous mixing prior to performing the experiment. The vessels were covered to avoid evaporation of solvent at elevated temperatures.

5.4.2.1.4 Fluid gel structure formation

Hot pre-gel solutions were cooled on a Kinexus Pro rheometer, which enabled precise application of cooling and shear rates. The final gel structures obtained after production were examined for the presence of gel particles and non-gelling medium and the ability to flow. In some gel structures, a non-gelling medium and ability to flow could not be identified and therefore those structures could not be called ‘fluid gels.' The term ‘sheared gels’ was used to unify terminology for all structures produced under shear.

5.4.2.1.5 Characterisation of sheared gel structures viscoelastic properties

Characterization of viscoelastic properties was performed 48 hours after mixed gel production using cone and plate 4/40 geometry. Small-amplitude oscillatory tests were used to characterize fluid gel structures viscoelastic behaviour. The amplitude sweep was used to determine the strength of interactions between material particles, microstructure resistance to strain deformation and to designate linear viscoelastic region. Strain amplitudes of 0.1-100 %

were applied at a constant frequency of 1 Hz. The point at which the storage modulus (G') changed by more than 10 % from linearity indicated the end of the linear viscoelastic region. The elasticity of the sheared gel microstructure was also evaluated. Values of the storage (G'), loss (G'') and complex (G^*) moduli were obtained using the frequency sweep test within the sample's viscoelastic region. Samples structural response to deformation was measured at frequencies between 0.1-10 Hz and values obtained at frequency of 1 Hz were used as comparison point. The values of loss tangent (δ) was calculated as a ratio of the energy dissipated (G'') to energy stored (G') per cycle of deformation. The cohesive energies of the structures were measured to quantify the strength of interactions between material particles and stability of the structure using **Equation 13:**

$$Cohesive\ energy = \frac{1}{2} G' \times \gamma^2 \quad (13)$$

The G' represents elastic modulus and solid component of the material. Generally higher G' values indicate more stable material. However length of the linear viscoelastic region γ needs to be also considered as the longer γ, the more robust and resistant to strain deformation is the structure.

5.5. Results and discussion

5.5.1. Viscosity increase and gelation temperatures of pure red algal polysaccharides structures formed under shear

Fluid gel structures of pure ι-carrageenan, κ-carrageenan, furcellaran and agarose were formed using a constant concentration of biopolymer, 0.5 % [w/w] and processed at the cooling rates of 1 ºC min^{-1} and shear rates of 400 s^{-1}. The increase in viscosity during gelation was monitored as a function of temperature to observe the effect of charge density of the

polysaccharide on the rate of ordering and aggregation of helices under shear. Flow curves associated with the formation of the fluid gels are compared in **Fig 5.1.** The viscosity and gelation temperatures are compared in **Table 5-1.**

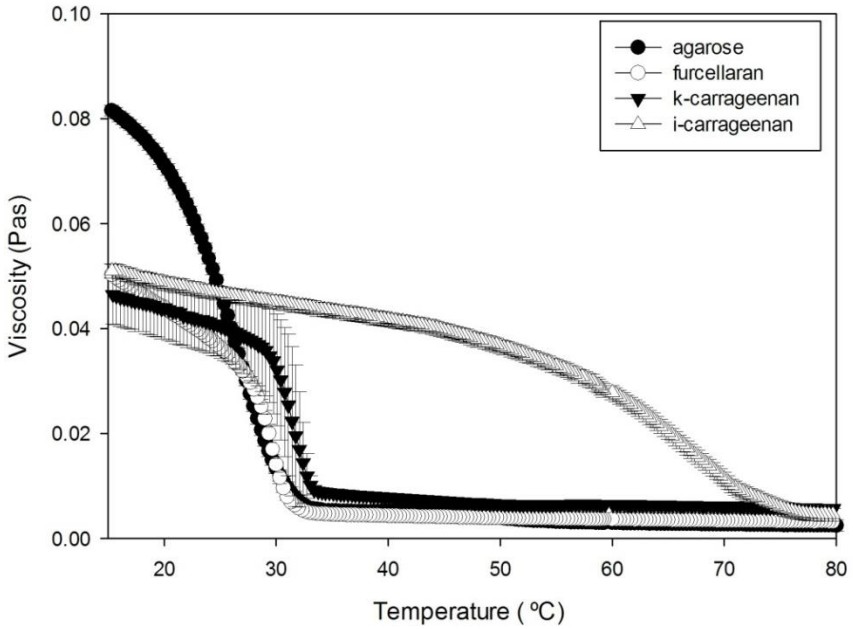

Figure 5.1 Increase in viscosity during red algal polysaccharides fluid gel structure formation as a function of temperature. Structures formed under a shear rate of 400 s^{-1} and a cooling rate of 1 ºC min^{-1}

Table 5-1 Changes in viscosity and gelation temperature during red algal polysaccharides fluid gel structure formation

Sample	Gelation onset temp (ºC)	Viscosity at onset (Pas)	Gelation offset temp (ºC)	Viscosity at offset (Pas)	Viscosity increase (Δŋ)
ι-carrageenan	80	0.005	45	0.040	0.035
κ-carrageenan	34	0.009	28	0.038	0.030
furcellaran	33	0.005	25	0.037	0.033
agarose	33	0.006	15	0.083	0.077

At the applied cooling rate, κ-carrageenan, furcellaran and agarose exhibited gelation onset at around 34 ºC, whereas ι-carrageenan ordering started at a much higher temperature, around 80 ºC. Differences in gelation onset temperature are result of different type of salts added to the pre-gel solutions. Additional investigations revealed that rapid increase in ι-carrageenan viscosity without salt addition and in presence of KCl occurred at around 50 ºC.

The viscosity increase during all charged polysaccharides (ι-carrageenan, κ-carrageenan and furcellaran) gelation was similar, around 0.03 Pas. This suggested that applied shear had similar effect on particles growth, regardless of polysaccharide type. In comparison, applied shear was insufficient to overcome gelation of the neutral agarose to the same extent as in case of charged polysaccharides. As a result the formed structure was more than two times as viscous with an increase in viscosity of around 0.08 Pas. These differences can be explained by the helix aggregation rate, which is related to the number of ester sulfate groups present on the disaccharide units in the polysaccharide chain. Lack of the sulfate groups on the disaccharide unit in agarose; therefore, there are no repulsions between double helices, more interactions between particles and a greater degree of their aggregation is possible compared to charged ι- carrageenan, κ-carrageenan and furcellaran. The optical light microscopy images of the structures were obtained compare the size of the aggregates. However images were insufficient to distinguish between the particles and water which created the red algal polysaccharide fluid gel structures due to the fact that 99.5 % [w/w] of the material was composed of water and therefore transparent. The example of the κ-carrageenan image is presented in **Fig 5.2.**

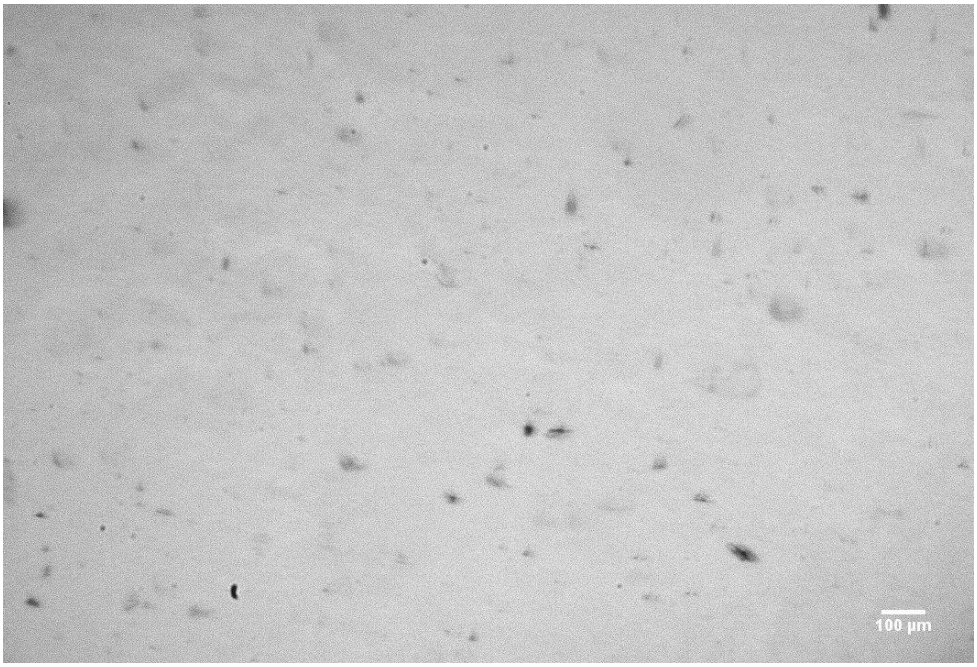

Figure 5.2 The optical light microscopy image of 0.5 % red algal polysaccharide fluid gel structure (κ-carrageenan)

The agarose fluid gel microstructure visibility was enhanced with iodine and is presented in **Fig 5.3.**

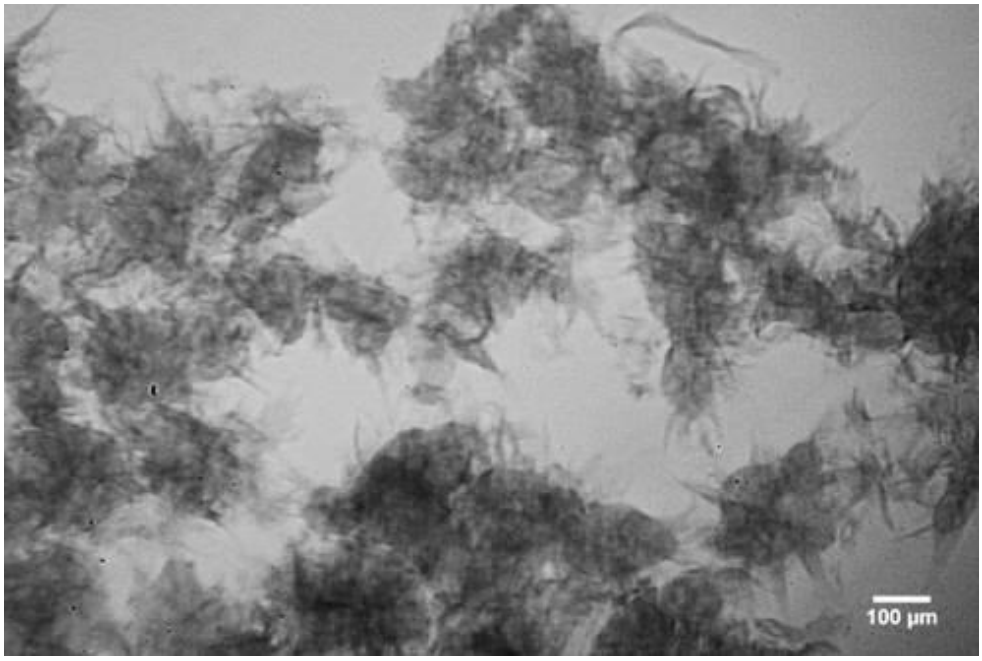

Figure 5.3 Agarose fluid gel microstructure

It was observed that application of shear during gelation of the agarose resulted in high volume of irregular particles with non-smooth surface which enhanced particle-particle interactions and as a result the viscosity of the structure.

Difficulties to distinguish between dispersed and continuous phase and physical structure properties could be compared using viscoelastic properties comparison.

5.5.2. Viscoelastic properties of pure red algal polysaccharide gels and fluid gel structures

Individual red algal polysaccharide gel and fluid gel structures were formed from the same primary solutions containing 0.5 % [w/w] of ι-carrageenan, κ-carrageenan, furcellaran or agarose. Fluid gel structures were formed using a cooling rate of 1 ºC min^{-1} and a shear rate of 400 s^{-1}. Small oscillatory frequency sweeps were used to measure the elastic modulus (G'), viscous modulus (G''), and loss tangent (tan δ) of the materials and investigate the effect of the chemical structure and the processing conditions (fluid gel) on the viscoelastic properties

of the structures. The measurements obtained at 1 Hz are illustrated in **Fig. 5.4** followed by detailed comparison of the viscoelastic properties presented in **Table 5-2.**

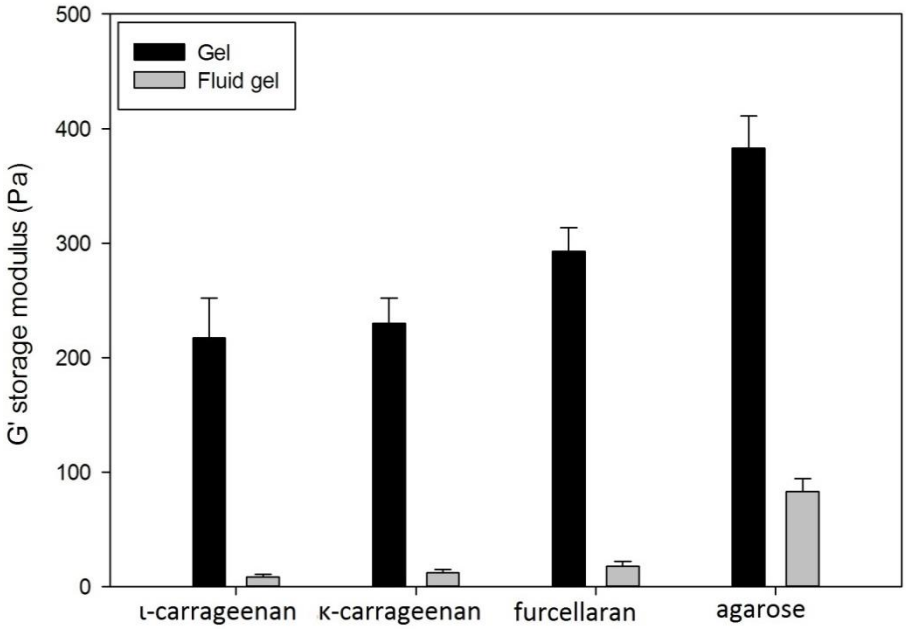

Figure 5.4 Comparison of gel strength (G') between quiescently cooled gel and sheared gel structures formed at the shear rate 400 s^{-1}. Red algal polysaccharides arranged in order of decreasing number of ester sulfate groups.

Table 5-2 Comparison of quiescent gel (G) and fluid gel structure (SG) viscoelastic properties: elastic modulus (G'), viscous modulus (G'') and loss tangent (tan δ)

Structure	**ι-carrageenan**		**κ-carrageenan**		**furcellaran**		**agarose**	
	G	**SG**	G	**SG**	G	**SG**	G	**SG**
G' Elastic modulus (gel elasticity %)	217	**8.4**(3.9%)	230	**12**(5.2%)	293	**18**(6.1%)	383	**83**(21.7%)
G'' Viscous modulus	2.8	**2.4**	22	**1.4**	33	**4**	78	**12**
tan *δ*	0.01	**0.29**	0.10	**0.12**	0.11	**0.22**	0.20	**0.14**

Gelation of quiescently cooled gel structures resulted in a three-dimensional network expanded across the entire liquid volume. The number and position of the ester sulfate groups

on the disaccharide units had a significant effect on the gel viscoelastic properties. The most charged ι-carrageenan gel had the lowest elasticity of 217 Pa whereas the neutral agarose gel structure was almost two times as elastic with a storage modulus of 383 Pa. The gel structure elasticity (*G'*) increased with decreasing polysaccharide charge which is the consequence of increase in network cross-link density.

In comparison, the strength of fluid gel structures decreased dramatically after formation under shear, as a consequence of less ability to store energy compared to quiescently cooled gels. Competition between structure formation forces and shear break up forces leads to the formation of two-phase system with a dispersed phase made of gel particles and a continuous phase formed from the non-gelling medium (the solvent not incorporated into gel particles). Fluid gel structures of charged polysaccharides stored less than 10 % and agarose less than 20 % of the deformation energy compared to the quiescently cooled gel structures, formed from the same primary solutions. Fluid gel structure is composed of particles and non-gelling medium which make it less elastic and less able to store energy compared to quiescently cooled gel structures. The elasticity of the fluid gel structures remained in the same order as those of gel structures with the lowest storage modulus, 8.4 Pa for the most charged ι-carrageenan fluid gel and the highest, 83 Pa for neutral agarose structure. The viscosity of the structures created from charged polysaccharides was comparable indicating similar size of the gelled particles, however their elasticity varied. The findings indicate that single red algal polysaccharide fluid gel structure strength increase with decreasing red algal polysaccharide charge density.

5.5.3. Viscosity and viscoelasticity of 8 % and 12 % [w/w] maltodextrin Paselli SA2 produced under shear

Solutions of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 were produced at the cooling rates of 1 ^{o} C min^{-1} and shear rates of 400 s^{-1}. The increase in viscosity was monitored as a function of temperature to observe the rate of ordering and aggregation of the maltodextrin under shear in the absence of red algal polysaccharide. The results obtained are presented in **Fig 5.5.**

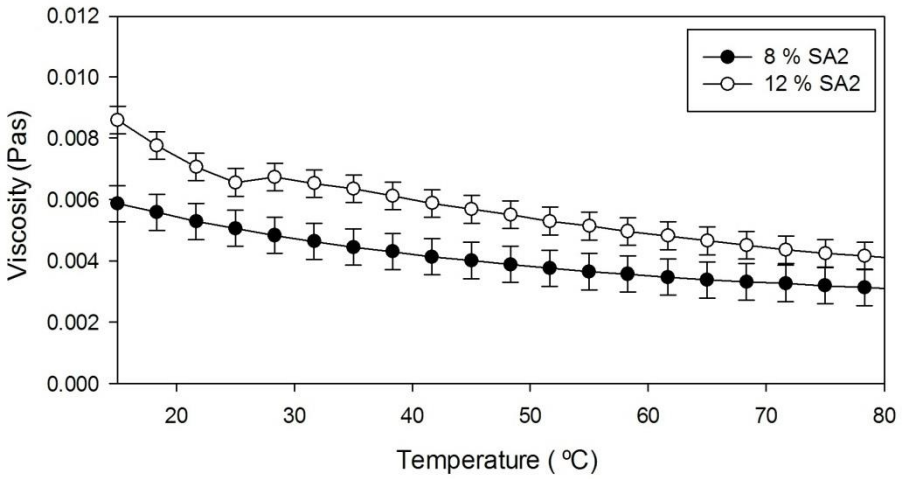

Figure 5.5 Flow curves of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 structure formation at the cooling rate 1 ^{o} C min^{-1} and shear rate 400 s^{-1}

It was observed that the overall increase in viscosity of maltodextrin Paselli SA2 pre-gel solutions was very low and at the end of the experiment, at the temperature of 15 ºC the viscosity of the 8 % [w/w] had only risen to 0.006 Pas, whereas 12 % [w/w] maltodextrin Paselli SA2 viscosity reached 0.010 Pas. The low final viscosities as well as lack of the rapid increase in viscosity during the experiment suggested that gelation did not occur during the experiment and at the end of the process both samples remained in a fluid state. The samples were then stored at a room temperature for a further 48 hours to observe if any further

changes in their physical state occured. Images of the samples immediately post-production and after 48 hours storage in the room temperature are compared in **Fig. 5.6** followed by and optical microscopy images of the microstructures are given in **Fig 5.7.** Comparison of viscoelastic properties of the structures is presented in **Table 5-3**

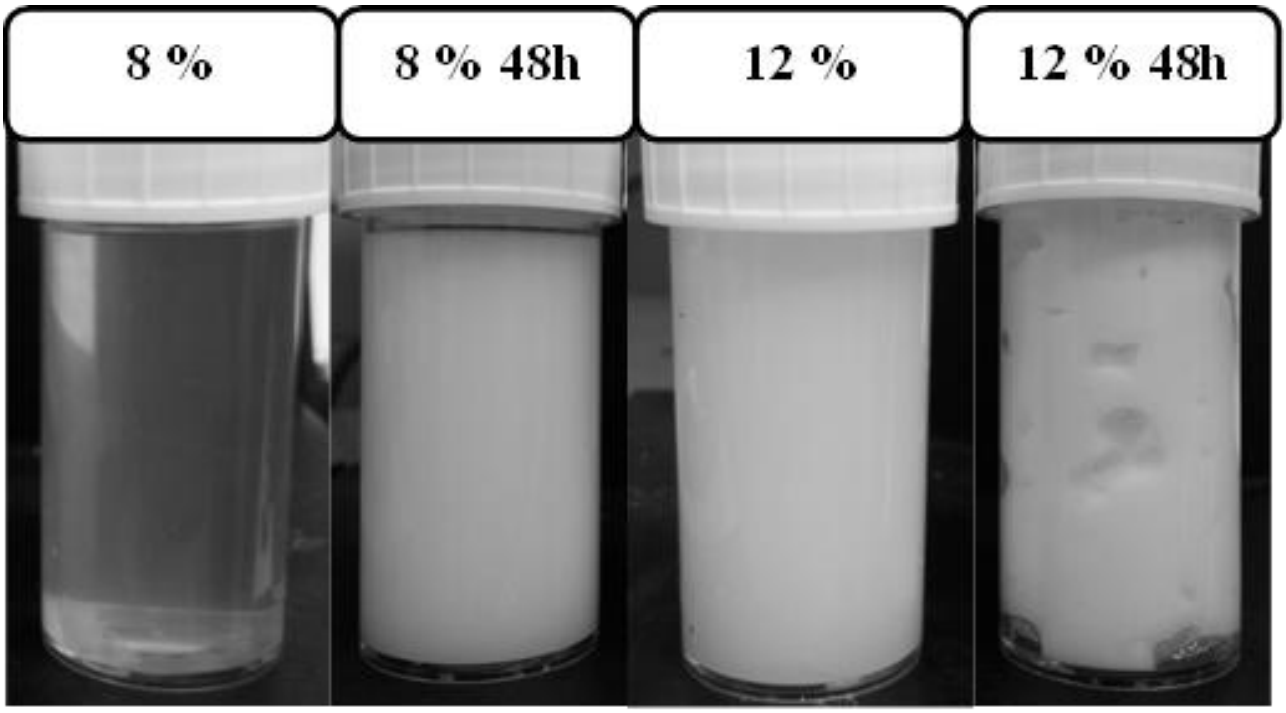

Figure 5.6 Comparison of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 sheared structures directly post production and after 48 hours storage at room temperature.

It was observed that the 8 % [w/w] maltodextrin sample was still a transparent liquid immediately post production. However, after 48 hours storage at room temperature, there was a visible transition from transparent to a white, turbid liquid, indicating aggregation of the maltodextrin molecules. In comparison, the 12 % [w/w] maltodextrin structure was a turbid liquid post production due to the higher concentration and after 48 hours of storage, a transition from turbid liquid to turbid gel could also be observed. To compare 8 % [w/w] and 12 % [w/w] maltodextrin structures in detail, viscoelastic measurements of elastic, viscous

and complex modulus were obtained 48 hours post production and results are presented in **Table 5-3**. Optical microscopy images of the structures are presented in **Fig. 5.7.**

Table 5-3 Comparison of viscoelastic properties of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 structures 48 hours post production

Sample	***G'*** **(Pa)**	***G''*** **(Pa)**	**tan *δ***	**Viscoelastic behaviour**
8 % SA2	0.4	0.5	1.25	'liquid like'
12 % SA2	1610	110	0.07	'solid like'

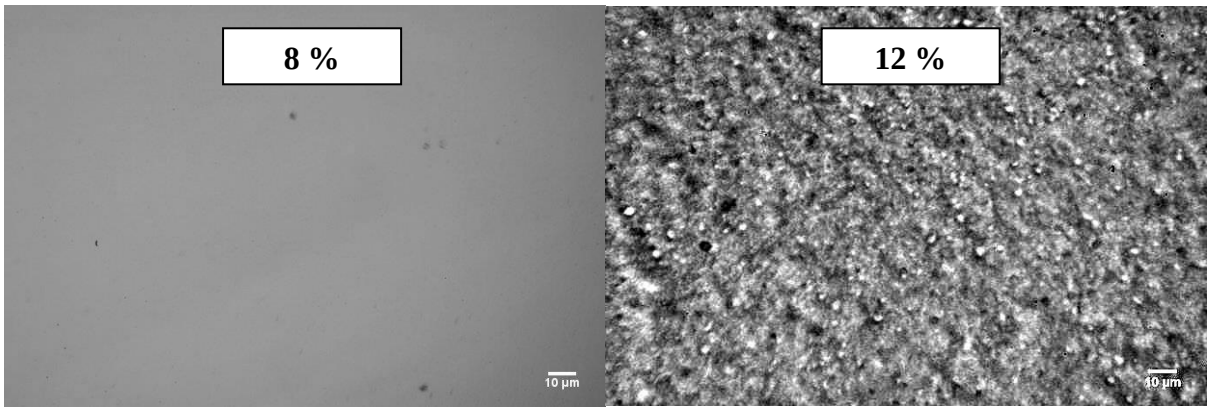

Figure 5.7 Comparison of optical microscopy images of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 structures 48 hours after production under shear

After 48 hours storage, it was observed that the ability of 8 % [w/w] maltodextrin to store energy was very poor. The moduli values close to 0 and loss tangent above 1 indicated that viscoelastic behaviour of the structure remained 'liquid-like'. However, the transition from a transparent to an opaque white liquid suggested that the maltodextrin solution was in a semi-diluted state in which some aggregation occurred, but the distance between aggregates was too great to form a continuous gel network. Optical microscopy images confirmed the small particle size in 8 % [w/w] maltodextrin structure. In comparison, the viscoelastic behaviour of

12 % [w/w] maltodextrin changed significantly during 48 hours post-production. The change from 'liquid-like' to 'solid like' viscoelastic behaviour with storage modulus (*G'*) significantly higher than loss modulus (*G''*) and loss tangent close to 0, suggested that the maltodextrin solution was above critical concentration allowing sol to gel phase transition to occur during storage. The resultant structure had a great ability to store energy reflected by a high storage modulus (*G'*) value of 1610 Pa. Optical microscopy images revealed that the 12 % [w/w] maltodextrin gel had an irregular, particulate structure with particles below 10μm in diameter.

5.5.4. ι-carrageenan/maltodextrin structures formed under shear

The chemical structure of ι-carrageenan is made of repeating disaccharide units with two ester sulphate groups on every disaccharide unit and is the most charged polysaccharide used in this study. As a consequence ι-carrageenan fluid gel elasticity was the lowest amongst all investigated polysaccharide structures, formed under the same conditions. However, the addition of maltodextrin may lead to mixed fluid gel structure formation and impact on the size, elasticity and interactions between ι-carrageenan particles. To investigate this hypothesis, mixtures of 0.5 % [w/w] ι-carrageenan, 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 in presence of calcium chloride were prepared. The process conditions were identical to the one used for single fluid gels, a shear rate of 400 s^{-1} and a cooling rate of 1 ºC min^{-1}. Changes in the viscosity during structure formation were monitored as a function of temperature to observe the effect of maltodextrin on the rate of aggregation and onset gelation temperature of ι-carrageenan under shear. Flow curves obtained are presented in **Fig 5.8.** The flow curve of 0.5 % [w/w] ι-carrageenan without maltodextrin and calcium were added as a comparison. The viscosity and gelation temperatures are summarised in **Table 5-4.**

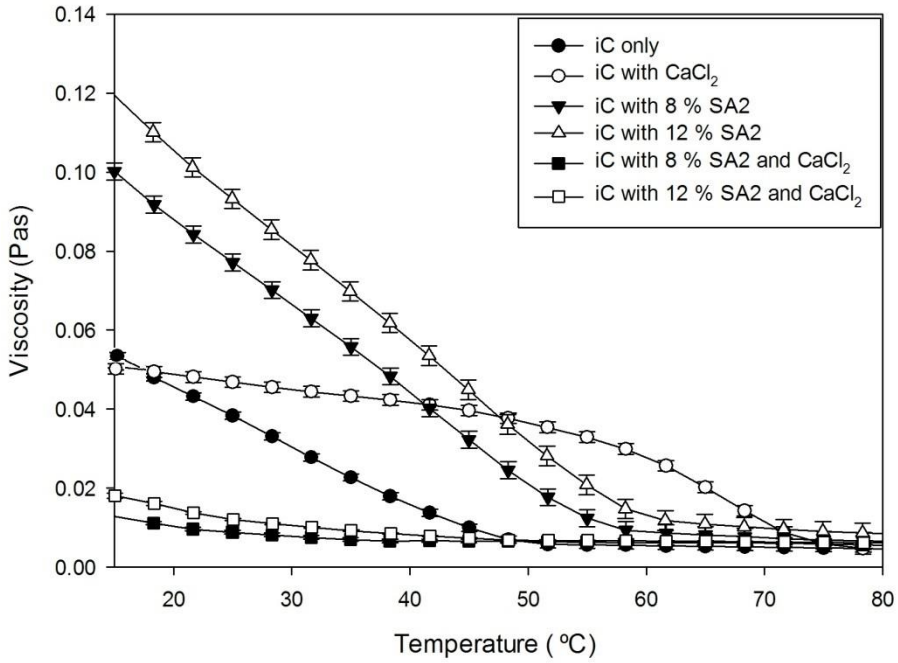

Figure 5.8 Formation of 0.5 % [w/w] ι-carrageenan structure under shear, in the presence of 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 and calcium. Structures were formed at a cooling rate of 1 º C min^{-1} and a shear rate of 400 s^{-1}

Table 5-4 Changes in viscosity and gelation temperature values during ι-carrageenan sheared gel structures formation

Sample	Initial viscosity at 80 ºC (Pas)	Final viscosity at 15 ºC (Pas)	Increase in viscosity (Δŋ)	Onset gelation temperature (ºC)	Increase in T_{gel} (Δ°C)
ιC	0.005	0.054	0.049	50	-
ιC $CaCl_2$	0.004	0.050	0.046	77	27
ιC 8 % SA2	0.007	0.100	0.093	60	10
ιC 12 % SA2	0.008	0.119	0.111	61	11
ιC $CaCl_2$ + 8 % SA2	0.005	0.013	0.008	-	-
ιC $CaCl_2$ + 12 % SA2	0.006	0.018	0.012	-	-

It was observed that ordering of the single 0.5 % [w/w] ι-carrageenan structure started at around 50 ºC and the structure viscosity at the end of the production process had increased to 0.054 Pas. In the presence of calcium, the onset gelation temperature of ι-carrageenan increased significantly to 77 ºC. The initial viscosity dropped slightly due to the decrease in ι-carrageenan charge, which resulted in less extended molecules. However, the final structure viscosity increased and was similar to the single ι-carrageenan structure which indicated that within the given process parameters, calcium had significantly increased the rate of helix ordering and aggregation but the size of the particles was restricted by the applied shear to the same extent as structure without calcium. In comparison, the addition of neutral maltodextrin Paselli SA2 resulted in an increase in gelation onset temperature to around 60 ºC which suggested that maltodextrin promoted helices ordering and aggregation compared to pure ι-carrageenan structure. A significant increase in viscosity of around 0.1 Pas and lack of plateau region indicated interactions between maltodextrin and ι-carrageenan which promoted gelation and formation of structure more resistant to the shear. When both calcium and maltodextrin were added to ι-carragenenan, abrupt viscosity increase which would indicate material gelation was not observed at any temperature during the formation process. Lack of the rapid increase in viscosity suggested that addition of calcium triggered a segregative phase separation and water redistribution occurred resulting in non-gelling maltodextrin forming a continuous phase which prevented ι-carrageenan gelation and formation of mixed fluid gel structure.

The viscoelastic properties of the ι-carrageenan/maltodextrin structures were characterised 48 hours post production to observe changes in material behaviour during storage. The strength of the structures represented by storage (*G'*) modulus is presented in **Fig 5.9.** Individual structures are added as a reference. The viscoelastic properties of the ι-carrageenan structure

individually and as a mixture with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 are summarised in **Table 5-5.**

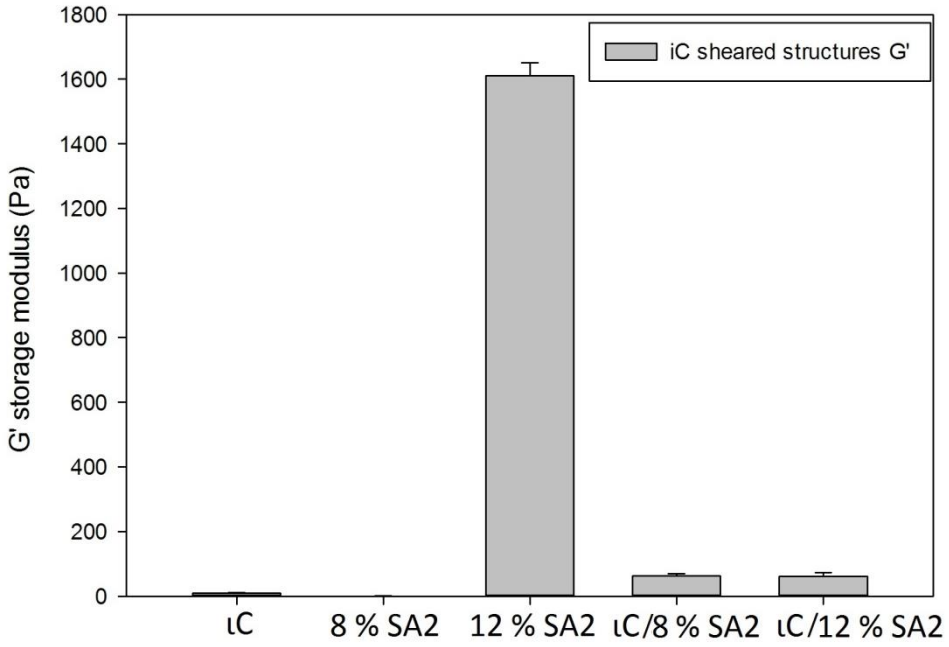

Figure 5.9 The strength of individual and mixed ι-carrageenan/maltodextrin structures represented by the storage (G') modulus. The structures properties were characterised 48 hours post production.

Table 5-5 Viscoelastic properties of 0.5 % [w/w] ι-carrageenan sheared gel structure individually and as a mixture with 8 % [w/w] and 12 % [w/w] maltodextrin Paselii SA2

Structure	**LVR (%)**	**G' (Pa)**	**G'' (Pa)**	**Tan δ**	**Cross point (%)**	**Cohesive Energy (J/m^3)**	**Viscoelastic behaviour**
ιC $CaCl_2$	20	8.4	2.4	0.28	-	1680	solid like
8 % SA2	-	0.4	0.5	1.25	-	-	liquid like
12 % SA2	2	1610	110	0.07	16	3220	solid like
ιC 8 % SA2	79	14	2.8	0.20	-	43687	solid like
ιC 8 % SA2 $CaCl_2$	10	66.4	14.2	0.21	-	3320	solid like
ιC 12 % SA2 $CaCl_2$	2.5	61.4	17.1	0.28	-	192	solid like

After 48 hours storage, it was observed that the strength of the ι-carrageenan structure mixed with 8 % [w/w] maltodextrin and calcium was greater in comparison to the single ι-carrageenan structure, 66.4 Pa, and 8.4 Pa respectively. Lack of the ι-carrageenan gelation during production process suggests that increase in elasticity was due to some maltodextrin aggregation in the continuous phase during storage. Further increasing the maltodextrin content in mixed systems, up to 12 % [w/w] contributed to structure softening and a decrease in strength along with cohesive energy and shift in viscoelastic behaviour towards more liquid-like indicating fewer interactions between components and weaker structure. Optical microscopy images of the ι-carrageenan/maltodextrin structures were obtained to observe the microstructure and distribution of phases after storage. The images illustrating the 0.5 % [w/w] ι-carrageenan /8 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] ι-carrageenan /12 % [w/w] maltodextrin Paselli SA2 are presented in **Fig 5.10.**

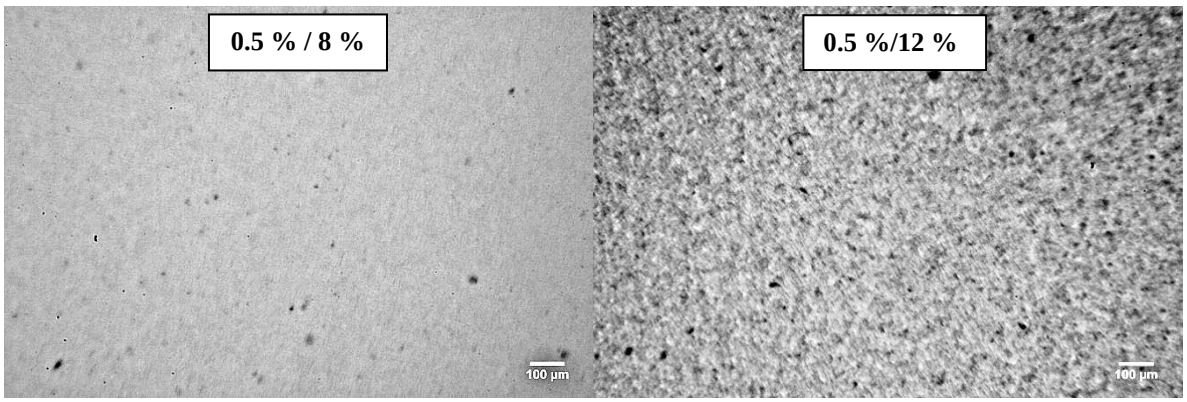

Figure 5.10 Optical microscopy images illustrating structure of 0.5 % [w/w] ι-carrageenan /8 % [w/w] maltodextrin Paselli SA2 (a) 0.5 % [w/w] ι-carrageenan /12 % [w/w] maltodextrin Paselli SA2 sheared gel systems.

White areas represent the ι-carrageenan phase, and the dark areas represent the maltodextrin phase. Mixtures of ι-carrageenan with 8 % [w/w] maltodextrin formed a homogenous structure, where distinguishing between two phases was not possible. Increasing the

maltodextrin concentration to 12 % [w/w] resulted in a phase separated structure with more visible, very finely dispersed microstructure which suggested that phase segregation during storage occurred via nucleation and growth mechanism.

5.5.5. κ-carrageenan/maltodextrin structures formed under shear

κ-carrageenan is made of repeating disaccharide units with one ester sulphate group on every unit. As a result, the gel structure is less charged and able to aggregate to a greater extent than gels made of ι-carrageenan. Hot solutions of 0.5 % [w/w] κ-carrageenan were mixed with solutions containing 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 and potassium chloride. Mixed structures were formed at the shear rate of 400 s^{-1} and a cooling rate of 1 ºC min^{-1}. Viscosity changes were measured as a function of temperature to observe the effect of maltodextrin on the rate of aggregation of κ-carrageenan as well as the onset gelation temperature. Flow curves obtained during the production process are presented in **Fig 5.11**. The flow curve of 0.5 % [w/w] κ -carrageenan without co-solutes was included for comparison. Changes in viscosity and onset gelation temperature observed during the formation of mixed structures are summarised in **Table 5-6**.

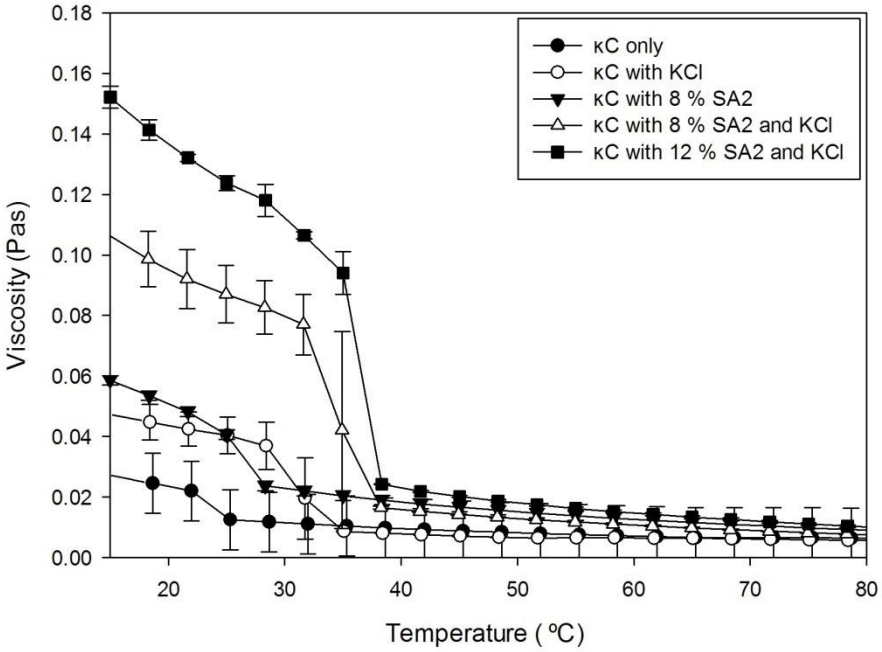

Figure 5.11 Flow curves of 0.5 % [w/w] κ-carrageenan mixed with KCl, 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2. Sheared gel structures were formed at a cooling rate of 1 º C min^{-1} and shear rate of 400 s^{-1}

Table 5-6 Changes in viscosity and gelation temperature values during κ-carrageenan sheared gel structures formation

Sample	**Initial Viscosity at 80 ºC (Pas)**	**Final viscosity at 15 ºC (Pas)**	**Increase in viscosity (Δη)**	**Gelation Onset T_{gel} (ºC)**	**Increase In T_{gel} (ºC)**
κC	0.0089	0.025	0.0161	23	-
κC KCl	0.0066	0.042	0.0354	32	9
κC 8 % SA2	0.016	0.059	0.0430	28	5
κC KCl + 8 % SA2	0.014	0.112	0.0980	37	15
κC KCl + 12 % SA2	0.018	0.149	0.1310	37	15

The addition of 0.1 % [w/w] potassium cations resulted in a significant increase in onset gelation temperature to around 32 ºC and an increase in viscosity during ordering and

aggregation to 0.035 Pas. Addition of 8 % [w/w] maltodextrin in the absence of potassium cations increased the gelation onset temperature to 28 ºC and the growth in viscosity was greater compared to the mixture with addition of potassium chloride only (0.043 Pas). When 0.5 % [w/w] κ-carrageenan was mixed with both potassium salt and 8 % [w/w] maltodextrin, the gelation onset temperature increased further to 37 ºC. The rate of ordering and aggregation also increased resulting in an increase in viscosity of 0.098 Pas. A further increase in the maltodextrin concentration up to 12 % [w/w] facilitated higher increase in viscosity up to 0.131 Pas. The results obtained suggested that maltodextrin contributed to κ-carrageenan helix ordering and aggregation both in the presence and absence of potassium ions. However in the presence of potassium, the increase in viscosity was greater, suggesting the formation of a larger effective aggregate size and/or volume fraction.

The viscoelastic properties of the sheared structures were characterised 48 hours post production to observe the effect of maltodextrin on structure strength and stability. Comparison of structure strength, represented by storage (G') modulus is presented in **Fig 5.12**. The viscoelastic properties of the sheared gel structure of 0.5 % [w/w] κ-carrageenan, individually and as a mixture with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2, are summarised in **Table 5-7.**

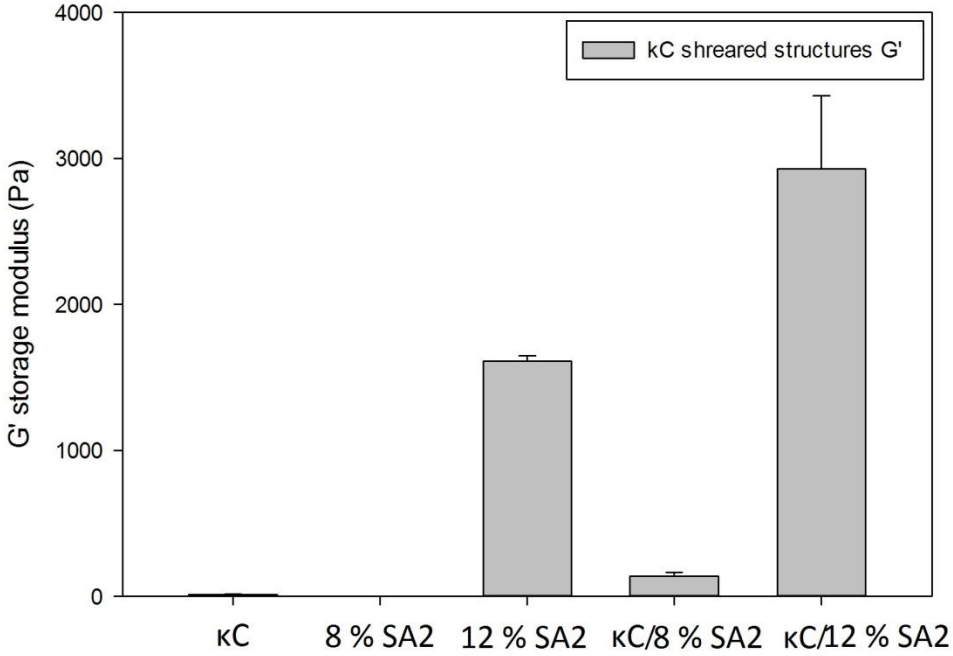

Figure 5.12 Structure strength represented by storage modulus (G') for 0.5 % [w/w] κ-carrageenan only sheared gel and as a mixture with 8 % [w/w] and 12 % [w/w] maltodextrin Paselii SA2

Table 5-7 Viscoelastic properties of a 0.5 % [w/w] κ-carrageenan only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2

Structure	**LVR (%)**	**G' (Pa)**	**G'' (Pa)**	**tan *δ***	**Cross point (%)**	**Cohesive Energy (J/m^3)**	**Viscoelastic behaviour**
κC	0.04	12	1.4	0.12	4	1.5	solid like
8 % SA2	-	0.4	0.5	1.25	-	-	liquid like
12 % SA2	2	1610	110	0.07	16	3220	solid like
κC KCl + 8 % SA2	4	134	24	0.18	20	1072	solid like
κC KCl + 12 % SA2	3	2930	242	0.08	13	13185	solid like

An increase in the 0.5 % [w/w] κ-carrageenan/8 % [w/w] maltodextrin Paselii SA2 structure strength (*G'*) from 12 Pa to 134 Pa was observed compared to single 0.5 % [w/w] κ-carrageenan structure. The elastic modulus is proportional to the square of the biopolymer

concentration. This indicated formation of mixed aggregates with increased κ-carrageenan particles aggregation in presence of maltodextrin. Part of the maltodextrin remained in the continuous medium and further increased its viscosity during storage which also had the effect on increasing the elastic modulus. Despite the increase in elasticity, structure remained as a fluid gel containing κ-carrageenan/maltodextrin aggregates dispersed in a non-gelling medium. A further increase in maltodextrin concentration up to 12 % [w/w] resulted in a significant rise in the storage modulus to 2930 Pa. The high storage modulus value suggested that the effective concentration of the maltodextrin in the continuous medium increased significantly after the majority of the water was incorporated into the mixed fluid gel aggregates and the amount of water in the non-gelling phase was very low. As a result maltodextrin concentration increased and solidification of the continuous phase occurred during storage. It is very likely that the formed structure consisted of κ-carrageenan/maltodextrin aggregates embedded in a solid maltodextrin continuous phase. Optical microscopy images of the sheared gel structures were obtained to examine the microstructure and phase distribution of 0.5 % [w/w] κ-carrageenan /8 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] κ-carrageenan /12 % [w/w] maltodextrin Paselli SA2 structures, 48 hours post production. Images are presented in **Fig 5.13.**

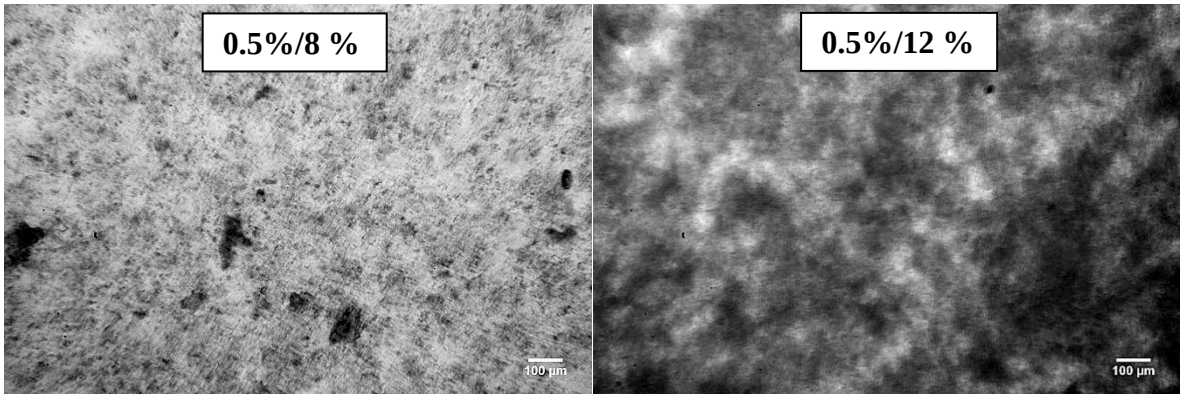

Figure 5.13 Optical microscopy images illustrating the microstructure of 0.5 % [w/w] κ-carrageenan /8 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] κ-carrageenan /12 % [w/w] maltodextrin Paselli SA2 sheared gel systems.

White areas on the image represent κ-carrageenan/maltodextrin aggregates, and dark areas represent maltodextrin not incorporated into the aggregates. Some maltodextrin aggregates can be observed in the continuous phase of the κ-carrageenan structure mixed with 8 % [w/w] maltodextrin. However, the background of the image remained white which confirmed that the continous phase of the structure was made of κ-carrageenan/maltodextrin aggregates and water and dispersed phase was made of maltodextrin aggregates. In comparison, when the maltodextrin addition in the mixture increased to 12 %, the background of the image shifted from white to dark, indicating phase inversion of the maltodextrin to the continuous phase. The images are in agreement with results obtained during the characterisation of viscoelastic properties and they confirm that addition of neutral component can increase structure viscosity and elasticity due to increase in the rate of ordering and aggregation during aggregates formation and increase in solids contents in the continuous phase of the structure.

5.5.6. Furcellaran/maltodextrin structures formed under shear

Furcellaran contains one ester sulphate group on alternate disaccharide units. As a result, it has a similar, but less charged structure than that of κ-carrageenan. This enables furcellaran

helices to aggregate to a greater extent. However, as previously observed in κ-carrageenan mixtures, the addition of maltodextrin can potentially affect furcellaran gelation under shear. To investigate this hypothesis, 0.5 % [w/w] furcellaran was mixed with potassium chloride, 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2. The structures were formed at a shear rate of 400 s^{-1} and at a cooling rate of 1 ºC min^{-1}. The viscosity was monitored during structures formation as a function of temperature to observe the effect of maltodextrin on the rate of aggregation and onset gelation temperature of the furcellaran samples. The results obtained during the production process are presented in **Fig 5.14.** The flow curve of fluid gel formation of the 0.5 % [w/w] furcellaran, without co-solutes, was added for comparison. The changes in viscosity and gelation temperature are summarised in **Table 5-8.**

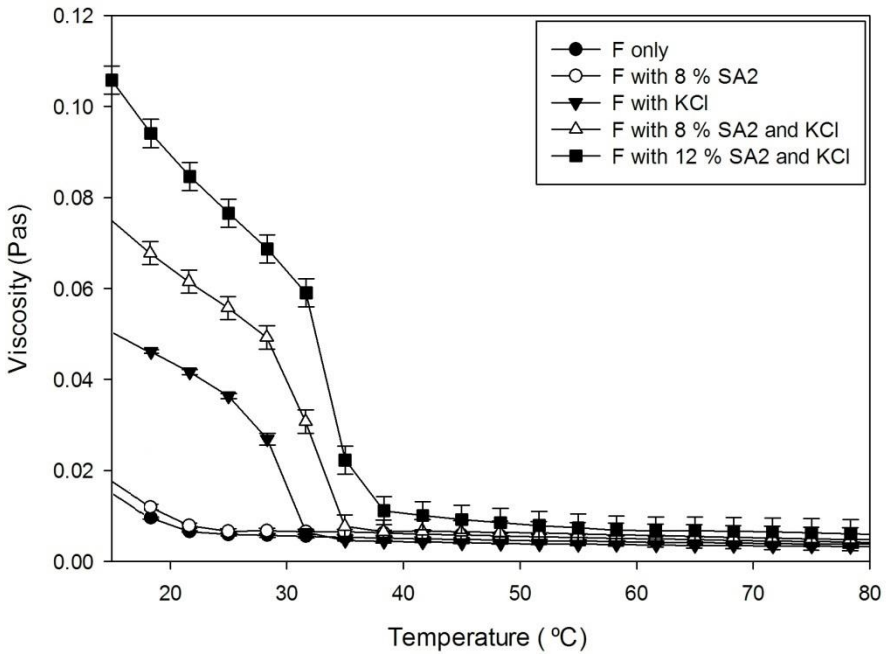

Figure 5.14 Formation of mixed 0.5 % [w/w] furcellaran / 8 % [w/w] maltodextrin Paselli SA2 and 12 % [w/w] maltodextrin Paselli SA2 structure under shear. The structures were formed under the cooling rate of 1 º C min^{-1} and a shear rate of 400 s^{-1}

Table 5-8 Changes in viscosity and gelation temperature during furcellaran sheared gel structure formation

Sample	Initial Viscosity at 80 ºC (Pas)	Final Viscosity at 15 ºC (Pas)	Increase in viscosity (Δŋ)	Onset gelation temperature (ºC)	Increase in T_{gel} (ºC)
Fu	0.004	0.016	0.012	20	-
Fu 8 % SA2	0.006	0.018	0.012	20	-
Fu KCl	0.004	0.050	0.046	32	12
Fu KCl + 8 % SA2	0.006	0.075	0.069	35	15
Fu KCl + 12 % SA2	0.008	0.106	0.098	37.5	17.5

During the formation of the 0.5 % [w/w] furcellaran fluid gel structure without co-solutes as well as structure with the addition of 8 % [w/w] maltodextrin Paselli SA2, no rapid increase in viscosity was observed. The overall increase in viscosity was subtle, only 0.012 Pas. This indicated no transition from sol to gel and therefore no formation of fluid gel aggregates. In the presence of potassium cations, furcellaran structure formation started at around 32 ºC and the increase in viscosity recorded during the ordering and aggregation stage was 0.046 Pas. When furcellaran was mixed with potassium ions and 8 % [w/w] maltodextrin, a further increase in the gelation onset temperature to 35 ºC was observed. The rate of aggregation also improved leading to the increase in viscosity to 0.069 Pas. A further increase in maltodextrin concentration in the mixture to 12 % caused another increase in the gelation onset temperature up to 37.5 ºC and the viscosity of the structure also rose to 0.098 Pas. The results obtained were similar to those for the κ-carrageenan/maltodextrin mixtures; however the recorded values were lower. This suggested analogous structure formation mechanism which involved increase in particle-particle interactions and incorporation of maltodextrin molecules into aggregates during mixed fluid gels structure formation. Addition of maltodextrin resulted in

increase in furcellaran rate of ordering and aggregation and more resistance to the shear. The excluded volume effect between mixture components after addition of maltodextrin also resulted in gelation onset temperature increase indicating less distance between furcellaran molecules.

To observe structure development post production, the viscoelastic properties of sheared gels were characterised after 48 hours. A comparison of the elasticity of individual and mixed furcellaran and maltodextrin structures, represented by elastic modulus (*G'*) is presented in **Fig 5.15.** The viscoelastic properties of a 0.5 % [w/w] furcellaran only sheared gel structure as well as in a mixture with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 are summarised in **Table 5-9.**

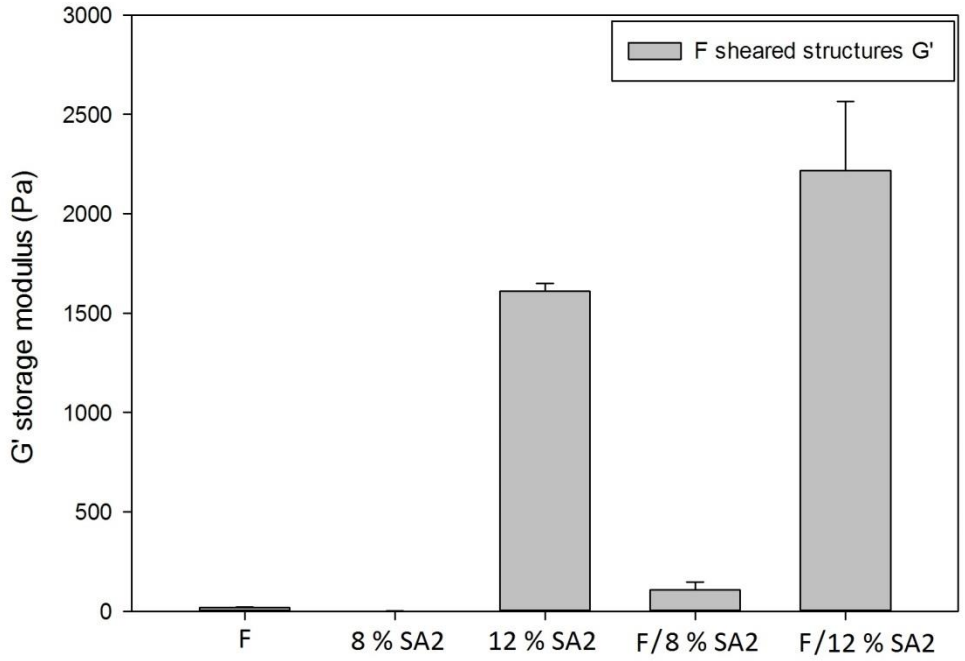

Figure 5.15 The strength of a 0.5 % [w/w] furcellaran only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2, represented by elastic modulus (G')

Table 5-9 Viscoelastic properties of 0.5 % [w/w] furcellaran only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselii SA2

Structure	LVR (%)	G' (Pa)	G'' (Pa)	Tan δ	Cross point (%)	Cohesive Energy (J/m^3)	Viscoelastic behaviour
Fu	1	18	4	0.22	6	9	solid like
8 % SA2	-	0.4	0.5	1.25	-	-	liquid like
12 % SA2	2	1610	110	0.07	16	3220	solid like
Fu KCl + 8 % SA2	4	107	12.4	0.12	45	856	solid like
Fu KCl + 12 % SA2	3	2216	128	0.06	16	4064	solid like

Similarly to κ-carrageenan/maltodextrin structures, when 0.5 % [w/w] furcellaran was mixed with 8 % [w/w] maltodextrin Paselli SA2, the structure strength (G') increased from 18 Pa to 107 Pa. This is mainly a consequence of furcellaran/maltodextrin aggregate formation. Partial aggregation of maltodextrin in the continuous phase, during storage also contributed to the increase in storage modulus, however structure still consisted of non-gelling part. A further increase in maltodextrin concentration up to 12 % resulted in significant increase in the structure strength up to 2216 Pa. The increase in elastic modulus value, which was closer to the 12 % [w/w] maltodextrin only value than to the 0.5 % [w/w] furcellan fluid gel structure indicated the formation of a continuous maltodextrin phase, which solidified during storage. The structure consisted of mixed furcellaran/maltodextrin aggregates trapped in a solid maltodextrin continuous phase. After 48 hours storage the character of the structure changed and due to the absence of a non-gelling continuous phase could no longer be described as a fluid gel structure.

The optical microscopy images of the sheared gel structures of 0.5 % [w/w] furcellaran mixed with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 were obtained to observe microstructure and phase distribution, after 48 hours storage. Images are presented in **Fig 5.16.**

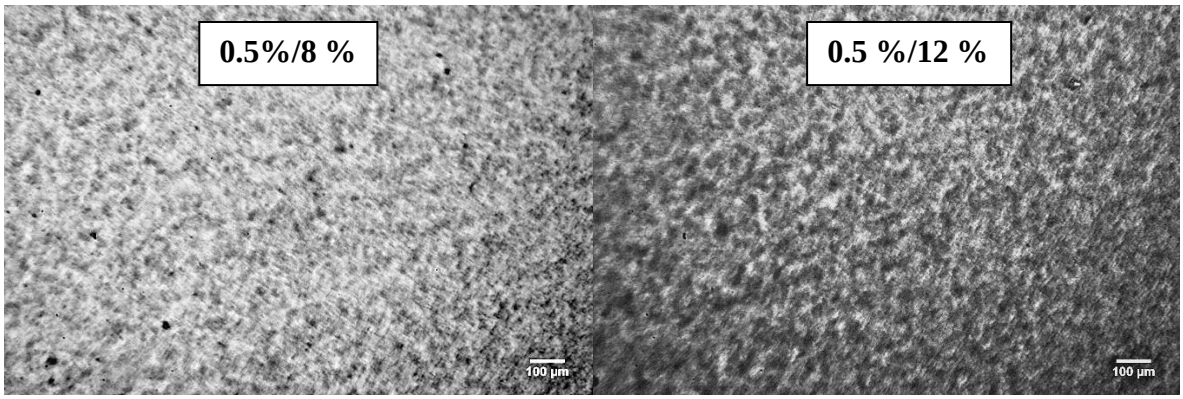

Figure 5.16 Optical microscopy images illustrating structure of 0.5 % [w/w] furcellaran /8 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] furcellaran /12 % [w/w] maltodextrin Paselli SA2 produced under shear, after 48 hours of storage.

White areas on the images are attributed to furcellaran/maltodextrin aggregates and water. Dark areas represent maltodextrin, which remained in continuous phase at the end of the formation process. Both images show structures with evenly distributed phases. In the furcellaran mixture with 8 % [w/w] maltodextrin, some maltodextrin aggregates were formed during storage. However, the white background of the image suggests that the volume of mixed aggregates and water dominated over maltodextrin, and the continuous phase did not solidify. The fluid gel structure contained a dispersed phase formed of κ-carrageenan/maltodextrin aggregates and maltodextrin aggregates suspended in the remaining water. When the maltodextrin concentration in the mixture was increased to 12 % [w/w], a change in the image background from white to dark, was observed. This indicated a continuous phase inversion of the maltodextrin. The high elastic modulus of the structure

indicated that maltodextrin was forming a solid continuous phase during storage. After 48 hours post production, the structure was made of furcellaran/maltodextrin aggregates trapped in a solid maltodextrin continuous phase.

5.5.7. Agarose/maltodextrin structures formed under shear

Agarose was the only uncharged polysaccharide used in this study as a comparison to helix forming charged polysaccharides. The absence of ester sulfate groups on the disaccharide units enables agarose to aggregate to the greatest extent, which was reflected by the highest increase in viscosity during structure formation under shear (0.078 Pas). To investigate the structure formation in the presence of maltodextrin, a solution of 0.5 % [w/w] agarose was mixed with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA. Mixed solutions were then subjected to a shear rate of 400 s^{-1} and a cooling rate of 1 ^{o}C min^{-1}. The viscosity during structure formation was monitored as a function of temperature to observe the effect of maltodextrin on agarose rate of ordering and aggregation and onset gelation temperature.

The flow curves obtained during the production process are presented in **Fig 5.17.** The 0.5 % [w/w] agarose flow curve without any co-solutes was added as a comparison. Changes in viscosity and onset gelation temperature are summarised in **Table 5-10.**

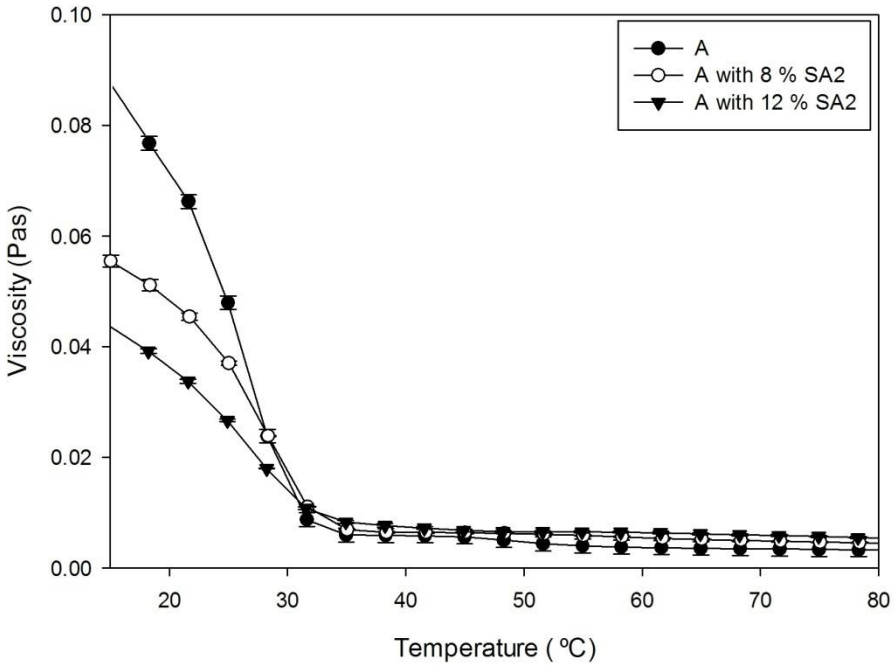

Figure 5.17 The formation of 0.5 % [w/w] agarose structures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2. Sheared gel structures were formed at the cooling rate of 1 ° C min^{-1} and a shear rate of 400 s^{-1}

Table 5-10 Changes in viscosity and gelation temperature values during red agarose sheared gel structures formation

Sample	Initial Viscosity at 80 °C (Pas)	Final Viscosity at 15 °C (Pas)	Increase in viscosity (Δŋ)	Onset gelation temperature (°C)	Increase in T_{gel} (°C)
Ag	0.005	0.083	0.078	32	-
Ag 8 % SA2	0.006	0.055	0.049	32	-
Ag 12 % SA2	0.006	0.043	0.037	32	-

When 0.5 % [w/w] agarose was mixed with 8 % [w/w] maltodextrin the rate of ordering and aggregation decreased dramatically, and viscosity increased only to 0.049 Pas. This indicated that maltodextrin was not incorporated into aggregates and instead caused decrease in agarose aggregate size. Increasing maltodextrin concentration in the mixture to 12 % [w/w] resulted in

a further decrease in the structure viscosity to 0.037 Pas and smaller agarose aggregates. Possible explanation is that maltodextrin presence in the mixture interfered with agarose helix association causing their separation and resulting in decrease in numbers of molecules forming aggregates. No effect of addition of maltodextrin on changes in the gelation onset temperature was observed.

Viscoelastic properties of the agarose/maltodextrin sheared structures were characterised 48 hours post production to observe the development of the structure during storage. Comparison of agarose and maltodextrin structure strength, expressed by storage modulus (*G'*) is presented in **Fig 5.18**. Viscoelastic properties of 0.5 % [w/w] agarose only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2 are summarised in **Table 5- 11.**

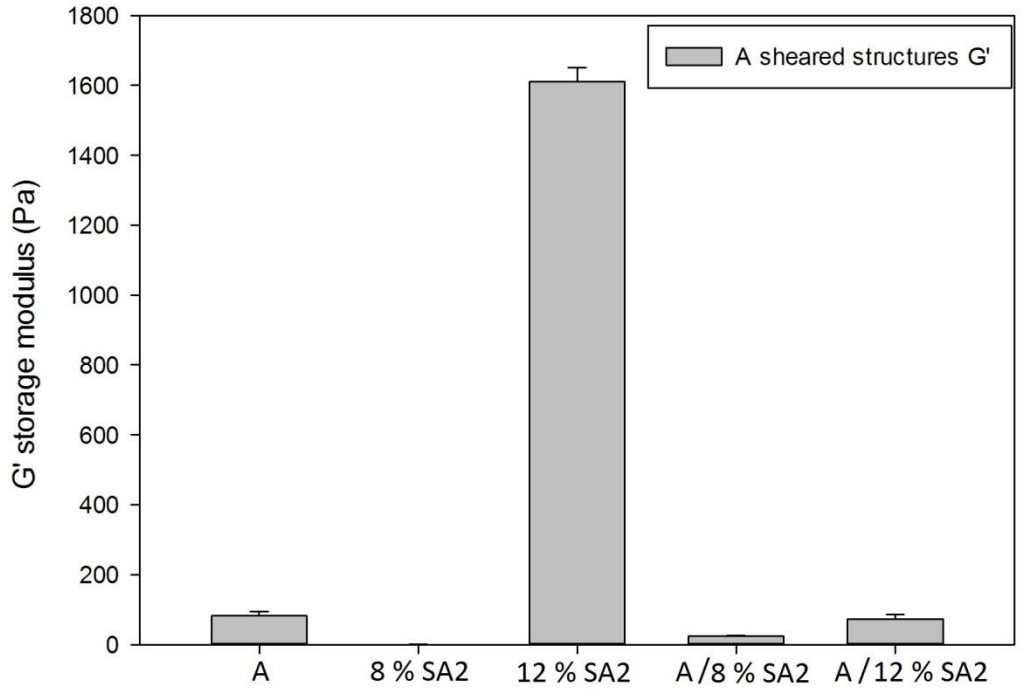

Figure 5.18 Storage moduli (G') of 0.5 % [w/w] agarose only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselli SA2

Table 5-11 Viscoelastic properties of 0.5 % [w/w] agarose only sheared gel structure and mixtures with 8 % [w/w] and 12 % [w/w] maltodextrin Paselii SA2

Structure	LVR (%)	G' (Pa)	G'' (Pa)	Tan δ	Cross point (%)	Cohesive Energy (J/m^3)	Material behaviour
Ag	0.4	83	12	0.14	2.6	6.6	solid like
8 % SA2	-	0.4	0.5	1.25	-	-	liquid like
12 % SA2	2	1610	110	0.07	16	3220	solid like
Ag 8 % SA2	0.7	24	2	0.08	10	6.0	solid like
Ag 12 % SA2	0.9	73	10	0.13	23	30	solid like

Single agarose fluid gel structure elasticity (*G'*) was 83 Pa. The addition of 8 % [w/w] maltodextrin decreased the ability of the structure to store energy almost four fold, to 24.4 Pa. The decrease in elastic modulus was mainly influenced by the formation of smaller agarose aggregates and reduction in the extent of helix aggregation. In the presence of 8 % [w/w] maltodextrin a weaker fluid gel structure was formed. The agarose/maltodextrin structure is very likely to contain agarose aggregates and maltodextrin aggregate suspended in the remaining, non-gelling water. Maltodextrin, which remained in the continuous phase, did not form a solid structure during storage due to insufficient concentration. Addition of 12 % [w/w] maltodextrin to 0.5 % [w/w] agarose resulted in higher structure strength of 74.9 Pa compared to the mixture with 8 % [w/w] maltodextrin. However, structure strength was still lower than the 0.5 % [w/w] agarose only fluid gel, which indicated that increase is more likely to be due to maltodextrin solidification in the continuous medium over the 48 hours.

Optical microscopy images illustrating sheared gel microstructure and phase distribution of 0.5 % [w/w] agarose with 8 % [w/w] maltodextrin Paselli SA2 and 0.5 % [w/w] agarose with 12 % [w/w] maltodextrin Paselli SA2 are presented in **Fig 5.19.**

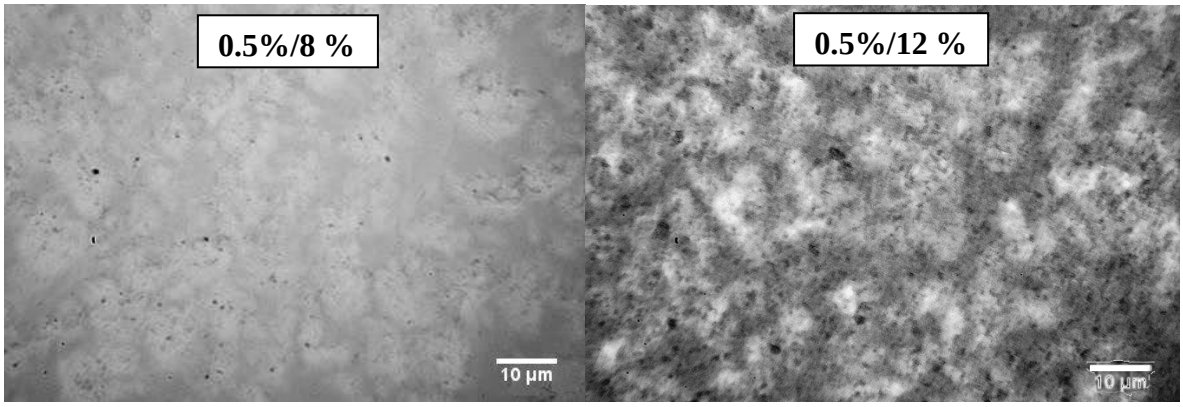

Figure 5.19 Optical microscopy images illustrating microstructure and phase distribution of 0.5 % [w/w] agarose /8 % [w/w] maltodextrin Paselli SA2 0.5 % [w/w] agarose /12 % [w/w] maltodextrin Paselli SA2 sheared gel systems after 48 hours of storage.

White areas on the image represent agarose/maltodextrin aggregates and water. Dark areas represent maltodextrin. It was observed that 0.5 % [w/w] agarose with maltodextrin Paselli SA2 formed phase-separated structures. Addition of 12 % [w/w] maltodextrin resulted in a decrease in the size of the agarose aggregates and increase in maltodextrin concentration in the continuous phase, indicated as darker colour of the background.

Discussion and conclusions

The summary of increases in viscosity during red algal polysaccharide/maltodextrin sheared gel structure formation is summarised in **Table 5-12.**

Table 5-12 Summary of increases in viscosity obtained during formation of mixed red algal polysaccharides/maltodextrin structures under shear

Sample	Increase in viscosity (Pas)
8 % SA2	0.006
12 % SA2	0.010
ι-carrageenan	0.048
$CaCl_2$	0.047
8 % SA2	0.093
8 % SA2+ CaCl	0.007
12 % SA2	0.111
12 % SA2+ CaCl	0.011
κ-carrageenan	0.016
KCl	0.035
8 % SA2	0.043
8 % SA2+ KCl	0.098
12 % SA2 + KCl	0.131
furcellaran	0.012
KCl	0.046
8 % SA2	0.012
8 % SA2 + KCl	0.068
12 % SA2+ KCl	0.098
agarose	0.078
8 % SA2	0.048
12 % SA2	0.037

An insignificant increase in viscosity during 8 % [w/w] and 12 % [w/w] maltodextrin structure formation indicated that no gelation occurred, and structures remained in a liquid state at the end of the fluid gel formation process. However, the presence of maltodextrin in the mixture with different charge density red algal polysaccharides was shown to have an effect on structure formation under shear. The observed effect was strongly associated with polysaccharide charge and addition of helix aggregation promoting ions

- In the mixture with the charged ι-carrageenan which contains two ester sulphate groups, no gelation occurred in the presence of maltodextrin when helix aggregation promoting calcium cations were added which suggests negative synergism between

components. The increase in helix aggregation rate was observed in the absence of calcium cations as components remained in one phase.

- In the mixture with κ-carrageenan which contains one ester sulphate group, the highest helix aggregation rate was observed in the presence of maltodextrin and potassium cation which is believed to be result of maltodextrin incorporation into fluid gel aggregates during structure formation. Increase in viscosity was also seen in the absence of potassium ions but not to the same extent as in the presence of KCl.
- In the mixture with furcellaran, containing one ester sulphate group per two disaccharide units, similarly to κ-carrageenan mixture, highest helix aggregation rate was observed in the presence of maltodextrin and potassium cations. No increase in viscosity was seen in the presence of maltodextrin and an absence of potassium ions.
- In the mixture with neutral agarose, the highest helix aggregation rate was observed in the absence of maltodextrin. The addition of maltodextrin decreased the aggregation rate.

At the end of the single component fluid gel formation, all of the red algal polysaccharide structures examined contained gel particles suspended in the non-gelling medium. When maltodextrin was added to the system, the solvent quality changed.

The investigation suggested that mixed fluid gel structure formation is affected greatly by red algal polysaccharide charge density, specific cation addition and neutral co-solute concentration (maltodextrin). All of those factors affected component compatibility, and the resultant structure was formed as the effect of it. The mixed fluid gel structure is formed if red algal polysaccharide gelation is not inhibited by non-gelling co-solute which forms continuous phase of the pre-gel solution due to phase separation prior to gelation. Rapid increase in viscosity during red algal polysaccharide gelation under shear results in gel

aggregates formation. Higher ordering and aggregation rate of mixtures compare to single fluid gel system suggest mixed aggregates formation as an effect of incorporation of maltodextrin into the aggregate structure. The increase in the rate of ordering and aggregation is also possible due to components incompatibility and excluded volume effect which would lead to localised increase in polysaccharide concentration and increase in particle-particle interactions promoting nuclei growth.

Resultant structures contained red algal polysaccharide/maltodextrin aggregates and maltodextrin dispersed in the non-gelling medium. However, the slow ordering of maltodextrin continued in the structure continuous medium during storage. Requiring the investigation of viscoelastic properties of the structures 48 hours post production. A summary of structure strength represented by storage modulus (*G'*) is presented in **Fig 5.20.**

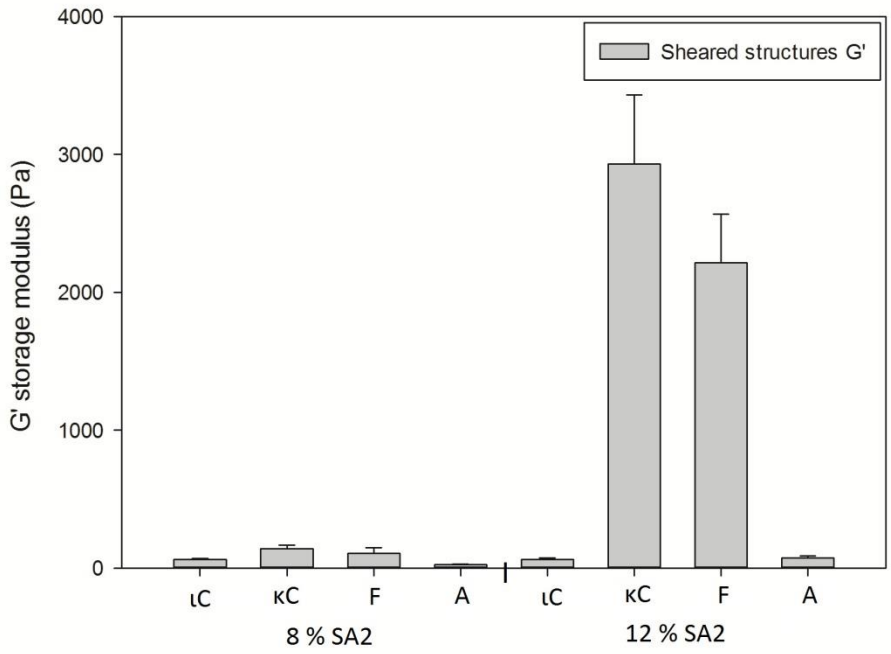

Figure 5.20 Summary of red algal polysaccharides/maltodextrin sheared structures strength represented by storage modulus (G')

After 48 hours post production, all sheared structures remained as fluid gels apart from the ι-carragenan mixtures where no gelation occurred and κ-carragenan and furcellaran mixtures with 12 % [w/w] maltodextrin where maltodextrin gelled during storage and formed a continuous phase which entrapped the aggregates. The gelled maltodextrin continuous phase formation significantly increased structure strength resulting in the strong, solid-like behaviour.

Summary of viscoelastic properties of mixed fluid gel structures is presented in **Table 5-13**

Table 5-13 Summary of mixed fluid gel structures viscoelastic properties

Structure	**LVR (%)**	**G' (Pa)**	**G'' (Pa)**	**Tan δ**	**Cross point (%)**	**Cohesive Energy (J/m^3)**
κC 8% SA2	4	134	24	0.18	20	1072
Fu 8 % SA2	4	107	12.4	0.12	45	856
Ag 8 % SA2	0.7	24	2	0.08	10	6.0
Ag 12 % SA2	0.9	73	10	0.13	23	30

In conclusion addition of maltodextrin resulted in:

- κ-carrageenan and furcellaran – a significant increase in storage modulus (*G'*), an increase in resistance to strain deformation and cohesive energy which suggest a stronger, more stable structure formation due to increased aggregation during formation and maltodextrin aggregation in the non-gelling phase during storage.
- agarose – decrease in storage modulus (*G'*), increase in resistance to strain deformation and increase in cohesive energy for mixture with 12 % which suggest that

maltodextrin interrupts helix ordering and aggregation during formation under shear, but structure stability increases during storage when maltodextrin aggregation occurs.

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Chapter 6. CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1. Conclusions

The aim of this thesis was to gain a better understanding of the formation of mixed fluid gel structures and correlations between formulation, process conditions, structure and rheological properties.

In chapter 3, κ-carrageenan mixtures with pregelatinized cross-linked waxy maize starch were investigated. The structure obtained during formation under shear is very likely to consist of dispersed phase: irregular aggregates of κ-carrageenan particles and starch granules and single starch granules. Continuous phase was made of not structured hydrocolloid and/or water. The investigation has shown that:

- κ-carrageenan/starch mixed fluid gels structures could be produced on a Kinexus Pro rotational rheometer using formulations with components concentration of: < 2 % [w/w] κ-carrageenan, < 4 % [w/w] pregelatinized cross-linked waxy maize starch, < 3 % [w/w] potassium chloride.
- The increase in viscosity (aggregation rate) during formation of κ-carrageenan/starch mixed fluid gel structure depends strongly on the formation process conditions, particularly shear rate and components concentration, particularly κ-carrageenan.
- A linear relationship between increase in viscosity as well as gelation onset temperature and increase in κ-carrageenan concentration in the presence of 2 % CLWM-starch was observed as a result of more interaction between κ-carrageenan molecules as well as κ- carrageenan and starch molecules.
- The addition of the starch between 0.5- 4 % [w/w] to the κ-carrageenan showed a linear relationship between the increase in starch concentration and increase in final structure viscosity, however, the effect was not significant in comparison to increase of the κ- carrageenan concentration in the mixture. This is an effect of a difference in

molecules conformation. κ-carrageenan as a linear, charged polysaccharide has more exteded molecular structure and occupies more volume compared to starch.

- The increase in shear rate applied during formation process resulted in a decrease in the final structures viscosity indicating shear thinning behaviour (structure breakdown in a shear field due to hydrodynamic forces). Resistance to flow investigations under various shear rates showed similar shear thinning behaviour between single and mixed with starch fluid gel structures.
- The increase in cooling rate applied during formation process resulted in an increase in final structure viscosity which was directly related to the presence of starch granules in the mixture as single κ-carrageenan fluid gel structures exhibited opposite behaviour. This behaviour could be a result of rapid κ-carrageenan gelation at high cooling rates, leading to immobilisation of greater number of starch granules in the aggregates. When the cooling rate was decreased, a reduction in final viscosity was recorded, as phase separation can occur faster than the gelation can take place.
- The presence of as little as 2 % pregelatinized CLWM-starch resulted in a significant 26 fold increase in the final viscosity compared to 0.5 % κ-carrageenan only fluid gels, made under the same conditions. This indicated that phase separated system triggered by excluded volume effect was formed and as a result of the rise in effective concentrations of the κ-carrageenan in the mixture. This enabled the formation of the bigger aggregates which has more significant effect on viscosity compared to single κ-carrageenan fluid gel. However, aggregates were too big to provide required fat-like mouthfeel.

In chapter 4, κ-carrageenan mixtures with maltodextrin Paselli SA2 were investigated. This mixture was considered as a blend of gelling polysaccharide (κ-carrageenan) with intermediate molecular weight polysaccharide (maltodextrin) exhibiting different gelation

kinetics. κ- carrageenan gel formation was rapid and started around 30 ºC. Maltodextrin gelation was slow and started below 20 ºC. As mixed aggregates were already formed, maltodextrin gelation contributed mainly to increase in continuous medium viscosity. The structure obtained during formation under shear is very likely to consist of dispersed phase: irregular aggregates of κ- carrageenan/maltodextrin and free maltodextrin chains, continuous phase: remained, not structured water.

The research results in this chapter have shown that:

- κ-carrageenan helix ordering and aggregation during structure formation under shear can be strongly influenced by the addition of maltodextrin Paselli SA2. Maltodextrin concentrations higher than 8 % [w/w] lead to segregative interactions between components. As an effect κ-carrageenan effective concentration increased and resulted in an increase in onset gelation and melting temperatures as well as greater viscosity increase during aggregates formation. However due to the lower maltodextrin molecular weight, increase in aggregates size was not as significant as in the case of κ-carrageenan/starch structures. The addition of 20 % of maltodextrin resulted in the 5 times more viscous structure than κ-carrageenan single fluid gel system.
- The type of the formed structure strongly depended on the amount of maltodextrin which remained in the continuous phase after fluid gel structure formation. The addition of more than 12 % of maltodextrin to the formulation resulted in continuous phase solidification as a result of enough maltodextrin molecules for gelation to occur during storage. Therefore, κ-carrageenan/maltodextrin mixed fluid gel structures formation was possible up to 12 % of maltodextrin as non-gelling continuous medium is required for this structure.

- Confocal laser scanning microscopy images of mixed fluid gels revealed that thermodynamic incompatibility between components increased with the increase in maltodextrin concentration in the system.
- Differential scanning calorimetry results also showed that structure thermal stability increased with 8 % of maltodextrin and above. At 15 % [w/w] maltodextrin phase inversion to continuous maltodextrin system occurred.
- Investigation of the process conditions revealed that an increase in the shear rate applied during the formation process decreased the final structure viscosity owing to reduced size of the aggregates. However, higher flow index indicates the more shear thinning behaviour of the mixed system. At the shear rates above 100 s^{-1} formed aggregates were smaller in comparison to κ-carrageenan single fluid gel.
- During the investigation of the process conditions significant importance of processing time which was related to the applied cooling rate was also observed. The size of the formed κ-carrageenan /maltodextrin aggregates increased with a decrease in cooling rate (prolonged formation time), owing to maltodextrin slower ordering rate in the continuous phase.

In chapter 5, ι-carrageenan, κ-carrageenan, furcellaran and agarose mixtures with maltodextrin Paselli SA2 were investigated. This mixture was considered as a blend of different charge density gelling polysaccharides derived from red algae with maltodextrin which exhibits different gelation kinetics. The mixed fluid gel structure is very likely to consist of dispersed phase: irregular aggregates red algal polysaccharide/maltodextrin and continuous phase of remained free maltodextrin and polysaccharide chains and/or a water.

The aggregates size is dictated by the dynamic equilibrium between two competing processes: small gel nuclei grow, controlled by the cooling rate and the coalescence and

break-up processes, mainly controlled by the shear field applied. The addition of maltodextrin in the mixture introduces an additional level of complexity due to components compatibility. The competition between macromolecules for space had an effect of dividing of the water between coexisting phases. The polyelectrolyte charge and addition of maltodextrin had a significant effect on the resultant structure which caused a change in the mixture position on the phase diagram illustrated in **Fig 6.1.**

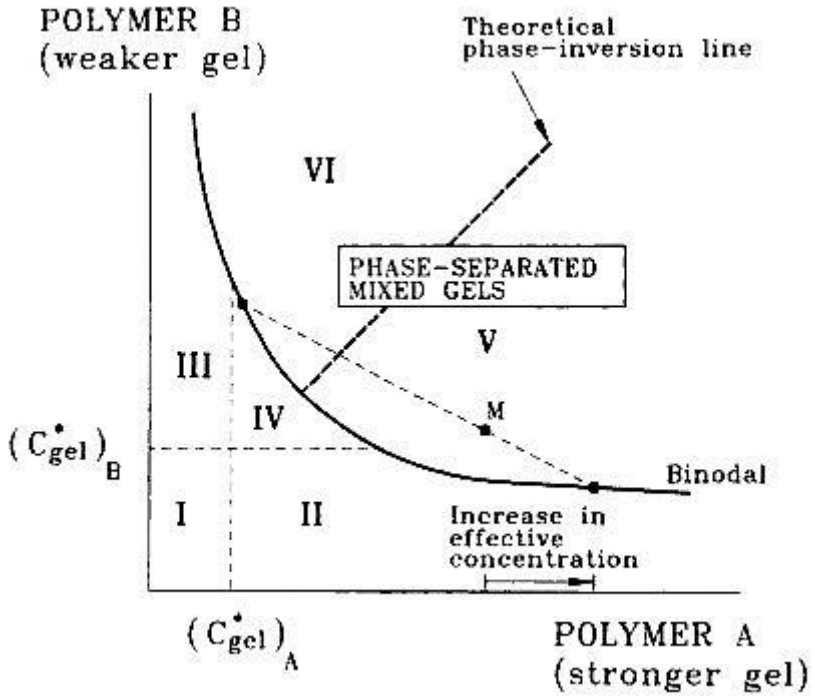

Figure 6.1 Phase diagram for mixed gels of strong and weak polymer (Image adapted from Tolstoguzov 1995)

Polymer A is represented by 0.5 % of red algal polysaccharides; polymer B is represented by 8 and 12 % maltodextrin Paselli SA2.

- Mixing of ι-carrageenan, containing two ester sulphate groups with maltodextrin and calcium resulted in no gelation. Redistribution of the water caused negative synergism and dilution of ι-carrageenan and formation of mixed solution (zone I) with both components below a critical concentration. ι-carrageenan highest helix aggregation

rate was observed in the absence of calcium cations. This is believed to be a consequence of the macromolecules forces to form one phase in order to render electroneutral character of the system. During storage, some maltodextrin aggregation occurred. However, the resultant structure was weaker and less stable compared to single ι- carrageenan fluid gel as indicated by an increase in loss modulus (*G''*), a decrease in resistance to strain deformation and decrease in cohesive energy. No mixed fluid gel structure formation was observed.

- Mixing of κ-carrageenan containing one ester sulphate group with maltodextrin, in the presence of potassium cations, resulted in an increase in helix aggregation rate. The increase was also observed in the absence of potassium ions but not to the same extent as in the presence of KCl. Synergistic effects are believed to be the effect of components incompatibility which resulted in more concentrated κ-carrageenan which formed the continuous phase of the mixture (zone V). Significant increase in storage modulus (*G'*) increases in resistance to strain deformation and cohesive energy which suggest stronger, more stable structure formation. Mixed fluid gel structure was formed only with 8 % of maltodextrin. The continuous medium of the mixture with 12 % maltodextrin solidified during storage, changing the type of the structure.
- Behaviour of the mixtures with furcellaran, containing one ester sulphate group on alternate disaccharide unit were similar to those of κ-carrageenan/maltodextrin and. The highest helix aggregation rate was observed in mixture with maltodextrin and in the presence of potassium cations. However, the increase was lower compared to mixtures with more charged κ-carrageenan. No gelation occurred in the absence of potassium ions. Similarly to κ-carrageenan, a significant increase in storage modulus (*G'*), increases in resistance to strain deformation and cohesive energy which suggest

stronger, more stable structure formation. Mixed fluid gel structure was formed with the addition of 8 % maltodextrin.

- The highest helix aggregation rate for neural agarose was observed in the absence of maltodextrin. In agarose/maltodextrin mixture, maltodextrin formed continuous phase (zone VI). This resulted in a decrease in aggregates size and as a consequence decreased storage modulus (*G'*). However, an increase in resistance to strain deformation and cohesive energy for mixture with 12 % of maltodextrin suggested some aggregation during storage. Mixed fluid gel structures were formed with 8 and 12 % of maltodextrin, but aggregates size decreased with increase in maltodextrin.
- Obtained results suggested that formation of mixed fluid gel structure from red algal polysaccharides is affected not only by specific cations addition but by neutral component concentration, such as maltodextrin which can influence the size of the formed aggregates. The aggregates size is resultant of components compatibility.

Conclusions summary:

- Mixed fluid gel structure will be formed if at least one of the polysaccharide in the mixture undergoes gelation during the formation process. It is also important that the ordering and aggregation of the mixture components occur at the same timescale as process in order to create fluid gel structure.
- Mixed fluid gel aggregates formation depends mainly on the polysaccharide which gelation occurs first.
- The polysaccharide which gelation does not occur during the production process may affects fluid gel aggregates size and viscosity of the continuous medium. The effect depends on the concentration of non-gelling polysaccharide and compatibility with the primary gelling polysaccharide in the mixture prior to gelation.

- Compatibility of the pre-gel mixture with specific cations and its concentration needs to be precisely established, as inhibition of gelation can occur if non-gelling polysaccharide forms continuous phase of the pre-gel solution, as it took place in mixture with ι-carrageenan.
- Red algal polysaccharide charge density is of great importance in mixed fluid gel structure design. The most significant increase in aggregates size is observed in mixture containing polysaccharide with one ester sulfate group on disaccharide unit. If red algal polysaccharide gelation is observed, aggregates size decrease with a decrease in red algal polysaccharide charge density. It is possible that smaller molecules of maltodextrin interact with red algal polysaccharide molecules during ordering and aggregation and stabilize helix by filling the gaps between helix and increase crosslink density of the aggregates in charged polysaccharides. The extent of aggregation of neutral agarose is decreased because lack of repulsion enables helix to greater aggregation without gaps to be filled by maltodextrin. Therefore, maltodextrin presence in between agarose helix causes their separation.
- Red algal polysaccharides fluid gel structure formation, in the presence of maltodextrin Paselli SA2 results in wide range of sheared gel structures with various viscoelastic properties. Mixtures composition and as a result rheological properties can be altered by changing processing conditions and/or formulation to match desired food application.
- Investigated mixed fluid gel structures were formed as a result of weak physical interactions (entrapment of additional component) rather than chemical interactions.

6.2. Future recommendations

This section aims to highlight areas of potential further research, based on the conclusions developed from this study.

1. **Expanding the understanding of structure – texture relationship in mixed fluid gel structure.** Rheological characterisation of the fluid gel structures is important in the design of production process, shelf stability and sensory perceptions such as texture and mouthfeel. Texture and mouthfeel are crucial in food product acceptability. Therefore, more extensive studies of structure – texture relationship by additional characterisation using texture profile analyses (TPA) and tribological analysis could be performed.
    - Mechanical parameters characterisation - texture profile analyses enable to quantify multiple textural parameters such as hardness, cohesiveness, adhesiveness, brittleness, chewiness, gumminess. Studies of structures separately and as a mixture provide information on the effect of the additional component on textural parameters and how to manipulate the ingredients proportions to achieve desired mechanical parameters of the structure.
    - Oral processing characterisation – tribology analyses enable to understand how the structure breaks down during oral processing. Mastification can also be simulated on Kinexus rheometers. It is possible that structures with the same rheological behaviour, response differently to oral processing and have different tribological behaviour. Characterisation of single fluid gel structures and their mixtures enable understanding of the impact of the additional component on the structure break down during oral processing.
2. **Detailed phase diagram construction and determination of the structures volume fraction**

The phase diagrams of the red algal polysaccharide/maltodextrin mixtures could be constructed to observe phase behaviour and volume distribution after mixing, over a range of component concentrations and investigate the boundaries between one phase system and phase separated systems formed quiescently and under shear. Various ranges of specific cations (K, Ca) could be examined to observe at which concentration phase separation is triggered.

3. **Determination of the amount of starch and maltodextrin incorporated into the aggregates and remained in the continuous phase and their contribution to the structure strength.**

   Centrifugation or other method of separation of the aggregates from continuous medium could provide information about the phase volume ratio. The examination of aggregates and continuous phase separately could provide information about the contribution of the phases to the structure strength as well as enable direct comparison of aggregates formed using various process conditions and/or formulations. The investigation of the amount of starch and maltodextrin in the continuous medium would be crucial to determine how much maltodextrin was incorporated into the mixed aggregates and is the higher ordering and aggregation rate result of maltodextrin-red algal polysaccharide interactions and mixed aggregates formation or increase in red algal particles interactions as an effect of components incompatibility and localised increase in concentration, resulting in enhanced particle growth.

4. **Visualization of the changes in the microstructure during processing - rheo-microscopy**

   The production of the fluid gel structures on the rheo-microscope would enable detailed observations and imaging of the structure formation in real time and at any point of the production process. This would be of considerable importance, especially

prior to gelation to observe if phase separation in the pre-gel mixture occurs and during aggregates formation to observe their assembly. Aggregates assembly visibility could be enhanced by using the fluorescent module or any other method which could help to distinguish between both components and enable to observe the aggregates and confirm mixed of single structure.

5. **More complex structure formation - addition of another component into the mixture**

   Food products are complex structures, composed of not only polysaccharides but also structural elements such as proteins or fats. Incorporation of the additional ingredient into the structure and making it more complex could provide information on the effect of different type of the component on the mixed fluid gel aggregates formation.

6. **Characterization of aggregates size and distribution during storage**

   Structures stability during storage could also be examined. Measurements of storage (G') and loss (G'') moduli over time, as well as the ratio between them (Tan δ), can provide information of structure viscoelastic behaviour. Changes towards more solid-like (aggregates size increase as a result of coalescence) or more liquid-like (aggregates size decrease as a result of disintegration) or no change (constant aggregates size, stable structure) could be observed. Consecutive optical microscopy images during storage period could illustrate changes in aggregates size and distribution and support viscoelastic data.

7. **Analyses of the structure on the Nanoscale and molecular scale**

   Studies of macroscopic structure behaviour can be supported by investigations of the structure formation on the nanoscale and molecular scale. Cryo-scanning electron microscopy (cryo-SEM) or transmission electron microscopy (TEM) could be used to image nanostructure morphology of the mixed fluid gels aggregates (starch granules

and biopolymer helical fibres). Images of the individual components should be obtained as a comparison. Nuclear magnetic resonance (NMR) spectroscopy could be used to investigate structures formation on a molecular scale and intermolecular interactions between two components building blocks. NMR allows quantifying what is immobilised in the gel network and the effect of the additional component on the biopolymer helix aggregation.