

The Effect of High Shear Mixers on Functionality of non-starch-based hydrocolloids

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The Effect of High Shear Mixers on Functionality of non-starch-based hydrocolloids

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Master Thesis in Food Technology

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Popular Abstract

Hydrocolloids are commonly used food additives that help in modification of viscosity, texture and stability of different food products, for example dressings, meat products, juice, dairy beverages, etc. The application for hydrocolloids is wide, but one of the problems associated with hydrocolloids is that it is difficult to disperse them homogeneously which limits its utilisation to the fullest. This problem can be overcome with the help of mixing hydrocolloid in a High Shear Mixer which facilitates efficient dispersion of hydrocolloid.

In this project, the aim was to understand the effect of High Shear Mixer on the properties like viscosity, stability and texture of each hydrocolloids. Various parameters like mixing time, temperature and speed of the mixer were altered and their effect on different concentration of hydrocolloid was observed. Also, behaviour of hydrocolloids was compared both in water and different food product for example apple juice and chocolate milk. The different hydrocolloids used in this project were Xanthan, CMC, Pectin, Gellan and kappa-Carrageenan, which were chosen based on their application in beverage and dairy products.

Hydrocolloid solution were prepared in a 25 litres High Shear Mixer by varying different parameters to understand the impact of mixing on the product. Both apple juice and chocolate milk containing hydrocolloids were also prepared in the High Shear Mixer. The produced samples were analysed with the help of a rheometer (viscosity and yield stress) and texture analyser (Stevens value). Stability tests were performed for chocolate milk with the help of centrifugation.

Altering parameters like mixing speed, temperature and time have an influence on the viscosity and texture of the hydrocolloid solution. In general, at low concentration of hydrocolloids in water, no effect of time, temperature and speed of mixing was observed, as the viscosity of the system was very low. While at higher concentration, mixing time and temperature were important parameters as it affected the both the viscosity and texture of the hydrocolloid solution. Further, the observed behaviour of hydrocolloids in water was different from their behaviour when they were dispersed in the food matrix. As hydrocolloids can be affected by different factors like pH, ions, sugar, proteins, etc. in food which influences their properties.

Preface

This master thesis project was performed in collaboration between Tetra Pak, Sweden and the Department of Food Technology and Nutrition, Lund University faculty of Engineering, LTH. The duration of this master thesis was from January 2019 until June 2019. The aim of this thesis was to investigate the effect of High Shear Mixer on different hydrocolloids.

We wish to thank our supervisor from Tetra Pak Dragana Arlov and from LTH Lars Nilsson for all their support, encouragement and advice during our work. We also want to thank Dan Johansson and Hans Bolinsson from LTH for the help they provided all through the practical work for this thesis. Finally, we would like to thank CP Kelco, Denmark for their contribution in providing information around hydrocolloids and their application.

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1. Introduction

1.1 Motivation

Hydrocolloids are used in various industries including food, pharmaceutical, cosmetic, etc. To utilise the full potential of the added hydrocolloid, a better understanding of their structure, thickening mechanisms, setting temperature, hydration mechanism, etc. should be known before their use. Each hydrocolloid has different properties and their behaviour with which it interacts with the different matrix like water or food, as they have different molecular interactions and chemical bonds to each other. The application for hydrocolloids is wide, but one of the problems associated with hydrocolloids is that it is very difficult to disperse them homogeneously for example CMC. Also, there is a risk of formation of fisheye, lump formation, etc. which poses a problem and are hard to overcome. With the use of High Shear Mixer, many of the above-mentioned problems with hydrocolloids can be overcome. The High Shear Mixer is a device which helps to efficiently disperse the hydrocolloid in a way that a consistent and lump-free product is produced.

1.2 Objectives

The objective of this project is to understand the effect of High Shear mixer (HSM) on the rheological properties like viscosity and texture on different non-starch-based hydrocolloids. Different parameters like speed, temperature and time of mixing are altered in the HSM and their effects are observed. Commonly utilized food hydrocolloids like CMC, Xanthan, Gellan, Pectin and Carrageenan were used both in aqueous environment and in food, and a comparison was done on the behaviour of each gum on different matrices.

1.3 Scope and limitation

The scope associated with this project includes understanding the behaviour of various non-starch-hydrocolloids and their interaction with water and food. It was a 20-week long project where the tasks to be done included literature research, design of experiments, running of trials, data analysis and finally writing the report. The limitation with this project was to narrow down the scope of the project to just 5 gums and the application associated were mainly beverages and dairy.

2. Background

2.1. High Shear Mixer

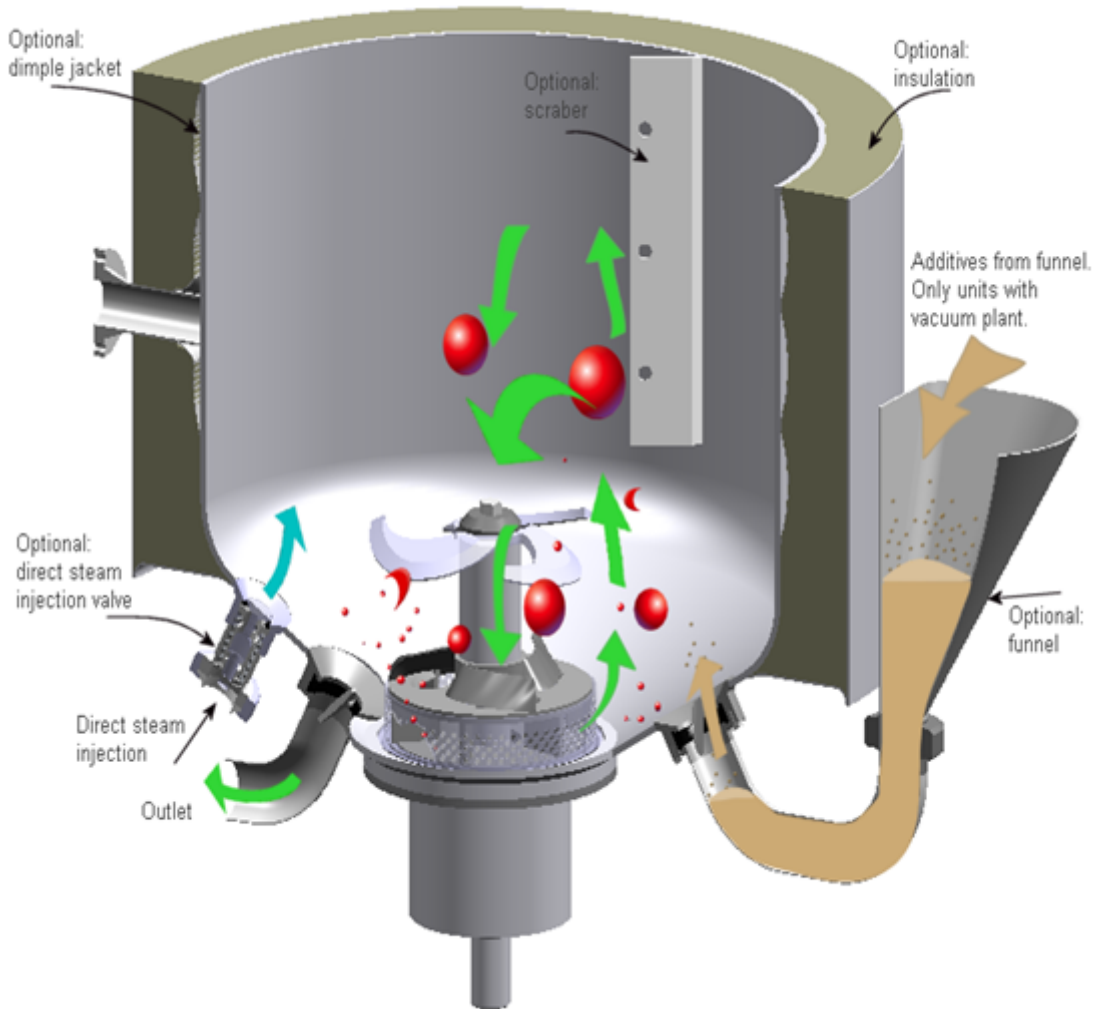


Figure 1: Interior of 25 litres Tetra Pak High Shear Mixer (Tetra Pak).

Batch Mixing is a process often used in food production where different ingredients in both powder and liquid forms are mixed together to obtain a consistent product. The Tetra Pak High Shear Mixer is a 25 litres vacuum mixing tank equipped with a rotor on the bottom along with a stationary stator unit shown in Figure 1. The dynamic stator in the mixer can be lowered to have high shear and raised to have low shear. A scraper is attached with the machine that improves the bulk flow of the product while mixing which can rotate in both clockwise and counter clockwise direction. The powder or any additive ingredients can be sucked into the tank with the help of vacuum, ensuring optimal dispersion and wetting of the powder in the system. Also, vacuum improves the reduction of air incorporation in the product, reducing foam formation. The product inside the tank can be heated with the help of either direct steam injection or heating from the jacket (Tetra Pak, 2019).

2.2. Basics of Hydrocolloids

Hydrocolloid is a group of polysaccharides and proteins with a very high molecular weight that aid in giving an improved texture to food in terms of viscosity, thickening, gelling, stability, mouthfeel, etc. They have an affinity towards the water and can be dissolved in water or can be dispersed well in a system with the help of shear forces. Hydrocolloids can be either linear or branched and, if they have side groups attached to the main structure it can have an influence on the gelling properties, solubility, sensitivity to ions, etc. It is widely used in food industries due to their capability to change the rheology of the food product by modifying texture and viscosity which changes the organoleptic properties of the food. Hydrocolloids are also used in stabilising of food product by entrapment of particles that are insoluble in continuous phase and not letting it separate. Thus, making it very popular to be used as a food additive. The hydrocolloids can be either naturally produced like from land plants (Pectin) or marine algae (Carrageenan) or can be obtained with the help microbial fermentation (Xanthan). To attain the full potential of hydrocolloid they should be dispersed and hydrated completely in a solution. Each hydrocolloid has different properties (molecular structure, charge, size, etc.) and thus, the hydration procedure differs for different hydrocolloids. For example, a few hydrocolloids require the presence of salt or sugar to be completely hydrated while the other requires a higher temperature (Phillips and Williams, 2014).

There is a wide variety of gums that are available for example guar gum, gelatine, agar, gum arabic, etc. Though, in this study the gums were narrowed down to only 5, which are most commonly used in Tetra Pak food products. The various hydrocolloids that are used in this study are Xanthan, CMC, Gellan, Pectin and kappa Carrageenan. Properties of each hydrocolloid is mentioned briefly in *Table A19*. A brief description of each hydrocolloid is given below:

2.2.1. Xanthan

Xanthan gum is an extracellular polysaccharide produced by the microorganism *Xanthomonas Campestris*.

Hydration of xanthan depends on the several factors. One of the factors is dispersion. Poor dispersion results in lump formation during mixing. Too much lumps in the mixer prevents complete hydration and reduces the functionality of the xanthan gum. It can be improved by using high shear mixers or using dry ingredients such as sugar, starch or salt as dispersants to separate the particles. The ratio of dispersant: Xanthan of 10: 1 can be used for this purpose (Phillips and Williams, 2014). Physical separation of xanthan particles with the help of dispersant improves the dispersion and hydration. Another factor which can affect the quality of hydration is the composition of the solvent (presence of salt and pH). Also, particle size is important for proper hydration. Smaller particles disperse and hydrate faster.

Xanthan is soluble in both cold and hot water. Xanthan molecules can form helix aggregates in the solutions. Xanthan solutions show shear thinning behaviour meaning that when the shear rate is increased, viscosity is reduced because the rod like and stiff aggregates orient in the direction of the applied shear and start to flow.

Typical used concentration as thickener in food application range from 0.05% to 0.3%. Effect of salts on viscosity of xanthan solutions depends on the concentration of xanthan gum in solution. Below approximately 0.2% gum concentration, monovalent salts such as sodium chloride cause a slight fall in viscosity. At higher gum concentrations, viscosity increases as salts are added (Phillips and Williams, 2014). The viscosity of xanthan solutions is sensitive to changes in pH below pH 4.0. (Phillips and Williams, 2014).

2.2.2. CMC

Sodium carboxymethylcellulose (CMC) is a type of modified cellulose which is soluble in water. This ability is achieved by introducing carboxymethyl groups along the cellulose chain to make the hydration of the molecule possible while dispersing in the water. The association of CMC molecules with water molecules result in the increase in viscosity. CMC exhibits good solubility in both cold and hot water, but higher temperatures can ease the dissolution of gum because of the lower viscosity.

The CMC solutions demonstrate shear thinning behaviour while shearing and will be regained when the shear is removed. The viscosity of the CMC solutions decreases by increasing the temperature but increases again when the temperature is decreased. Longer heating time, especially if the temperature is above 100 °C may result in loss of viscosity. The viscosity of the solutions is stable within a pH range between 4-11. It can be used 0.05% to 0.5% in beverage applications. (Phillips and Williams, 2014).

2.2.3. Pectin

Pectin is a part of the group heteropolysaccharide containing a majority of galacturonic acid units and they are present in the cell wall of all plants. Pectin can be naturally found in various fruits in different quantity and quality. Major sources for pectin are citrus peels, apple pomace, residues left from extraction of apple juice, etc. There are different types of pectin present based on different molecular composition. There are two types of Pectin based on their degree of esterification, i.e. High methyl ester (HM) pectin and Low methyl ester (LM) pectin. In HM Pectin, 50 and more carboxyl groups are methylated while, for LM Pectin the degree of esterification is less than 50. The gelling mechanism for each of the gum is different as LM pectin forms gel in the presence of divalent cations for example calcium, while HM Pectin requires presence of both lower pH and sugar to form gels. In this study, based on the application HM pectin is used for acidic beverages.

Efficient gelation of HM pectin is highly dependent on a balance of different factors like pH, calcium concentration, pectin concentrations and if any sequestrants are present. The gelling mechanism of pectin is highly dependent on the hydrogen bonding between the carboxyl group and the alcohol groups and the hydrophobic interactions between the methyl-ester groups creating junction-zones. Optimum dissolving temperature and pH for pectin is in the range of 50-80 °C and 3-5 respectively. For effective use of pectin, it is important to disperse the solid particles before dissolving which can be accomplished with the help of High shear mixer. (Phillips and Williams, 2014).

2.2.4. Gellan gum

Gellan gum is a naturally produced polysaccharide that is an effective gelling agent which is used in the food and beverage industry. It is secreted by the micro-organism called *Sphingomonas elodea*. Gellan gum tends to form gels at very low

concentrations (0.1-0.12%) when the solutions are cooled from a higher temperature to lower in the presence of cations, for example Calcium that promote gel formation. (Imeson, 2010).

There are two different types of gellan gum based on the production method Low Acyl (LA) gellan and High Acyl (HA) gellan. HA gellan forms elastic, non-brittle gums but LA gellan forms non-elastic, brittle gums (Phillips and Williams, 2014). Gellan gum used in this study is HA gellan.

HA Gellan gums requires higher temperature of about 70 °C to 80 °C to get completely hydrated in the system. Hydration temperature of gellan gum is reliant on different factors like concentration of the gum and the type of ions present in the solution. Ions like sodium and calcium, if present in the solution prolongs the hydration of gellan molecules.

2.2.5. Carrageenan

Carrageenan is high molecular weight linear polysaccharide extracted from red seaweeds. Carrageenan consists of galactose units and sulphate groups between them in the molecular chain. Carrageenan molecules have both hydrophilic part due to the sulphate groups and hydrophobic regions because of the 3,6-anhydrogalactose in the structure.

The main carrageenan types are lambda, kappa and iota which have various dissolution and thickening properties according to their chemical structure (Hoeffler, 2004).

One of the best-known synergies is demonstrated between kappa carrageenan and milk proteins. In milk, kappa carrageenan interacts with positively charged amino acids in the surface of kappa-casein in the protein. The kappa carrageenan levels as low as 0.02% can be used to stabilize the dispersions in dairy products, for example the cocoa particles in chocolate milk (Phillips and Williams, 2014). Based on this property, kappa carrageenan was chosen to be studied in this project.

kappa carrageenan is soluble in hot water. At 70 °C, the coil-helix conformation occurs and aggregation between the helices result in the viscosity increase of the solution. Cooling the solution down to setting temperature which is 40 °C is of importance for gel formation in kappa carrageenan. kappa carrageenan requires cations such as potassium ions to form a gel. Kappa carrageenan is heat stable in neutral pH but it loses the viscosity in high temperature and low pH due to the hydrolysis of galactose chain.

2.3. Functionality of Hydrocolloid

2.3.1. Rheology

Rheology is a study of the flow and deformation of fluids which displays a time-dependent effect to varied stress. Fluids are distinct from solids as fluids have a property to deform constantly when a stress is applied, while solids have an elastic behaviour and can withstand stress. Rheology plays an important role in the food industry for sensory attributes like the texture of the final product, mouthfeel, design of the plant (the types of equipment to be used like pumps, pipes, etc.), stability of the product, functionality of the ingredients, etc. (Beaudoin, 2001).

Fluids can be categorised as either Newtonian or Non-Newtonian as shown in Figure 2. Newtonian Fluids show a linear relationship between shear stress and shear rate. The viscosity of a Newtonian fluids remains constant at different shear rate. Example of some Newtonian fluid is water, oil, sugar solution, etc. While Non-Newtonian fluids

are the fluids that do not have a linear relationship between shear stress and shear rate. Shear stress can either increase or decrease as a function of shear rate. Non-Newtonian fluids can be further classified as shear thinning (Pseudoplastic) and shear thickening (Dilatant). Example of Non-Newtonian shear thinning fluid is ketchup and shear thickening fluids is corn-starch in water.

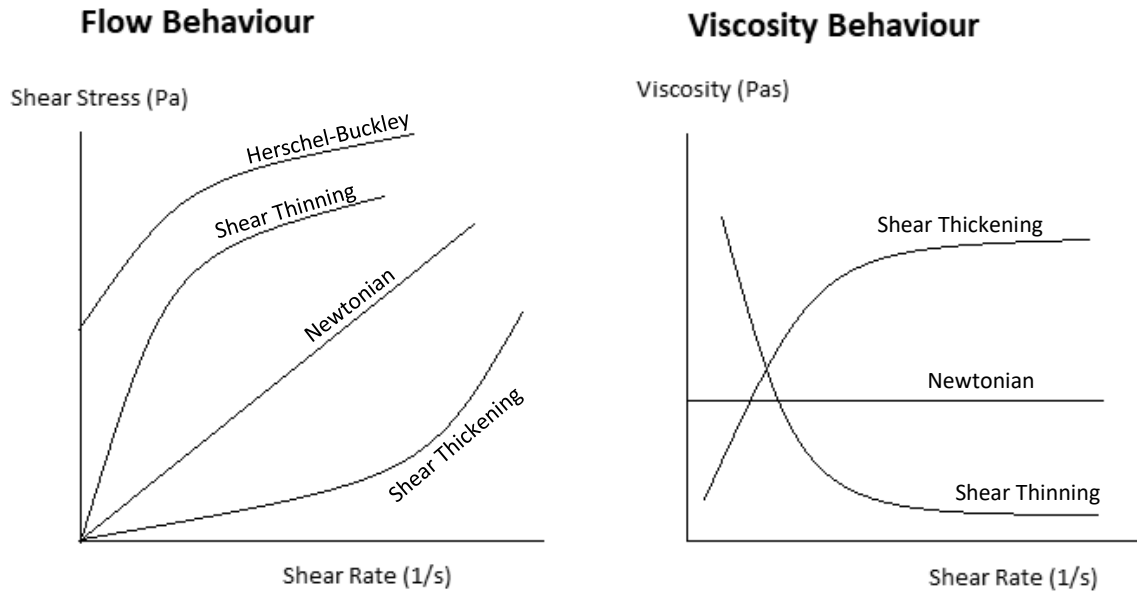


Figure 2: Flow behaviour and viscosity behaviour of fluids.

2.3.1.1. Viscosity

Viscosity is the measurement of the resistance to the flow of a fluid. According to Newton's Law of Viscosity, shear stress is directly proportional to the velocity gradient. Power Law characterizes the linear relationship between the logarithm of shear rate and logarithmic of shear stress. This law is expressed with two parameters (k and n value) as equation below:

$$\zeta = K\gamma^n \dots\dots\dots \text{(Equation 1)}$$

In the above equation, ζ is shear stress and γ is shear rate, K is the consistency coefficient and n are the power law exponent. n value helps in the determination of the flow behaviour of the fluid. If the value of n equals to 1 it shows that the fluid has Newtonian properties, when n is less than one it shows shear-thinning behaviour while it shows shear thickening properties if n is more than one.

2.3.1.2. Yield Stress

Yield stress is the stress that is required for the fluid to start flowing. Yield stress can be measured using different methods for example Amplitude Oscillatory measurements. The advantage of using this method is that it helps in understanding both the elastic component (G') and the viscous component of the sample (G'').

In hydrocolloid solutions, when the shear is applied, the stored energy is due to the stretching of chains but when there is frictional movement in the chains there is energy loss. When a lower frequency is applied to the system, the stress is due to the translational motion of the molecules which does not deform the molecules and thus

the viscous component (G'') dominates in the system. At higher frequencies, molecules are distorted and the difference between elastic component (G') and viscous component (G'') value reduces as shown in *Figure 3 b*). For hydrocolloid gel, the molecules have a three-dimensional continuous polymer network which can be stretched and deformed. The network bonding forces would keep the structure from the transitional movement; thus, the elastic component of the system dominates as shown in *Figure 3(a)*, the crossover point where both the viscous component and elastic component meet is the corresponding yield stress of the sample. (Heldman, Lund and Sabliov, 2007)

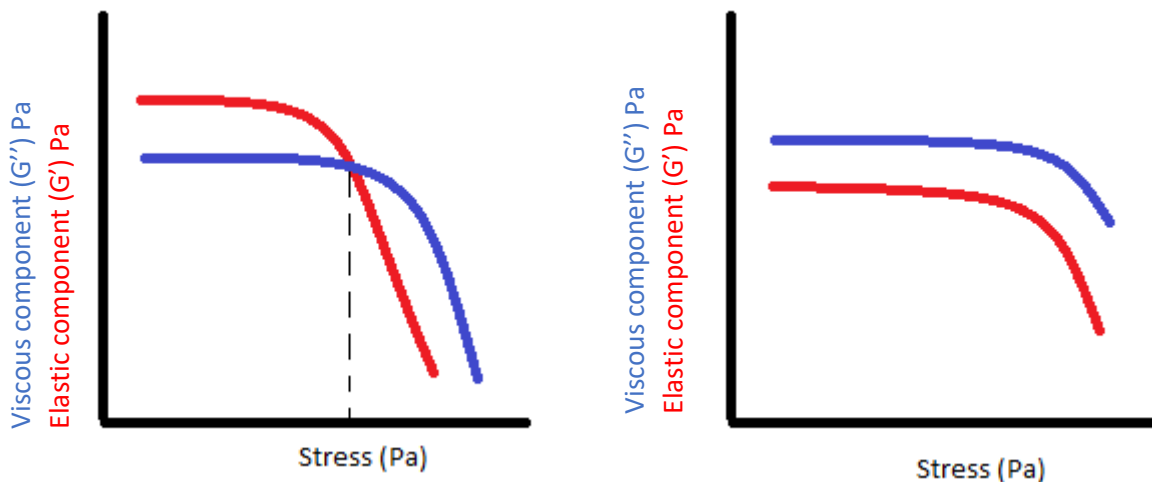


Figure 3: Illustration of viscous component (G'') and elastic component (G') in two cases: a) Elastic component is higher than the viscous component and the yield stress can be determined from the point of crossover. b) Viscous component higher than elastic component depicting no yield stress.

2.3.2. Stability

Most of the dispersions are thermodynamically unstable. The factors affecting the stability is the particle size of the dispersed phase or the density difference in the phases present in the system. The interaction between the phases determines the stability of the system. Interactions could either be attractive or repulsive. There is different type of instability that occurs in a dispersion:

- Creaming or Sedimentation is a separation caused due to the density difference leading to either upward or downward motion of the particle.
- Ostwald Ripening is caused when smaller droplets aggregate together thus forming a larger droplet.
- Coalescence is a process in which the droplets fuse together with other droplets.
- Flocculation is the aggregation of the droplets which is caused due to the collision. (Beckett, 1996)

Chocolate milk is a suspension meaning that cocoa particle (solid particles) is suspended in milk (continuous phase). As cocoa particle is hydrophobic in nature, they do not dissolve in milk and thus, if not mixed they end up as a sediment. To avoid the cocoa particle to sediment, hydrocolloids are added to slow down the process of sedimentation.

3. Material and methods

3.1. Hydrocolloids

All the hydrocolloids used for this study were based on beverage and dairy application. The different hydrocolloids were chosen in cooperation with CP Kelco, Denmark.

Table 1 gives a brief summary of the application notes of different hydrocolloids that were used for the thesis work. The information about was provided by CP Kelco.

Table 1: Application notes around each of the hydrocolloids used.

Hydrocolloid	Type	Applications	Functionality
High Acyl Gellan	KELCOL HM-B	Neutral pH dairy beverages like chocolate milk	Easy to disperse and hydrate, stabilizer and thickener
High Methyl Ester Pectin	GENU VIS	Acidic beverages	Thickener
Xanthan	KELTROL T plus	Beverages	Stabilizer, thickener
Kappa Carrageenan	GENULACTA® (K-100)	Neutral protein drinks Chocolate milk	Suspends cocoa particles, Prevents cream separation
Carboxymethyl Cellulose	CEKOL 30000	Beverages	Thickener

3.2. High Shear Mixer

All samples were prepared in Tetra Pak High Shear Mixer unit B120-25VA. This is a batch mixer with a rotor head of 120 mm diameter and 25 litres in batch volume. According to Figure 4 a), the mixer comprises of a bench with a screen on the right side and a jacket on the left side. Figure 4 b) shows the rotor and stator device and Figure 4 c) shows the standard stator seen from the side. The stator in 4 b) is placed in a lower position but it can be raised and lowered during the process for obtaining low and high shear respectively. The settings for the mixer are shown in Table 2.



Figure 4: Tetra Pak High Shear Batch Mixer (25 litres), on the left side of the mixer is the vessel and on the right side is the display unit. b) (top view) rotor and stator, c) Stator with slots 5x14mm

Table 2: High Shear Mixer Settings applied in the trials.

Parameter	Setting
Mixer Speed (%)**	70% or 90%*
Agitator Speed (%)	100%*
Pressure (mbar)	800 mbar
Temperature Jacket (°C)	20 °C
Temperature Product (°C)	20 °C

*where 70% refer to 18.2 m/s. and 100% refers to 26.0 m/s.

** Mixer speed was varied for different trials.

3.3. Rheometer

Rheology measurements that include analysis of yield stress and viscosity were performed using Rheometer (Kinexus pro+, Malvern Instruments, UK, Worcestershire) shown in Figure A1 in Appendix. The samples prepared in the High Shear Mixer are cooled down to room temperature and then the rheology measurement is run on the samples.

For measuring the yield stress with oscillatory amplitude sweep strain-controlled method, bob geometry used was V21 SC0001 SS and the serrated cup used was PC25 A0008 AL depicted in Figure 3 (a). 17.5 ml of samples were filled in the cup and the intended sequence in rheometer was run. The condition of the sequence is displayed in the Table 2. During the test, the samples are exposed to oscillatory motion. Amplitude Oscillatory strain-controlled method was applied where the sinusoidal strain is applied to the sample and the stress is measured. From the results obtained with the help of Rheometer, logarithmic of shear stress is plotted against the elastic (G') and viscous component (G''). The point at which both

the plots crosses over is the observed yield stress of the sample as shown in *Figure 3 a*).

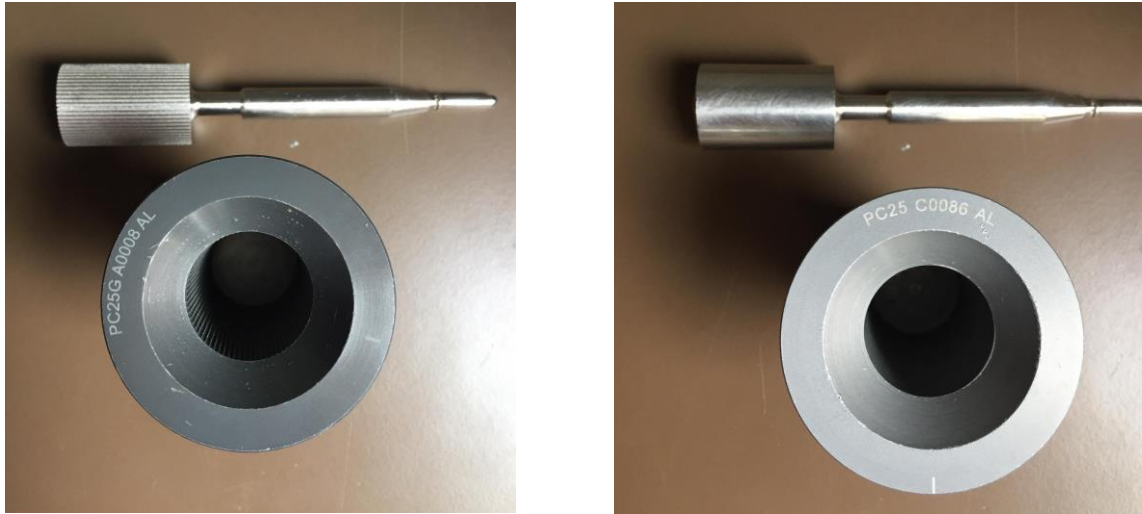


Figure 3, a) Serrated bob and cup used for measuring yield stress (Oscillatory Amplitude Sweep) b) Smooth bob and cup used for measuring viscosity.

Table 2: Settings applied on the rheometer while measuring the yield stress.

Parameter	Setting
Temperature	20°C
Start shear strain	1.00*10 ⁻³ strain
End Shear strain	10 strain
Frequency	1 Hz
Samples per decade	40

For measurements of K and n values (Equation 1), bob geometry used was C25 SC0053 SS and the cup used was PC25 C0086 AL (*Figure 3 (b)*). 17.5 ml of samples were filled in the cup and the intended sequence in rheometer was run. The condition of the sequence is displayed in the *Table 3*. From the result, shear stress is plotted against shear rate in a logarithmic scale, from which n and K value are extracted from the Power law equation.

Table 3: Settings applied on the rheometer while measuring the viscosity.

Parameter	Setting
Temperature	20 °C
Pre shear	100 s ⁻¹ (5 minutes)
Start Shear rate	20 s ⁻¹
End Shear Rate	1000 s ⁻¹
Sample Points	10

In order to be able to compare viscosities easier when varying mixing time, the apparent viscosity at a shear rate of 100 s⁻¹ is used. This is because 100s⁻¹ is the shear rate is used when the fluid flow through the pipes. (STEFFE and MORGAN, 1987)

3.4. Texture Analysis

The gelling properties of the samples were observed by measuring the Stevens values using a Texture Analyser (TA-XT2i, Stable Microsystems, UK, Godalming) shown in *Figure 4 (a)* with a disc plunger probe with a diameter of 40mm (*Figure 4 (b)*). The force was applied with the help of probe to the glass beaker containing 100 ml of gum solution. The compression force was recorded as a function of time. The conditions for the texture analyser are given below in the *Table A1*. The Stevens value was obtained as the average values of the compression force between the 5-11 s (Cedergårdh, F., 2014)

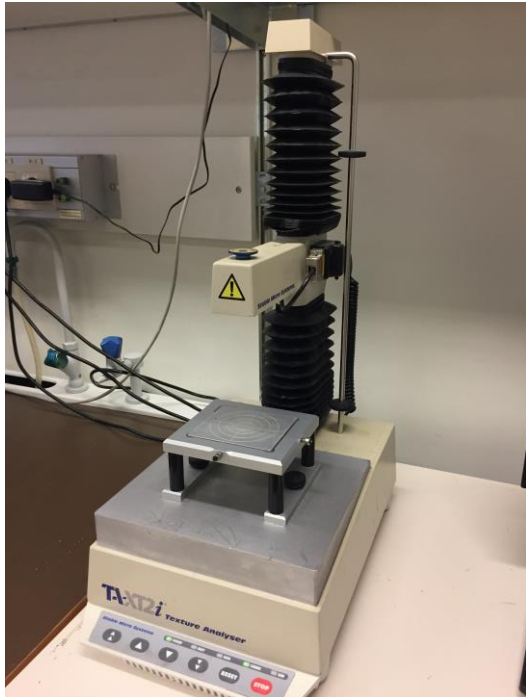


Figure 4: a) Texture Analyser (TA-XT2i, Stable Microsystems, UK, Godalming). b) Disc Plunger Probe used for the texture analysis.

3.5. Homogenizer

Homogenization is used for Chocolate Milk to prevent creaming or sedimentation of the fat and cacao particles, also it helps in the stability of the chocolate milk. High-Pressure Homogenizer consists of a high-pressure piston pump which forces the fluid to pass a narrow gap at a very high speed. This pressure causes the particles in the chocolate milk to become uniform giving stability to the final product.

For a homogenized product, high pressure Panda PLUS homogenizer is used (*Figure A3(a)*). It is a small size table top unit used for liquid products. The product is filled in the hopper continuously, which is then pumped to the two-stage homogeniser with the help of suction valve. Post the homogenisation, the product comes out of the pipe and can be collected in a beaker. The conditions used for the homogenization of chocolate milk are 180 bars at 70°C.

3.6. Stability test

Stability properties of the chocolate milk was checked by using the Centrifuge Allegra X-15R (*Figure A3(b)*). The process of sedimentation of cocoa particles can be accelerated with the help of mechanical stress in centrifugation method.

The chocolate milk samples were filled up to 10 ml in the centrifuge tubes and the tests were run at 1500 rpm for 15 minutes at 20°C. After the centrifugation, the tubes were taken out and with the help of a scale, the height of the sediment deposited was noted. Method development for stability test is given in Appendix section 7.1.4.

3.7. Test Plan - Hydrocolloids in water

Table 4: Different Parameters used for making samples in High Shear Mixer.

Gum	Type	Concentration (%)	Temperature (°C)	Speed (%)
Gellan	KELCOL HM-B	0.10	60	70
		0.15	80	90
		1		
Xanthan	KELTROL T plus	0.05	20	70
		0.10	60	90
		1		
Carrageenan	GENULACTA® (K-100)	0.02	70	70
		0.5		
		1.0		
CMC	CEKOL 30000	0.05	20	70
		0.10	60	90
		1.00		

The test plan included varying four different parameters and testing the functionality of the hydrocolloids. The four parameters that were varied are rotor speed, temperature, hydrocolloid concentration and mixing time as shown above in *Table 4*. Thus, all the trials were designed in a way that one of the parameters was changing and the rest of the parameter were constant. Initially, all the trials were performed in lower concentration as the anticipated food matrix was beverages and dairy. But later, to understand the functionality of hydrocolloids better, higher concentration was included as well.

For each hydrocolloid, different test conditions were followed according to their best practice properties. For example, xanthan and CMC can be dissolved in both cold and hot water and thus the temperature used for these gums were 20°C and 60°C. While, gellan needs higher temperature for dispersion and thus the tests were performed at 60°C and 80°C. And for carrageenan, it was dispersed at 20°C and then heated to 70°C and cooled down while mixing in the High Shear Mixer. The test plan designed for each gum is different and are mentioned separately in the Result and Discussion section.

3.7.1. Preparation of the gum solutions

For the preparation of the solution of CMC, Xanthan and Gellan, the samples were prepared by filling the Tetra Pak High Shear Mixer with 15 litres of water. If the temperature used for the sample preparation is above room temperature, steam is applied onto the jacket of the mixer in order to reach the desired temperature. Further, the hydrocolloid is added according to the required concentration. To

facilitate the dispersion of gum in the system and to avoid the sticking of gum on the surface or the scraper, for the first thirty seconds only the mixer is turned on with stator down. To have a vacuum in the system, a pressure of 800 mbar is set. After 30 seconds, we turn on the agitator speed to 100%. Samples are taken at different mixing times (as shown in *Table 9, 12 and 16* for Xanthan, CMC and Gellan respectively). After the trial, the mixing tank is emptied and cleaned. *Figure 5* below depicts the procedure:

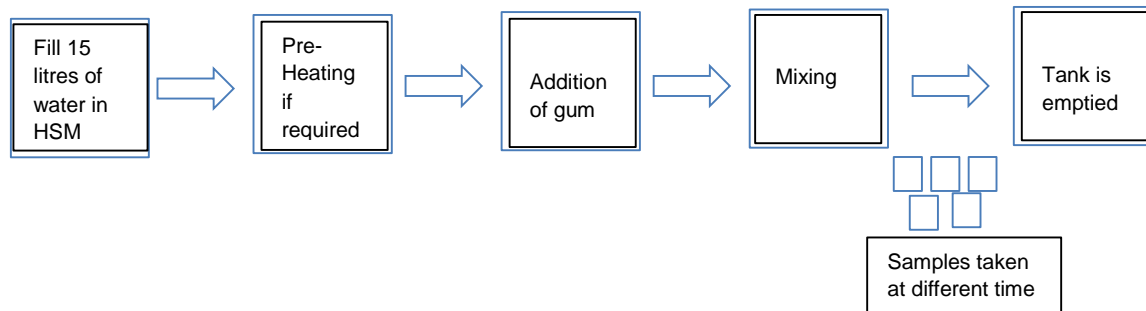


Figure 5. Procedure for the solution of CMC, Xanthan and Gellan Gum

The preparation method for carrageenan was different due its different dispersion characteristics. For the preparation of solutions with carrageenan, 15 litres of water were added in the High Shear Mixer. The gum was added to the water at 20°C and mixed in the mixer. A few samples were collected to check the dispersion of the gum. Further, steam was injected onto the jacket and the system was heated up to 70°C. Keeping the temperature 70°C the system was mixed for 18 minutes and samples were taken at different mixing times (shown in *Table 17*). After 18 minutes of mixing, the system was cooled from 70°C to 30°C and samples were collected. After the trial, the mixing tank is emptied and cleaned. *Figure 6* below depicts the procedure:

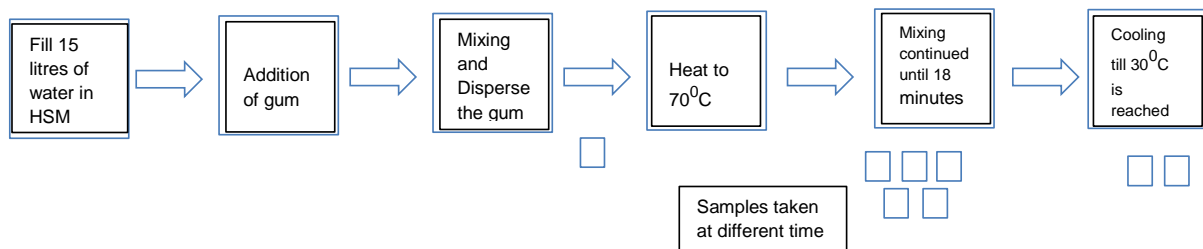


Figure 6. Procedure for the solution of Carrageenan Gum

3.8. Hydrocolloids in Food-matrix

Table 5: Different hydrocolloids used for the preparation of Apple juice and chocolate milk.

Gum	Type	Concentration (%)	Food Matrix
CMC	CEKOL 30000	0.05	Apple Juice
Pectin	GENU VIS	0.20 2	Apple Juice
Xanthan	KELTROL T plus	0.05	Apple Juice
Carrageenan	GENULACTA® (K-100)	0.02	Chocolate Milk
Gellan	KELCOL HM-B	0.12	Chocolate Milk

The test plan used for food matrix is mentioned in *Table 5*. The preparation of samples was done at mixer speed of 70%. Viscosity measurement and stability test were performed on the samples obtained from chocolate milk. For apple juice only viscosity measurement was performed.

3.8.1. Apple juice

Apple juice is made in a 15-litre batch in High Shear Mixer, ingredients used are mentioned in *Table 6*, the steps followed are as shown in *Figure 7*:

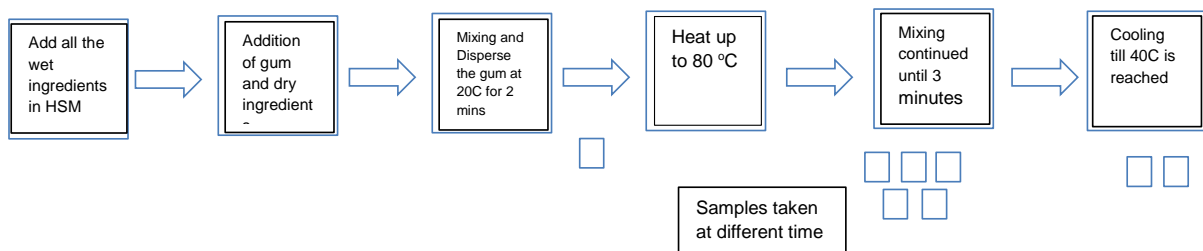


Figure 7. Procedure for the making apple juice for different hydrocolloids.

For making the apple juice, first, all the wet ingredients (water, apple juice concentrate, citric acid) were poured in the High Shear Mixer. After which dry ingredients like sugar and the gum were added. Mixing of all the ingredients was performed in the mixer at 20 °C for 2 minutes with mixing speed of 70% and samples are taken. The mixing was continued while the system is heated up to 80 °C (3 minutes) and mixing is continued until 3 minutes between which the samples at

different time are collected (as shown in *Table 15*). After 3 minutes, the system is cooled down until 40 °C is reached and a final sample is collected. Different concentration of gum was used for making apple juice like CMC (0.05%), Xanthan (0.05%) and Pectin (0.2%).

Table 6: Ingredients used in making Apple juice.

Component	%	In 15 litres
Apple juice concentrate	18	2.7 litres
Sucrose	2	300g
Water	80	12 litres
Citric Acid (50% w/v)	0.3	45ml

3.8.2. Chocolate Milk

In the *Table 7*, ingredient list is mentioned to make the chocolate milk. The two gums used for making chocolate milk were Carrageenan and Gellan. Different methods were used to make the water solution for gellan and carrageenan. Thus, to compare the behaviour of gum in water and food, the methods used by respective gums in water were used in making chocolate milk as well. The methods used in each case are as follows:

3.8.2.1. Chocolate milk with carrageenan

For making chocolate milk with carrageenan (0.02%) shown in *Figure 8*, in the mixer first add the milk. Further, all the dry ingredients including sugar, cocoa powder and gum is added. Mixing and dispersion of gum is done at 20°C for 2 minutes and samples are taken in between and homogenized. Then heat is applied in the mixer and the system is heated to 70°C while mixing (2 minutes). Mixing is continued until 18 minutes and samples are taken at different times (10, 30, 60 seconds, 3, 18 minutes) and are homogenized. After 18 minutes of mixing, the system is cooled from 70 °C to 30 °C and samples are collected and homogenised.

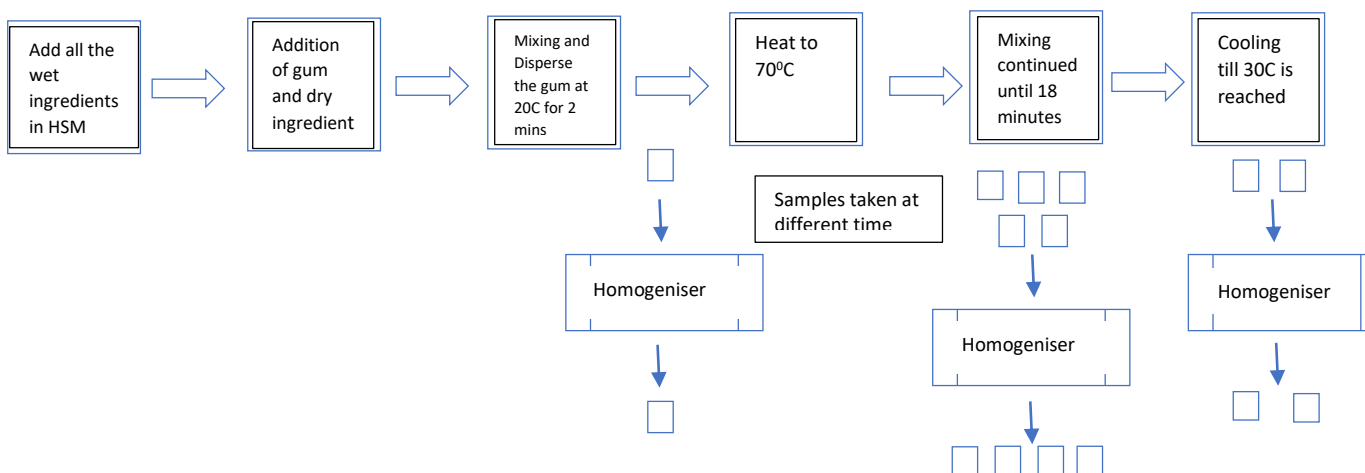


Figure 8. Procedure for making chocolate milk with Carrageenan Gum.

3.8.2.2. Chocolate milk with gellan

For making chocolate milk with gellan (0.12%) shown in *Figure 9*, in the mixer first add the milk. Further, all the dry ingredients like sugar and cocoa powder are added. Mixing is done and heat is applied in the mixer and the system is heated to 60°C (2 minutes). When 60°C is reached, gellan is added in the mixer. Mixing is continued until 18 minutes and samples are taken at different times (10, 30, 60 seconds, 3, 18 minutes) and are homogenized. After 18 minutes of mixing, the system is cooled from 60°C to 30°C and samples are collected and homogenised.

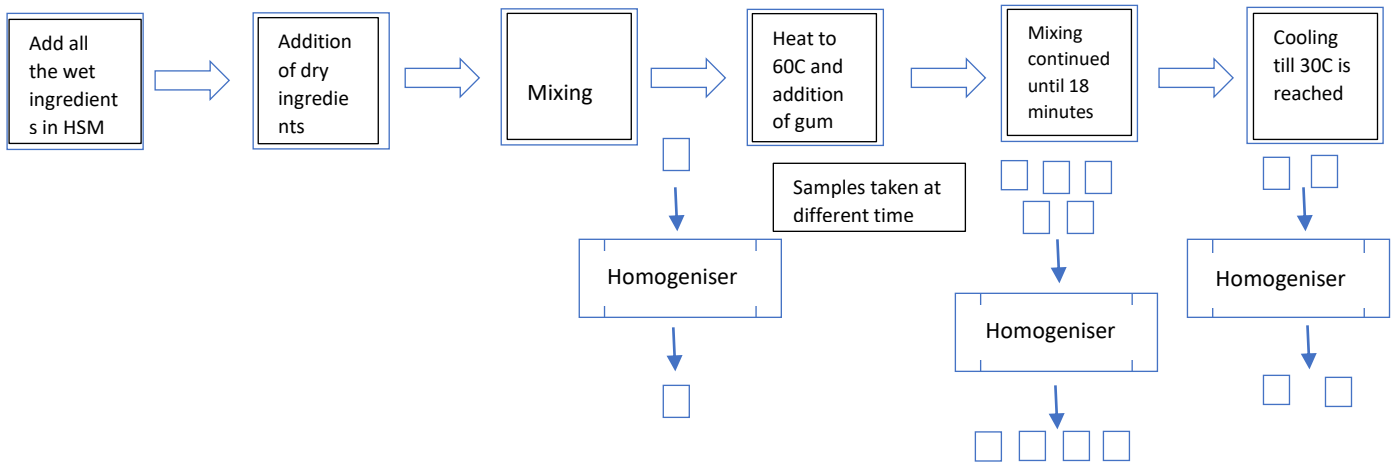


Figure 9. Procedure for making chocolate milk with Gellan Gum.

Table 7: Ingredients used in making Chocolate Milk.

Ingredient	%	In 15 litres
Milk 3% fat	90,48	13.57 litres
Cacao Powder	1.5	225 grams
Sugar	8	1200 grams

4. Result and Discussion

Sources of Error:

Plausible source of error must be considered during the measurements and the goal is to eliminate as many sources of error that can be possible. One of the errors that was considered is the measurement error. Measurement error is the variation between a quantity that has been measured and its true value. This may include random error or the error which is expected in the experiment due to small human error. Or it includes systematic error which is caused by the machine due to mis-calibration. Thus, to overcome this error, triplicates of samples were performed, and two standard deviation was done on the average of the values achieved. If two standard deviation is performed on the distribution it is in about 95% confidence interval. Batch to batch error was also calculated while reiterating 5 batches. (Bevington, 1991) The method used to calculate Batch to batch variation and measurement error is shown in Appendix section 7.2.

The result from the analysis are given in *Table 8*. Batch to batch variation was larger in comparison with the measurement error.

Table 8: Source of error.

Method	Standard Deviation (%)	Confidence interval
Viscosity Batch to batch variation (5 batches)	6.5	95%
Viscosity Measurement Error (3 samples)	1.8	95%
Texture Analyser Measurement Error (3 samples)	2.97	95%

4.1. Xanthan

4.1.1. Water matrix

For the water-matrix the concentration chosen for xanthan was based on the beverage application (0.05% and 0.1%) to compare the functionality of gum in water and food. However, to understand the functionality of Xanthan at higher concentration, 1% of xanthan solution was prepared as well. Test conditions used in the case of Xanthan is given below in *Table 9*.

Table 9. Test conditions for Xanthan solutions in water-matrix

Concentration (%)	Temperature (°C)	Mixing speed (%)	Time (s)
0.05	20	70	180-600-1200
		90	180-600-1200
	60	70	180-600-1200
		90	180-600-1200
0.1	20	70	180-600-1200
		90	180-600-1200
	60	70	180-600-1200
		90	180-600-1200
1	20	70	10-30-60-180-1080
	60	70	10-30-60-180-1080

4.1.1.1. Low concentrations

4.1.1.1.1. Viscosity

Effect of mixing speed and mixing time: To understand the effect of mixing speed and time, plots were made based on apparent viscosity (at 100s^{-1}) as a function of time. Figure 10 depicts the effect of speed on the apparent viscosity of 0.05% and 0.1% xanthan solutions at 20°C during the mixing time.

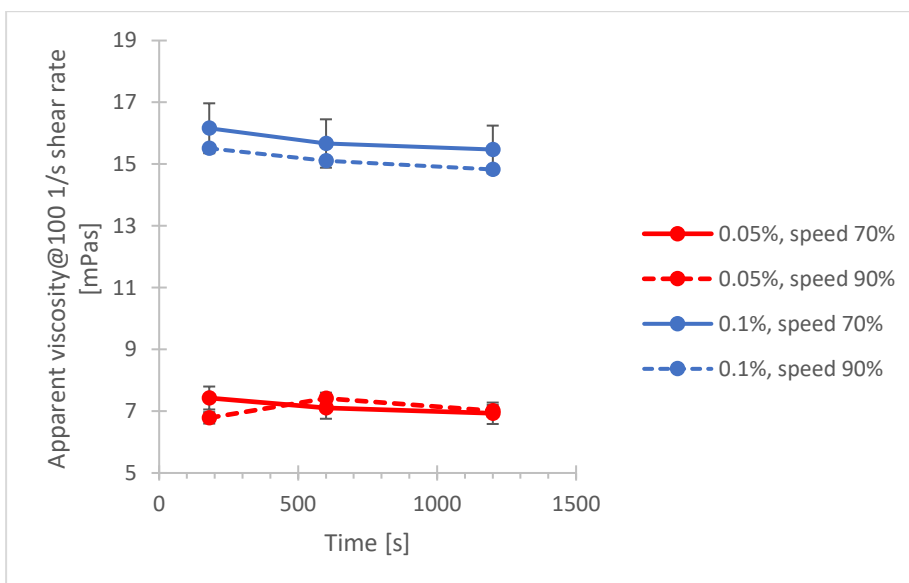


Figure 10: Apparent Viscosity of 0.05% xanthan solution at 100 1/s versus mixing time at 20°C at two speeds, 70% and 90%. Error bar is showing standard deviation error with 95% confidence interval.

According to the Figure 10, the curves at different mixing speeds (i.e. 70% and 90%) lie in the measurement error range. Thus, depicting that there is no effect of shear speed on Xanthan. It can also be observed that as the mixing time is increased, no loss in viscosity is seen.

Therefore, both mixing time and speed do not have any effect on the viscosity of the Xanthan solution at low concentrations.

Effect of temperature: In order to test the effect of temperature on the viscosity of the xanthan solution, plots were made based on apparent viscosity (at 100s^{-1}) versus function of time. *Figure 11* shows the effect of temperature on apparent viscosity of 0.05% and 0.1% xanthan solutions prepared at 70% speed.

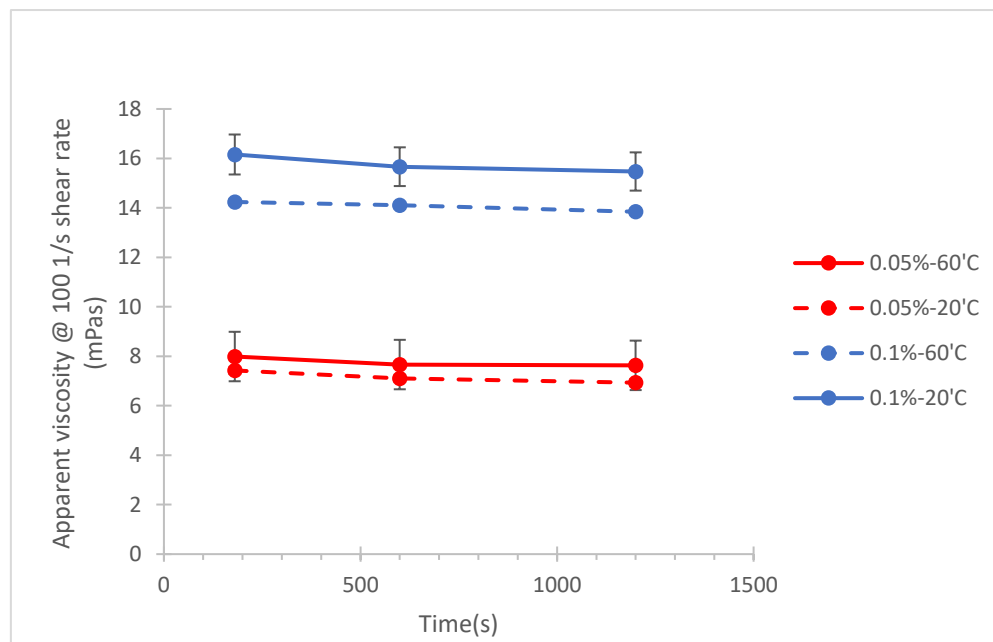


Figure 11: Apparent Viscosity of 0.1% xanthan solution at 100 1/s versus mixing time at two temperatures 20^o C and 60^o C and at 70% speed. Error bar is showing standard deviation error with 95% confidence interval

At a constant mixing speed of 70%, there is no impact of temperature on the viscosity of 0.05% xanthan solution observed, while, for 0.1% solution a slight decrease in viscosity is observed when increasing the temperature from 20^o C to 60^o C. Therefore, it can be concluded that mixing at higher temperature gives less viscosity as the hydration and the dissolution of the xanthan molecules is faster in higher temperature when compared to low temperature.

4.1.1.2. High concentrations:

4.1.1.2.1. Viscosity

To understand the effect of temperature and time on the viscosity of xanthan solution at higher concentration, apparent viscosity (at 100 1/s) versus time is plotted. In *Figure 12*, the apparent viscosity of 1% xanthan solution is illustrated during mixing at two different temperatures, 20^o C and 60^o C and at 70% mixing speed.

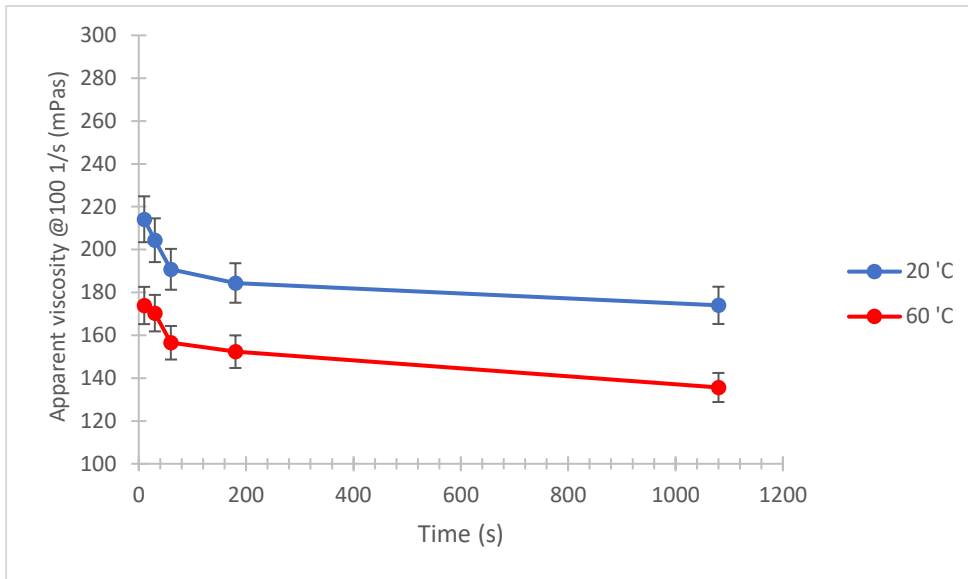


Figure 12: Apparent Viscosity of 1% xanthan solution at 100 1/s versus mixing time at two temperatures 20 °C and 60 °C and rotor speed of 70%. Error bar is showing standard deviation error with 95% confidence interval.

From *Figure 12*, with an increased range of mixing time, a gradual decrease in viscosity is observed and the highest value for viscosity is obtained at 10 seconds of mixing. After 180 seconds of mixing, the viscosity remains stable. The decrease in viscosity with increase in time of mixing can be due to the molecular structure of xanthan. Xanthan can form helix aggregates in the solution that occupies a high volume in the solutions, which result in high apparent viscosity (Phillips and Williams, 2009). This viscosity is achieved in just 10 seconds of mixing of Xanthan solution in the High Shear Mixer. Xanthan helical aggregates orient themselves in the direction of the flow while mixing for longer time, which result in the decrease of the apparent viscosity. The viscosity will be regained when putting at rest. The timescales for this phenomenon can range from minutes, in the case of destruction of the structure to hours in rebuilding (Barnes, 1997). Therefore, it can be assumed that although xanthan aggregates can regain the viscosity at rest, it takes longer time for rebuilding the destroyed structure by mixing over time. Also, it can be concluded that since xanthan makes the viscosity at initial dispersion time (10 seconds to 30 seconds) and it loses viscosity by shearing for longer time, there was no viscosity change observed in *Figures 10 and 11* in lower concentrations because the samples were taken after 180 seconds of mixing.

The viscosity of 1% xanthan solution prepared at 60 °C is lower than 20 °C. The reason can be assumed that the xanthan molecules are free to move in the solution at higher temperature and thus, the hydration and the dissolution of the xanthan molecules is faster in higher temperature when compared to low temperature. As, the gum molecules dissolve in continuous phase, the viscosity decreases. Therefore, by increasing dissolution rate of the Xanthan molecules in the water due the higher temperature, the viscosity of the solution reduces.

4.1.1.2.2. Texture Analysis

The Stevens value of 1% xanthan solution were obtained using the texture analyser. The Stevens value as the function of mixing time was plotted as shown in *Figure 13*.

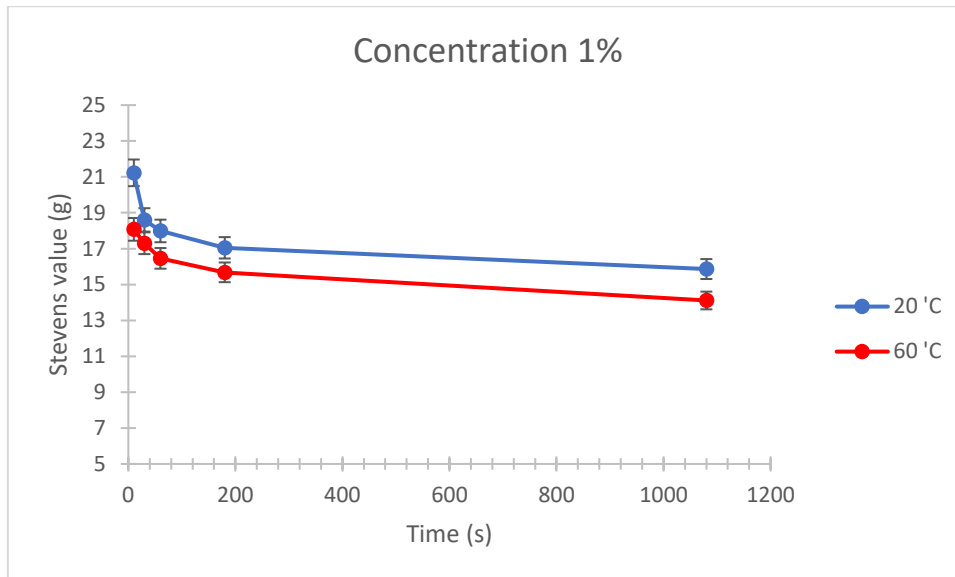


Figure 13: Stevens value of 1% xanthan solution versus mixing time at two temperatures, 20 °C and 60 °C and mixing speed of 70%. Error bar is showing standard deviation error with 95% confidence interval.

By increasing the mixing time there is a reduction in Stevens value. This behaviour follows the viscosity result and the explanation is given in Section 4.1.1.2.1.

4.1.1.2.3. Yield Stress

Yield stress of 1% xanthan solution which was mixed for 10 seconds, at 70% speed and 60 °C, was measured by using Amplitude oscillatory sweep method in rheometer. The result is depicted in *Figure 14*.

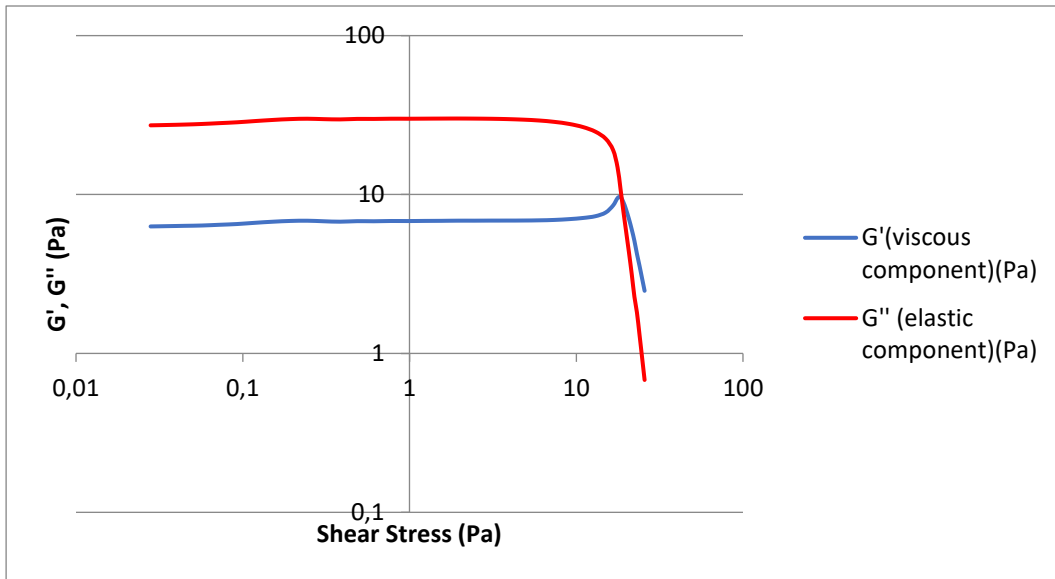


Figure 14: Amplitude Oscillatory sweep strain controlled of 1% xanthan solution mixed for 10 seconds at rotor speed of 70% and 60 °C

According to the Figure 14, when stress is applied to the solution, elastic component (G') dominates the viscous component (G''). At shear stress of 12.5 Pa, where the elastic component and viscous component crosses over, the differences between elastic and viscous component reduces, the formed network breaks down and the solution starts to flow. This point can be interpreted as yield stress (Heldman, Lund and Sabliov, 2007).

4.1.2. Food Matrix

The food-matrix used in the case of Xanthan is Apple juice. Table 10 displays the test conditions for preparation of apple juice containing xanthan in the High Shear Mixer. Apple juice was continuously mixed while altering the temperature during the preparation. Samples were taken at different mixing times and at different stages of preparation.

Table 10: The test conditions for apple juice containing xanthan

Xanthan concentration (%)	Mixing speed (%)	Temperature (°C)	Mixing time(s)
0.05	70	20	10-120
		80	250-270-300-360-420
		40	600

Figure 15 shows the apparent viscosity of apple juice containing 0.05% Xanthan gum during mixing time. The samples were collected during the process of making the apple juice in order to understand the effect of temperature and mixing time on

apparent viscosity of apple juice. Initial mixing is done at 20 °C for about 120 seconds, then the system is heated from 20 °C to 80 °C and is cooled down to 40 °C.

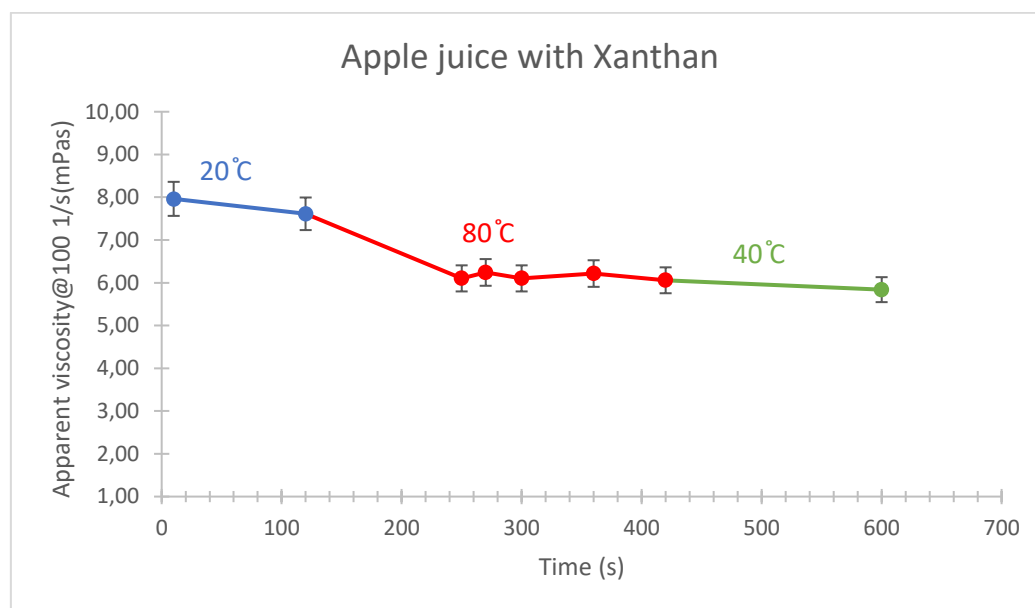


Figure 15: Apparent viscosity of Apple juice containing 0.05% xanthan gum at 100 1/s versus mixing time in different Temperatures (20 °C, 80 °C and 40 °C). Error bar is showing standard deviation error with 95% confidence interval.

In Figure 15, at 20 °C, where the dry ingredients are being dispersed in continuous phase, the viscosity of the apple juice is the highest. As the system is heated from 20 °C to 80 °C while mixing, a decrease in viscosity is observed. The viscosity of the apple juice further remains stable after reaching 80°C.

Table 11 depicts the apparent viscosity of the apple juice containing 0.05% xanthan after 180 seconds of mixing.

Table 11: Viscosity of xanthan solution and apple juice

Matrix	Time(s)	Concentration (%)	Temperature (c)	pH	Apparent viscosity @ 100 1/s (mPas)
Water	180	0.05	60	7.8	8.00
Apple juice	180	0.05	80	2.8	6.06

As shown in Table 11, the viscosity of the apple juice containing 0.05% xanthan after 3 minutes of mixing is lower than the viscosity of 0.05% Xanthan solution. This loss in viscosity is be due to the low pH of the juice. Xanthan is a charged polymer, thus there is an electrostatic repulsion between the side chains, which make the xanthan molecules to occupy large volume in the solutions. Changes in the pH can impact the charge density of Xanthan molecules. Low pH can affect the carboxylate groups in Xanthan to convert from ionised to un-ionised form. Consequently, the electrostatic repulsion between xanthan side chains reduces, which results in a more compact

molecular structure and therefore, less volume occupation of the molecules which causes a loss in the viscosity (Phillips and Williams, 2009).

Apple juice was prepared at 80 °C and it is assumed that the loss of viscosity because of the low pH can be more in high temperature due to the acceleration of acid hydrolysis of the polysaccharide (Phillips and Williams, 2009). This can be the reason of viscosity loss when heating from 20 °C to 80 °C while preparation of apple juice (*Figure 15*) because there was no effect of temperature observed on the viscosity of 0.05% of Xanthan in water matrix.

In conclusion, the viscosity of 1% of xanthan in water decreased by increasing the time of mixing from 10 seconds to 60 seconds, and after 60 seconds the viscosity of the system remains stable. However, at low concentrations since the samples were taken after 180 seconds of mixing, thus, there was no effect of mixing time observed. Mixing speed had no effect on xanthan solution at low concentration. Temperature of mixing had no influence on 0.05% xanthan solution while the viscosity decreased for 0.1% and 1% of xanthan solution when increasing the temperature. Also, the elastic component for 1% Xanthan solution is higher than the viscous component. Finally, when xanthan is dispersed in apple juice, loss in viscosity was observed when compared to water matrix due to low pH of the juice.

4.2. CMC

4.2.1. Water Matrix

For water-matrix, the concentration chosen for CMC was based on the beverage application (0.05% and 0.1%) to be able to compare the functionality of gum in water and food. However, to understand the functionality of CMC at higher concentration, 1% of CMC solution was prepared as well. Test conditions used in the case of CMC is given below in *Table 12*.

Table 12: Test conditions for CMC solutions in water.

Concentration (%)	Temperature (°C)	Speed (%)	Time (s)
0.05	20	70	180-360-1080
		90	
	60	70	
		90	
0.1	20	70	180-360-1080
		90	
	60	70	
		90	
1	20	70	10-30-60
	60		180-1080

4.2.1.1. Low Concentration

4.2.1.1.1. Viscosity

The effect of mixing speed and mixing time: For understanding the effect of mixing speed and mixing time on the viscosity of the CMC solution, plots were made

based on apparent viscosity (at 100s^{-1}) versus mixing time. *Figure 16* depicts the apparent viscosity of 0.05% and 0.1% CMC solutions during the mixing time at $60\text{ }^{\circ}\text{C}$.

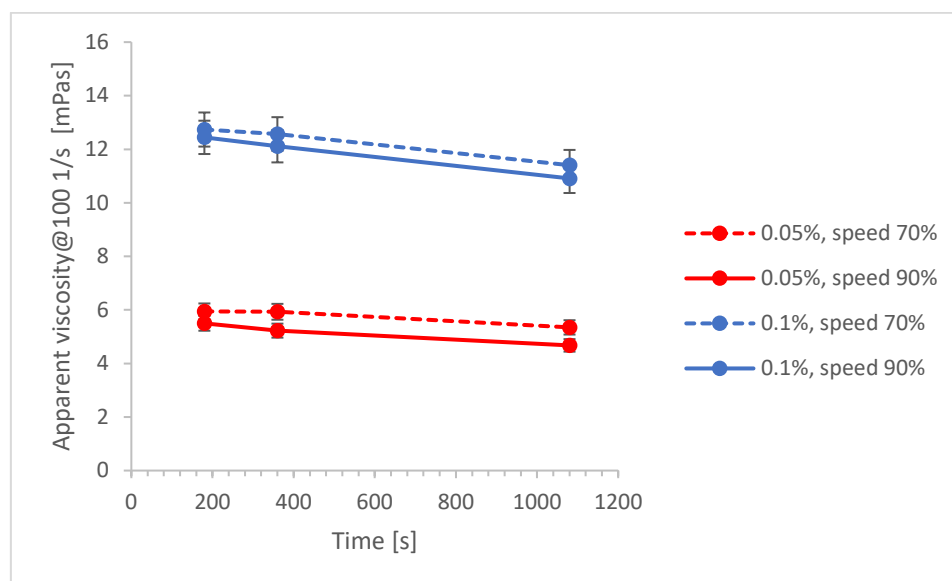


Figure 16: Apparent Viscosity of 0.05% and 0.1% CMC solution at 100 1/s versus mixing time at two mixing speeds, 70% and 90% at $60\text{ }^{\circ}\text{C}$. Error bar is showing standard deviation error with 95% confidence interval.

According to the *Figure 16*, at each concentration (0.05 and 0.1%), it is observed that there is no effect of mixing time on the viscosity. Also, the curves at different mixing speeds (i.e. 70% and 90%) lie in the error range, showing there is no effect of mixing speed on the viscosity of 0.05% and 0.1% CMC solutions.

Effect of temperature: For testing the effect of temperature on the viscosity of the CMC solution, plots were made based on apparent viscosity (at 100s^{-1}) versus function of time. In *Figure 17*, the effect of temperature on the apparent viscosity of 0.05% and 0.1% CMC solutions is displayed when prepared at 70% of mixing speed.

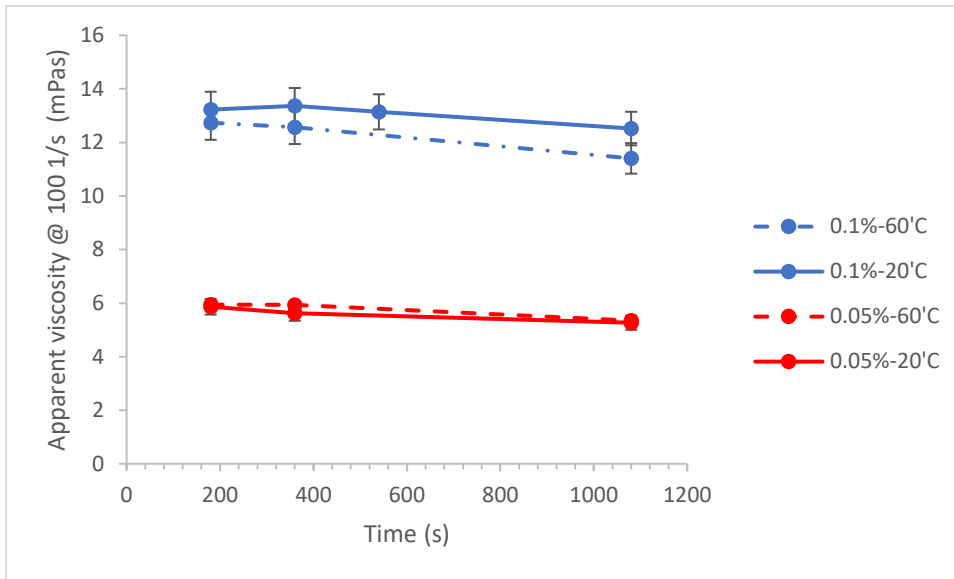


Figure 17: Apparent Viscosity of 0.05% and 0.1% CMC solution at 100 1/s versus mixing time at two temperatures, 20 °C and 60 °C and 70% speed. Error bar is showing standard deviation error with 95% confidence interval.

According to the *Figure 17*, in each concentration the curves at different temperatures (i.e. 20 °C and 60 °C) lie in the error range, depicting that there is no effect of temperature on the viscosity of 0.05% and 0.1% CMC solutions.

From the results observed above, there is no effect of time, speed and temperature on the viscosity of CMC solutions at low concentrations.

4.2.1.2. High Concentration

4.2.1.2.1. Viscosity

To understand the effect of temperature and time on the viscosity of CMC solution at higher concentration, apparent viscosity (at 100 S⁻¹) versus time is plotted. In *Figure 18*, the apparent viscosity of 1% CMC solution is illustrated during mixing time at two different temperatures, 20 °C and 60 °C and at 70% mixing speed.

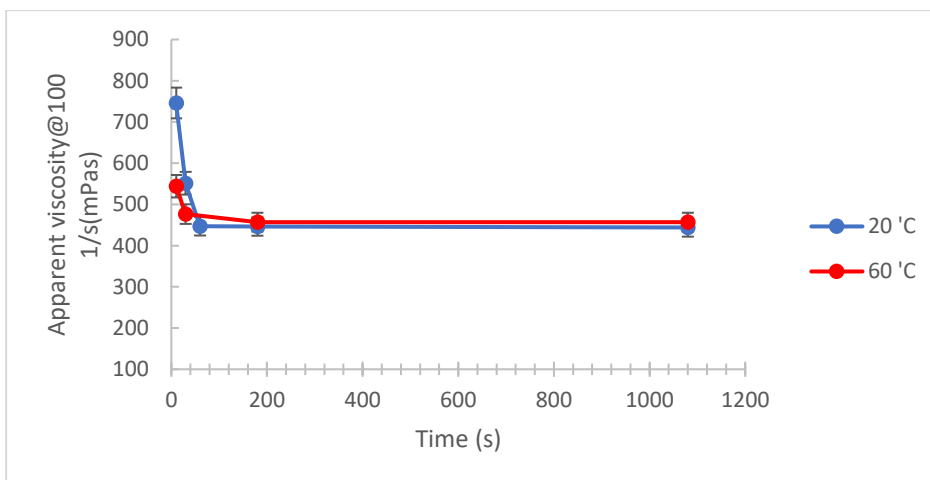


Figure 18: Apparent Viscosity of 1% CMC solution at 100 1/S versus mixing time at 70% speed at different temperatures (20 °C and 60 °C). Error bar is showing standard deviation error with 95% confidence interval.

As it is shown in *Figure 18*, in 1% of concentration of CMC solution, at 20 °C there is an abrupt loss in viscosity, during the initial time of mixing (up to 60 seconds) and then the viscosity remains stable. However, at 60°C, there is a gradual decrease in viscosity (up to 30 seconds) observed and after that the viscosity remains stable.

At initial mixing time (up to 60 seconds), the viscosity of the solutions prepared at 60°C is lower than viscosities at 20°C. While, after 60 seconds of mixing there is no difference in viscosity of the CMC solutions observed at two different temperatures (20°C and 60°C).

CMC is an anionic gum due to the presence of carboxymethyl groups. When CMC is dispersed in water, the polar groups will participate with the Hydrogen bonding of water. As the polymer is highly charged, the electrostatic repulsion between molecules arises. As a result, the polymer swells occupying a large apparent volume fraction and hence increase in viscosity is observed (Hoeffler, A.,2004). This peak viscosity is reached in initial mixing time. Further mixing results in the orientation of CMC molecules in the direction of the flow in the solution and thus, a decrease in the viscosity is observed from 10 seconds to 60 seconds. This can also be the reason why there was no viscosity change in low concentration, since no samples were taken before 180 seconds (depicted in *Figure 16 and 17*).

In higher temperature, the viscosity of 1% CMC solution is lower. The reason can be since in high temperature dissolution of CMC occurs more rapidly in the solution. Thus, the viscosity is lower at higher temperature (60 °C), when compared to the viscosity at lower temperature (20 °C).

4.2.1.2.2. Texture Analysis

Figure 19 shows the Stevens values of CMC solutions during the mixing time at temperatures 20 °C and 60 °C.

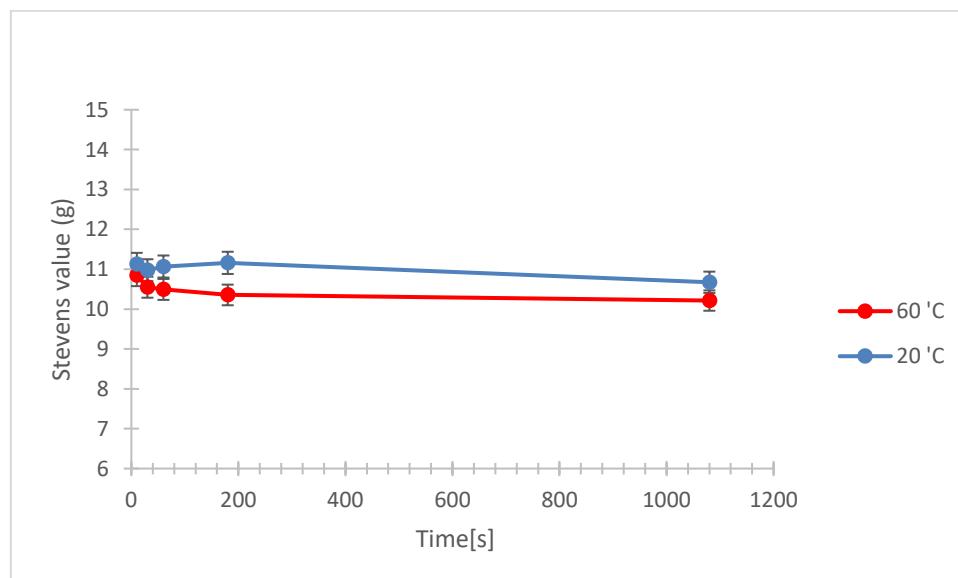


Figure 19. Stevens value for 1% CMC solutions as the function of time at 20°C and 60 °C, at 70% speed. Error bar is showing standard deviation error with 95% confidence interval

According to the *Figure 19*, at a specific temperature, there is a no effect of mixing time observed on Stevens values of CMC solutions. The reason speculated is due to the ability of CMC molecules to regain their structure at rest.

Preparation of the gum in higher temperature gives a lower Stevens value to the CMC solutions. The reason is because of the rapid dissolution of the molecules in higher temperature.

4.2.1.2.3. Yield Stress

Yield stress of CMC solution prepared at 10 seconds of mixing was measured by using Amplitude oscillatory sweep method in rheometer. The result is depicted in *Figure 20*.

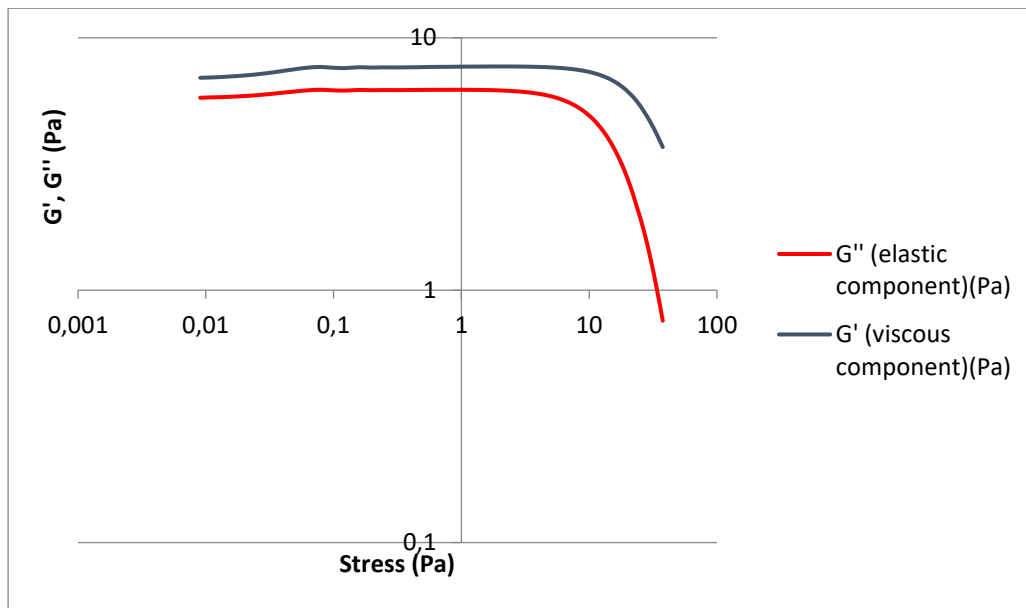


Figure 20: Amplitude Oscillatory sweep strain controlled of 1% CMC solution mixed for 10 seconds at speed of 70% and 60 °C

As shown in *Figure 20*, viscous component (G') in CMC solution is dominating the elastic component (G''). Also, there is no cross-over point seen for G' and G'' , showing that there is no yield stress in 1% CMC solutions.

4.2.2. Food Matrix

The food matrix used for CMC is Apple juice. *Table 13* displays the test conditions for preparation the apple juice containing CMC in the High Shear Mixer. The apple juice was continuously mixed in High Shear Mixer while altering the temperature during the preparation. Samples were taken at different mixing times at different stages of preparation.

Table 13: The test conditions for apple juice containing CMC

CMC concentration(%)	Mixing speed (%)	Temperature (°C)	Mixing time(s)
0.05	70	20	10-120
		80	250-270-300-360-420
		40	600

Figure 21 shows the apparent viscosity of apple juice containing 0.05% CMC during the mixing time. The samples were collected during the process of making the apple juice in order to understand the effect of temperature and mixing time on apparent viscosity of apple juice. Initial mixing is done at 20 °C for about 120 seconds, then the system is heated from 20°C to 80°C and is cooled down to 40°C.

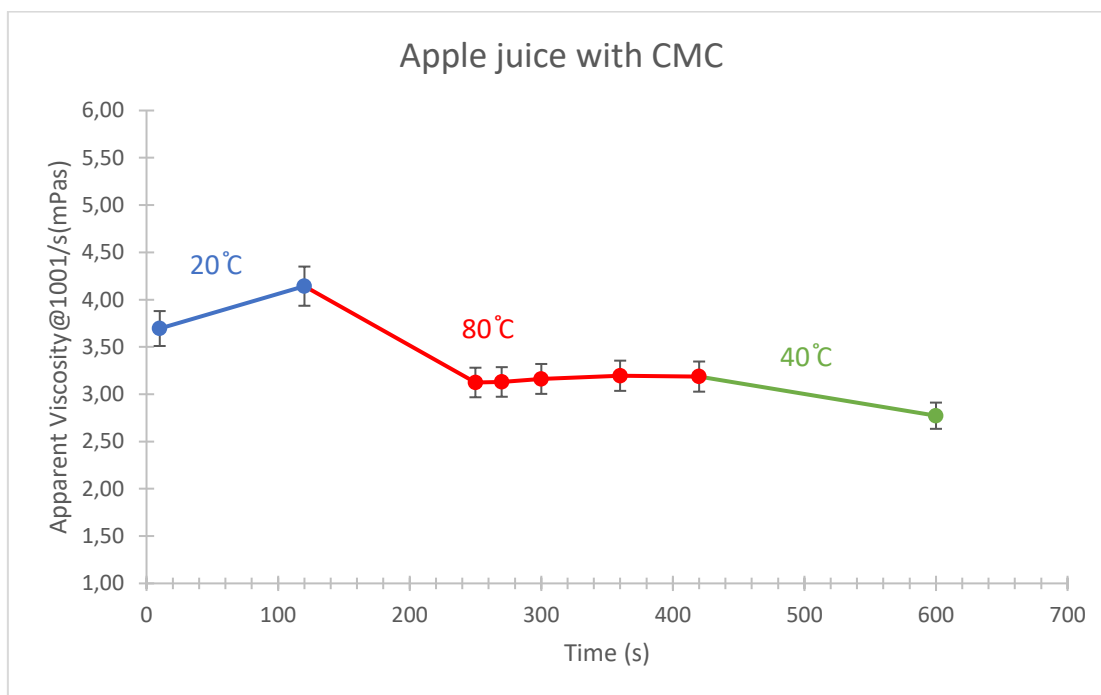


Figure 21: Apparent Viscosity of Apple juice containing 0.05% CMC at 100 1/s versus mixing time in different Temperatures (20 °C, 80 °C and 40 °C). Error bar is showing standard deviation error with 95% confidence interval.

According to the Figure 21, at 20 °C, where the dry ingredients were being dispersed in the aqueous phase, there is no change in viscosity observed as the points lie in the measurement range. When the system is heated from 20 °C to 80 °C, there is a drastic drop in viscosity and after that the viscosity remains stable.

Table 14 depicts the apparent viscosity of the apple juice containing 0.05% of CMC and 0.05% CMC in water after 3 minutes of heating while mixing.

Table 14: Apparent viscosity of CMC solution and apple juice.

Matrix	Time(s)	Concentration (%)	Temperature (c)	pH	Viscosity @ 100s ⁻¹ (mPas)
Water	180	0.05	60	7.8	5.94
Apple juice	180	0.05	80	2.8	3.19

The viscosity of the apple juice at 180 second of mixing at 80° C is lower than CMC solution mixed for 3 minutes at 60°C. The reason for the decrease can be due to the pH differences. The pH of apple juice is 2.8 while the pH of the tap water was about neutral (7.8). pKa value for CMC is reported as 4.3 (Metodiev Zhivkov, 2013). In other words, in solutions with pH lower than 4, the CMC molecules start to lose their charges and thus, CMC is not completely soluble in water. Resulting in a decrease in volume occupation of CMC and decrease in viscosity of apple juice Moreover, the apple juice was prepared in higher temperature (80 °C) than water matrix (60 °C), but as it was observed in the water matrix, there is no effect of temperature on the viscosity of the 0.05% CMC solutions after 3 minutes (*Figure 17*), the viscosity loss in apple juice cannot be considered due to the temperature.

Overall, the viscosity of 1% of CMC in water decreased by increasing the time and temperature of mixing from 10 seconds to 60 seconds. After 60 seconds the viscosity of the system remains stable. At low concentrations since the samples were taken after 180 seconds, thus, there was no effect of mixing time, mixing speed and temperature on the viscosity. No yield stress was observed for 1% CMC in water as the viscous component of CMC was higher than the viscous component. Apple juice containing 0.05% CMC showed lower apparent viscosity comparing to 0.05% CMC in water, the viscosity loss was due to the low pH in apple juice.

4.3. Pectin

4.3.1. Water-matrix

In order to understand the effect of pectin in water, 0.2% pectin solution was prepared in water. Shear Stress was plotted as a function of shear rate as shown in *Figure 22*.

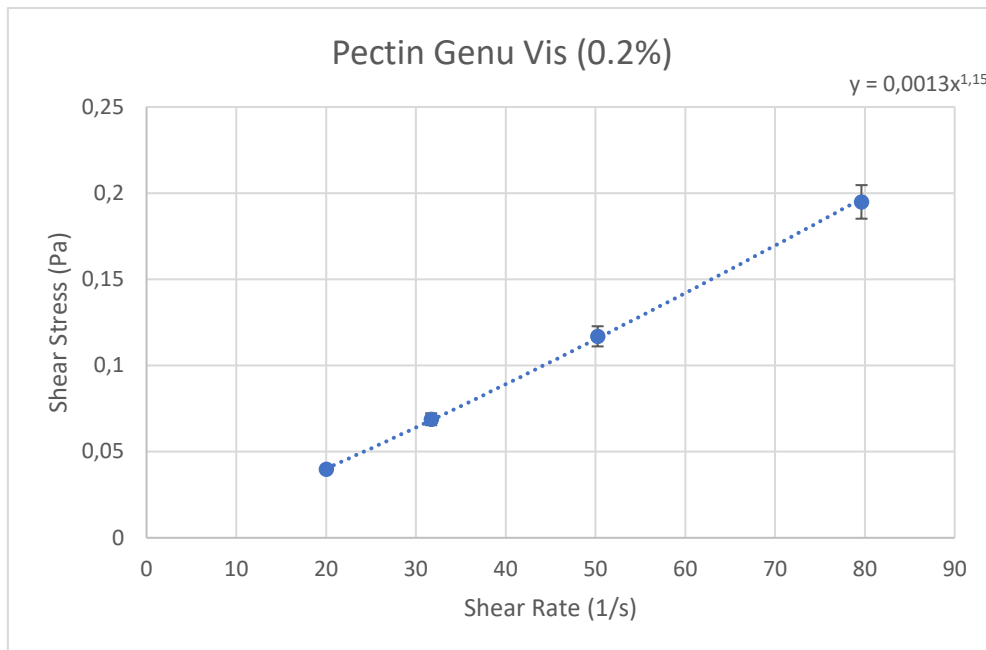


Figure 22: Plot of Shear Stress (Pa) versus Shear Rate (1/s) for Pectin Genu Vis (0.2%) in water. Error bar showing the Standard deviation error with 95% confidence interval.

Viscosity analysis was done on the samples (*Figure 22*) obtained after three minutes of mixing in High Shear Mixer at 60 °C. From the Power Law equation, K value and viscosity obtained was very low, i.e. 0.0013 and the 2,6 mPas respectively. The viscosity after the addition of the gum was very close to that of water (1,37 mPas). High methyl ester pectin forms a network only at lower pH and in the presence of sugar. (Phillips and Williams, 2014) Due to which, there was no viscosity changes seen in the pectin-water solution. Thus, further trials with varying temperature, speed and concentration were not done on water-matrix for Pectin.

4.3.2. Food Matrix

The food matrix trials done with Pectin were on apple juice. The reason why the chosen food matrix was apple juice is due to the reason that Pectin works best at lower pH and in product containing sugar, thus making apple juice a suitable food matrix to work with. Commercially pectin is used in a concentration range of 0.1 to 0.3% (*Table 1*), but in these experiments 2% concentration of pectin was used for understanding the behaviour of pectin at higher concentration. Test conditions for pectin in food matrix is given in *Table 15*.

Table 15. Test conditions for apple juice using Pectin.

Concentration (%)	Mixing speed (%)	Temperature (°C)	Time (s)
0.2	70	20	10, 120
		80	240, 250, 270, 360, 420
		40	600
2	70	20	10, 120
		80	240, 250, 270, 360, 420
		40	600

4.3.2.1. Low Concentration

Figure 23 shows the apparent viscosity of apple juice containing 0.2% Pectin as a function of mixing time. The samples were collected during the process of making the apple juice in order to understand the effect of temperature and mixing time on apparent viscosity of pectin in apple juice. Initial mixing is done at 20°C for about 120 seconds, then the system is heated from 20°C to 80°C and is cooled down to 40°C.

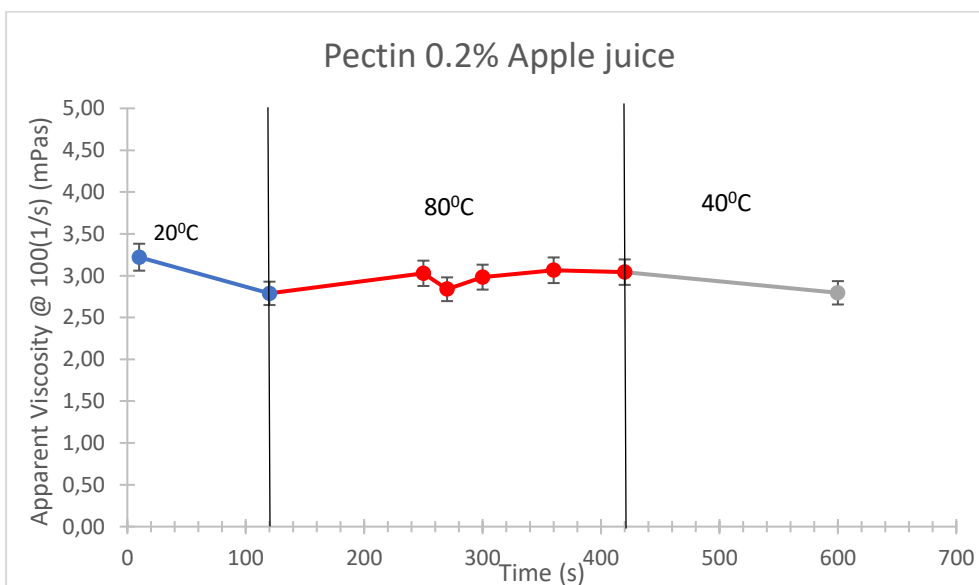


Figure 23: Apparent Viscosity @ 100 (1/s) (mPas) versus time (sec) for Pectin (0.2%) at different Temperatures (20°C, 80°C and 40°C). Error bar is showing the Standard deviation error with 95% confidence interval.

From Figure 23, at lower concentration the apparent viscosity of apple juice is very low i.e. in the range of about 3 mPas. There is no change in viscosity seen when the system was heated and cooled down. Rheometer used (Malvern) for measuring the viscosity when using bob and cup equipment, does not give precise values for low viscous fluids. For such low viscosities a double-gap is recommended. Thus, it can be concluded that the values obtained for apple juice with pectin at lower concentration might not be precise as the viscosity is very low.

4.3.2.2. High Concentration

4.3.2.2.1. Viscosity

Figure 24 shows the effect of apparent viscosity as a function of mixing time for pectin in High Concentration (2%). The samples were collected during the process in order to understand the effect of temperature and mixing time on apparent viscosity of pectin in high concentration. Initial mixing is done at 20°C for about 120 seconds, then the system is heated from 20°C to 80°C and is cooled down to 40°C.

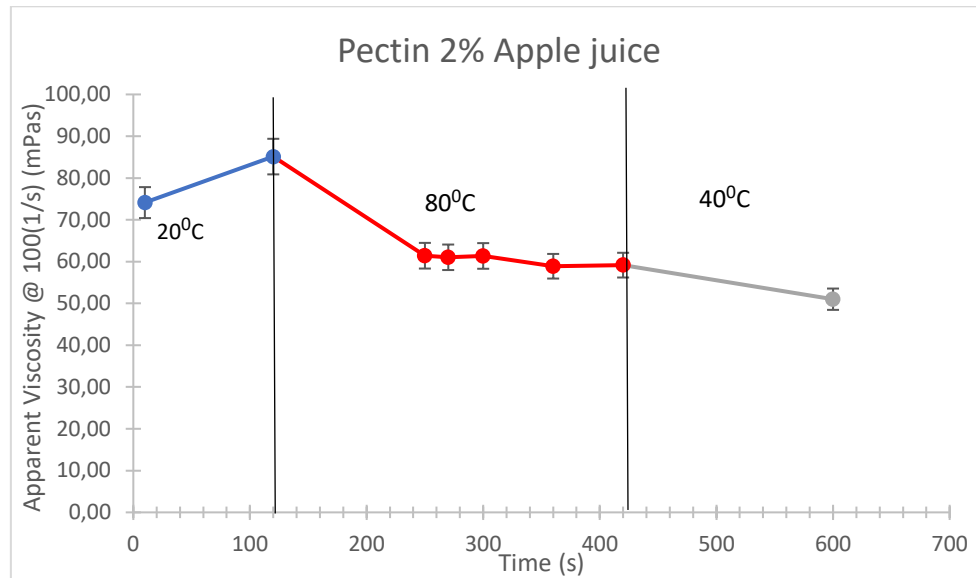


Figure 24: Apparent Viscosity @ 100 (1/s) (mPas) versus time (sec) for Pectin (2%) at different Temperatures (20°C, 80°C and 40°C). Error bar showing the Standard deviation error with 95% confidence interval.

At higher concentration of pectin (2%), the highest viscosity was seen after 2 minutes of mixing as shown in Figure 24. This increase in viscosity arises due to pectin molecules occupying a fraction of volume in the sample. The association of HM pectin (eventually leading to gel formation) is caused by a reduction in water activity (caused by sugar) promoting association of the polymer chains which rather want to interact with themselves than to the solvent molecules. The other contribution is a reduced pH which further diminishes the interaction with the solvent molecules and thus, promotes chain association.

After heating was applied and 80 °C was reached there was a drop in the viscosity from around 85 mPas to 65 mPas. When the temperature increases, there is a decrease in the hydrogen bond of water and sucrose in the system, which in turn, results in decrease of the specific volume or the occupied volume by the pectin molecule, causing the viscosity drop in the solution. (Muhidinov et al., 2010) (Lopes da Silva, Gonçalves and Rao, 1994) Thus, it can be concluded that viscosity of pectin is highly dependent on change in temperature. When heat is applied to the pectin slurry, decrease in viscosity is observed.

For the final product, when it was cooled in the High Shear Mixer from 80 °C to 40 °C, the viscosity reduced from 60 mPas to 51 mPas respectively. It is speculated that, while cooling the solution, continuous shear is applied on the product which causes the breaking of the network that is created with pectin in the food matrix.

4.3.2.2.2. Texture

Figure 25 shows the plot of Stevens value versus time for Pectin at 2% concentration. Stevens value was measured for all the samples collected during the preparation process to understand the effect of temperature or time of mixing.

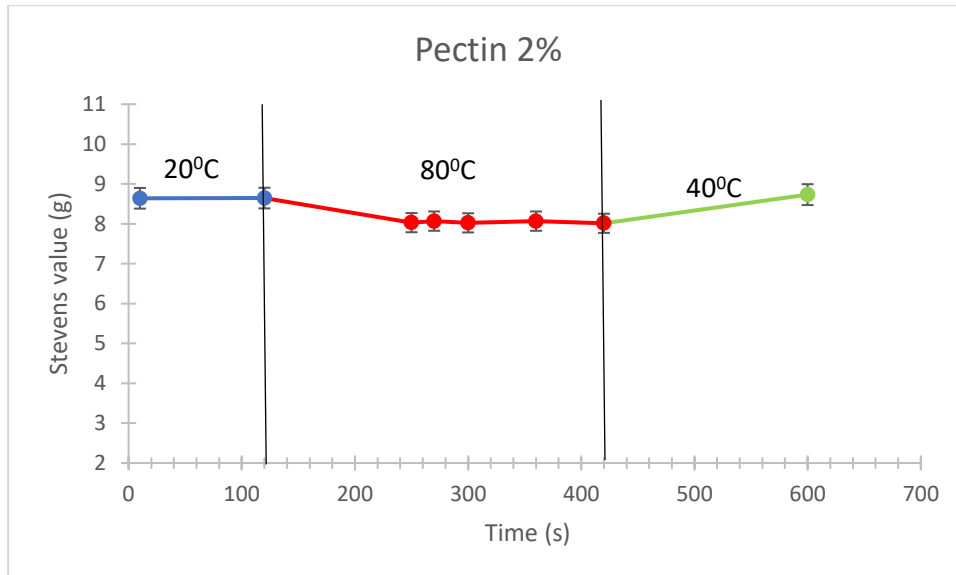


Figure 25: Stevens value (g) versus time (sec) for Pectin (2%) at different Temperatures (20 °C, 80 °C and 40 °C). Error bar is showing the Standard deviation error with 95% confidence interval.

There is a slight decrease in the Stevens value when the Pectin solution is heated from 20 °C to 80 °C as shown in Figure 25. The same trend is seen with the apparent viscosity of the solution. But, when the product is cooled in the High Shear Mixer, we see a slight increase in Stevens value of the sample after cooling down from 80 °C to 40 °C, while in the Figure 24, it is observed that the viscosity of the sample at 40 °C is the lowest. The possible explanation for this could be, the measurement error while measuring the viscosity or the texture of the samples.

Pectin requires presence of sugar and low pH to form a network and thus, in water matrix Pectin showed a very low viscosity, close to that of water. Thus, no trials were performed in water matrix. However, in apple juice, at low concentration i.e. 0.2% of pectin which is used commonly for commercial products, no effect of mixing time, temperature and speed was observed. Though, at high concentration of pectin (2%), with an increase in temperature, decrease in viscosity was observed. Also, there was a slight reduction in apparent viscosity observed as the mixing time increases.

4.4. Gellan

4.4.1. Water Matrix

For the water-matrix the concentration chosen for gellan was very low as the application to be tested on gellan was chocolate milk and the recommended concentration of gellan in chocolate milk is 0.12%. But, to understand the functionality of gellan at higher concentration, 1% of gellan was prepared as well. Test conditions used in the case of gellan is given below in Table 16.

Table 16: Test conditions for Gellan solutions in water.

Concentration (%)	Temperature (°C)	Speed (%)	Mixing Time (s)
0.1	60	70	10, 30, 180, 360, 1080
		90	180, 360, 1080
	80	70	180, 360, 1080
		90	180, 360, 1080
0.15	60	70	10, 30, 180, 360, 1080
		90	180, 360, 1080
	80	70	180, 360, 1080
		90	180, 360, 1080
1	60	70	10, 30, 60, 180, 1080
	80	70	10, 30, 60, 180, 1080

4.4.1.1. Low concentration

4.4.1.1.1. Viscosity

Effect of Mixing speed: In order to test the effect of mixing speed on the viscosity of the gum solution, plots were made based on apparent viscosity (at 100s^{-1}) versus function of time. *Figure 26* depicts the viscosity of the gum solution at different concentration and different speed at a constant temperature of $60\text{ }^{\circ}\text{C}$.

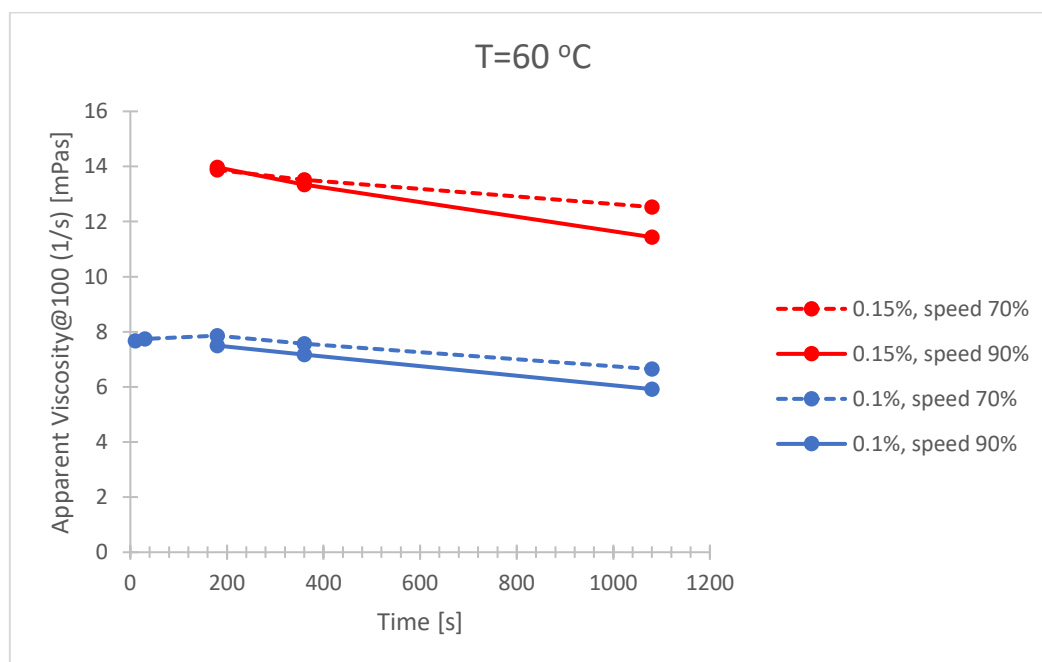


Figure 26. Apparent Viscosity as a function of time at Temperature 60°C for various concentration of gellan gum solution at different mixing speed. Error bars showing Standard deviation error with 95% confidence interval.

From *Figure 26*, it can be observed that at different speed of the mixer there is no significant difference in viscosity of the gum solution for each of the concentration. Also, the curves at different shear speed (i.e. 70% and 90%) lie in the measurement error range. Thus, depicting that there is no effect of mixing speed on gellan. Because we did not see any difference in speed for lower concentration, we decided to only run one speed i.e. 70%.

Effect of Temperature: In order to test the effect of temperature on the viscosity of the gum solution, plots were made based on apparent viscosity (at 100s^{-1}) versus function of time. *Figure 27* depicts the viscosity of the gum solution at different concentration and different temperature at a constant shear speed of 70%.

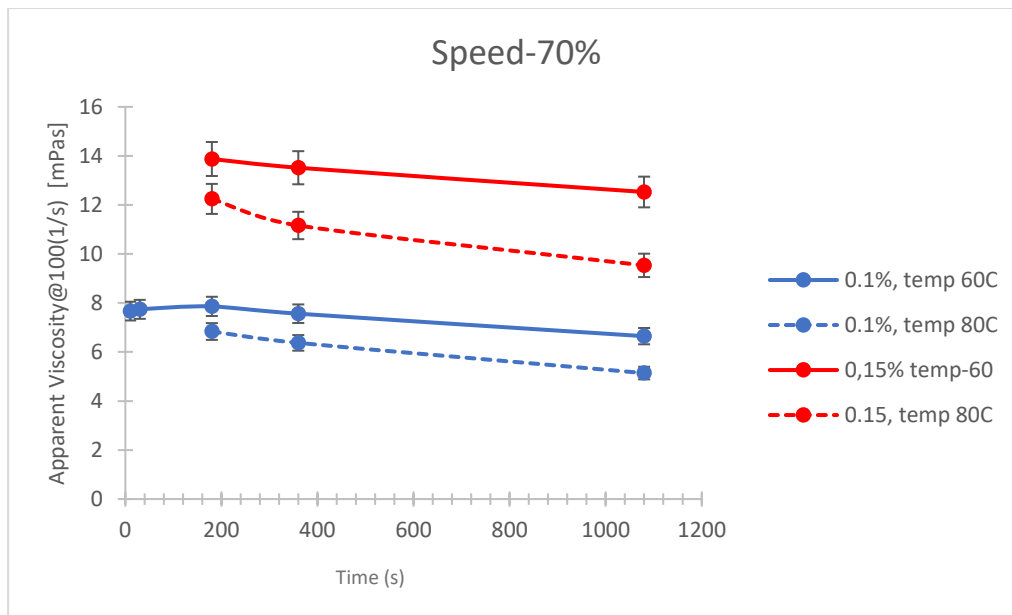


Figure 27. Apparent Viscosity as a function of time at Speed 70% for various concentration of gellan gum solution at different temperature. Error bars showing Standard deviation error with 95% confidence interval.

From *Figure 27*, it is observed that at higher temperature, the viscosity of the gum solution is affected by temperature. The viscosity of the gum solution at higher temperature is lower because the increase in temperature causes the aggregates formed by gellan molecule to orient itself in the direction of the flow and thus results in reduced viscosity of the final product (AlsaidYagoub and Osman M. Nur, 2013). Also, the curves at different temperature (i.e. $60\text{ }^{\circ}\text{C}$ and $80\text{ }^{\circ}\text{C}$) do not lie in the measurement error range.

Effect of mixing time: *Figure 26, 27* depict the apparent viscosity at 100 s^{-1} versus time for different concentration (lower concentration: 0.1%, 0.15%) for gellan. The trials are done at two different temperatures $60\text{ }^{\circ}\text{C}$ and $80\text{ }^{\circ}\text{C}$ and two different speeds 70% and 90%. The basic findings from the above graphs are that between 10 seconds and 360 seconds the viscosity is constant and then there is a decrease in viscosity after 360 seconds of mixing. When the gum is hydrated in the solution it starts forming a gel network which gives an increase in the viscosity. But, due to the

continuous shearing of the gellan-slurry in the mixer, the network created by gellan is broken continuously and thus loss in viscosity is observed.

4.4.1.2. High Concentration

4.4.1.2.1. Viscosity

To understand the effect of temperature and time on the viscosity of gellan solution at higher concentration, apparent viscosity (at 100s^{-1}) versus time is plotted. *Figure 28* depicts the apparent viscosity of gellan at 1% concentration for two different temperature 60°C and 80°C .

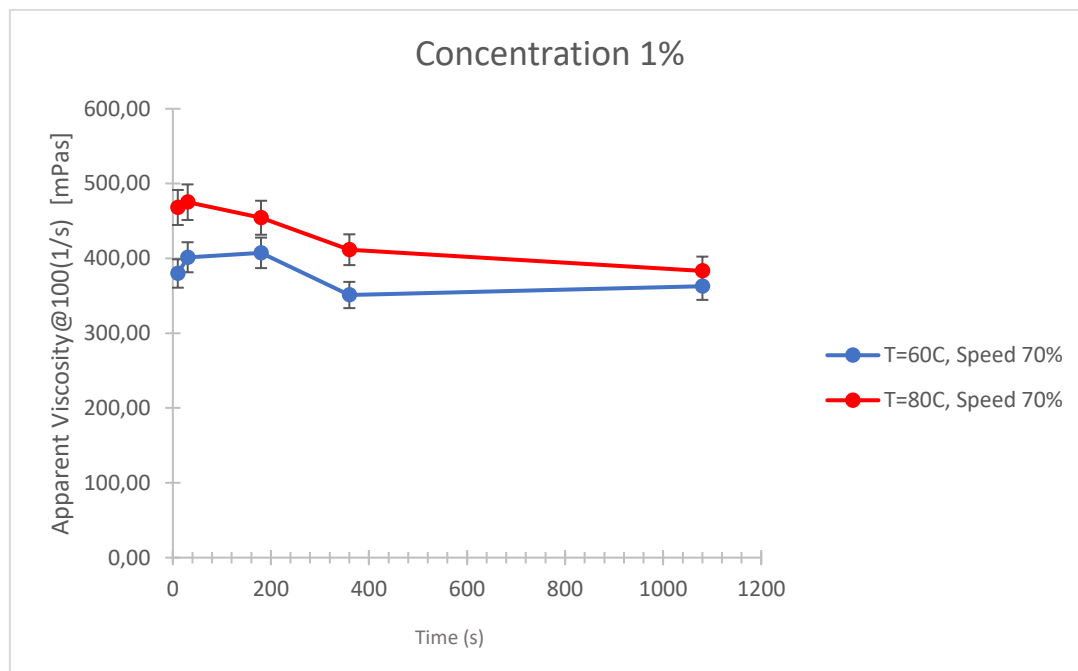


Figure 28: Apparent Viscosity @ 100 (1/s) (mPas) versus time(s) for Gellan at high concentration (1%) for two different temperature. Error bars showing Standard deviation error with 95% confidence interval.

At higher concentration of gellan, viscosity at 80°C is higher than that at 60°C (*Figure 28*). The reason behind the increase in viscosity at higher temperature for 1% gellan is because the amount of gellan added to the system is very large and at high temperature gellan solubilises and hydrates completely forming the gel where coil-helix transition occurs. Though at lower temperature, due the large volume there is an incomplete hydration of gellan added in the system, as the hydration temperature for High Acyl gellan gum is above 70°C (Phillips, G. and Williams, P., 2014).

From *Figure 28*, with the increase in time of shearing, over a time period between 10s and 180s there is no decrease in viscosity observed. Between 180s and 360s there is a drop-in viscosity observed. A similar trend was observed in the low concentration as well in Section 4.4.1.1.

4.4.1.2.2. Texture

Figure 29 shows the plot of Stevens value versus time at two different temperature 60°C and 80°C for 1% concentration of gellan.

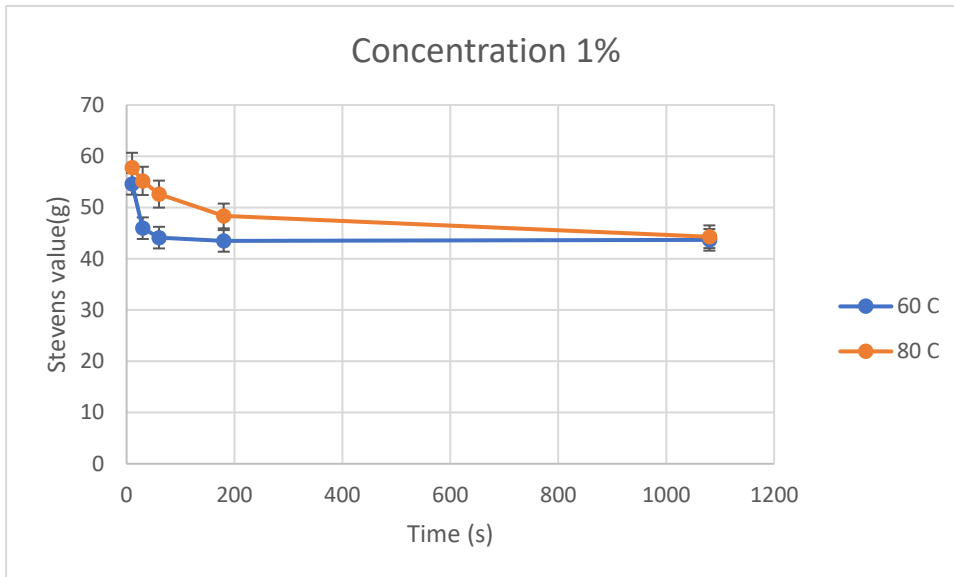


Figure 29: Stevens value versus time for Gellan at 1% concentration made at two different Temperature (60°C and 80°C). Error bars showing Standard deviation error with 95% confidence interval.

The Stevens value certainly reduces with the increase in time of shearing as seen in Figure 29. The Stevens value observed at higher temperature (80 °C) is higher when compared to lower temperature (60 °C). The curves at different temperature (i.e. 60 °C and 80 °C) do not lie in the measurement error range, other than the first and the last point. A similar trend can be seen with the change in viscosity with respect to time depicted in Figure 28. The highest Stevens value is obtained after mixing or dispersing the gum for less than 100 seconds and post that there is a reduction in the texture seen. This loss in texture is due to the long-time of mixing of the gum-solution continuously breaks the gel formed by gellan molecules.

4.4.1.2.3. Yield Stress

Yield stress measurement was done on samples of gellan with a concentration 1% for two different temperature 60°C and 80°C as shown in Figure 30 and 31.

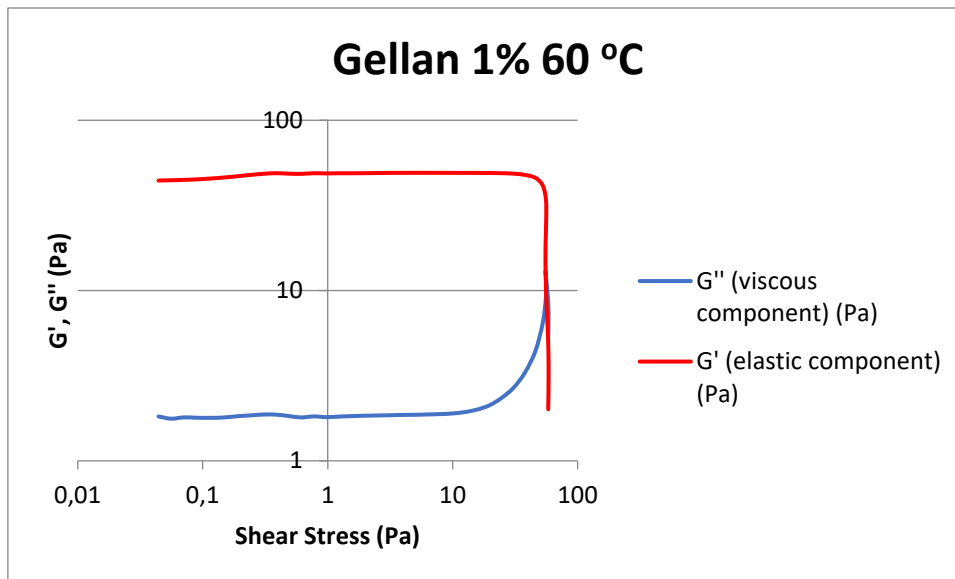


Figure 30: Oscillatory Amplitude Sweep Curve for Gellan 1% concentration at 60C.

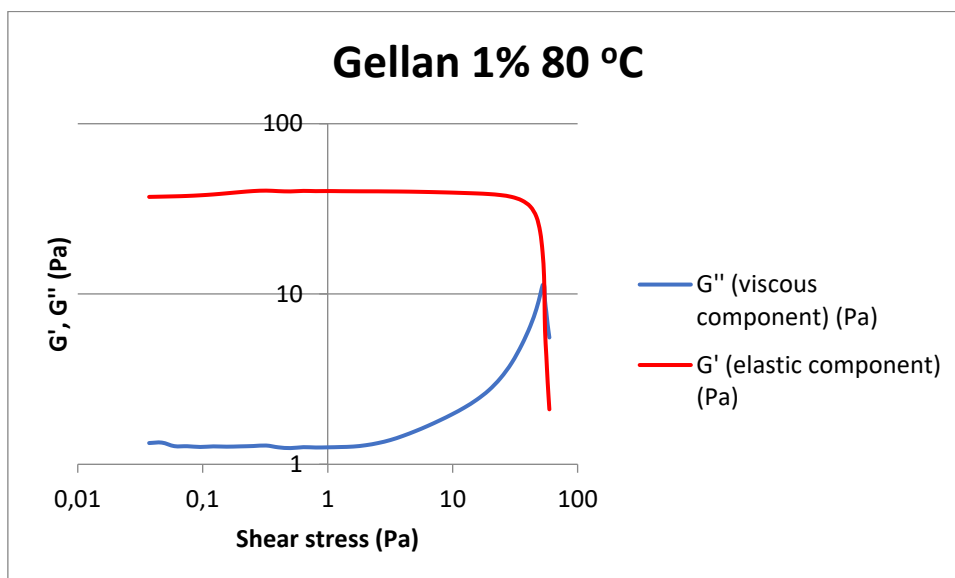


Figure 31: Oscillatory Amplitude Sweep Curve for Gellan 1% concentration at 80C.

In both *Figure 30 and 31*, we see that the elastic component is higher than the viscous component which signifies that the material has a more elastic behaviour or structure than flowing properties, i.e. it has more solid properties than liquid properties at rest. The yield stress value for gellan at 60 °C and 80 °C are 53.7 Pa and 55.6 Pa respectively. The same trend was seen for the apparent viscosity for gellan at 1% shown in Section 4.4.1.2.

4.4.2. Food-Matrix

Gellan has a wide application is neutral pH beverages and thus it is used in Chocolate milk for stabilising the cacao particles. The amount of gellan used in chocolate milk is very low, i.e. 0.12%. To understand the functionality of gellan in chocolate milk, viscosity and stability tests were performed and a comparison between food and water matrix was done.

To understand the behaviour of gellan in food matrix and water matrix, plots were made based on the apparent viscosity versus time for gellan in water matrix (0.1% and 0.15%) and gellan in chocolate milk (0.12%) as shown in *Figure 32*. Also, both homogeneous and non-homogeneous chocolate milk containing gellan were plotted to observe the effect of homogenization on gellan molecules.

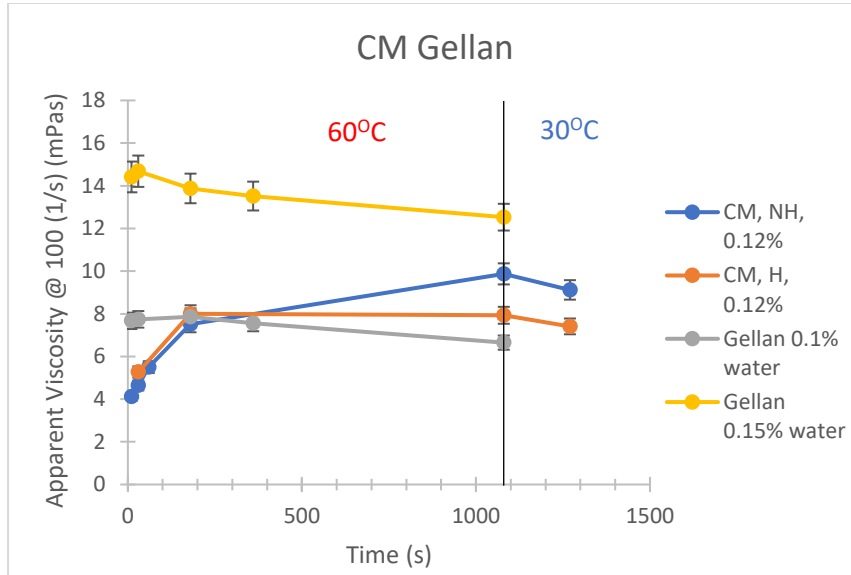


Figure 32: Apparent Viscosity @100 s(1/s) as a function of time for a) CM, NH, 0.12% (Chocolate Milk, Non-homogenised, 0.12% gellan), b) CM, H, 0.12% (Chocolate Milk, Homogenised, 0.12% gellan), c) Gellan 0.1% in water, d) Gellan 0.2% in water. Error bars showing Standard deviation error with 95% confidence interval.

In order to understand the dispersion behaviour of gellan molecules in water and food matrix, *Figure 32* is zoomed in, which can be seen in *Figure 33*.

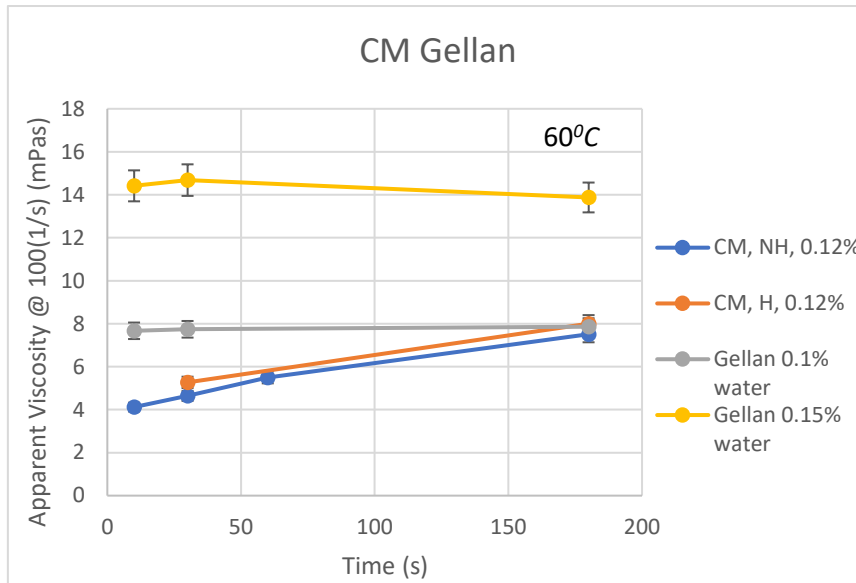


Figure 33. Zoomed in depiction of figure X above. Apparent Viscosity @100 s(1/s) as a function of time for a) CM, NH, 0.12% (Chocolate Milk, Non-homogenised, 0.12% gellan), b) CM, H, 0.12% (Chocolate Milk, Homogenised, 0.12% gellan), c) Gellan 0.1% in water, d) Gellan 0.2% in water at 60°C. Error bars showing Standard deviation error with 95% confidence interval.

Initial Dispersion: In Figure 33, the viscosity produced by 0.12% of gellan in milk matrix is lower than that of 0.10% in water matrix for up to about 200 second of mixing. Gellan is dispersed directly in the milk and it gets hydrated during heating. Though, dissolved solids interfere or inhibit in the hydration process of the gum (Phillips, G. and Williams, P., 2014). This can be seen in the curves above as lower viscosity is observed for chocolate milk in the beginning of the process. But after mixing it for longer time and at 60 °C, network can be achieved and increase in viscosity is observed (Figure 32).

Hydration: In Figure 32, the viscosity of gellan (0.12%) in the milk matrix increases after about 200 seconds of mixing, while there is a slight reduction, or no change observed in viscosity of gellan (0.1%) in the water matrix. As the mixing time increases the gum starts getting hydrated in the food matrix and helix formation of gellan molecules occurs, causing an increase in the apparent volume fraction and therefore, increasing the viscosity of the system.

Cooling: When the temperature is reduced in the mixer from 60 °C to 30 °C there is a slight reduction in the viscosity of the chocolate milk (Figure 32). Cooling the milk while continuous shear is applied on the product causes the breaking of the network that is created with gellan in the food matrix (Sworn, Sanderson and Gibson, 1995).

Homogenisation: In the above Figure 32 and 32, there is a difference in the viscosity of homogenized and non-homogenized samples of chocolate milk containing 0.12% of gellan. It is expected that when chocolate milk undergoes homogenization, there is an increase in the viscosity of the chocolate milk because the size of the particle dispersed in the chocolate milk reduces after homogenization which thus, contributes to the increase in the viscosity of the product (Clark et al.,

2009). But, in this case, it is seen that the viscosity of chocolate milk is higher when it is not homogenized and lower when the homogenisation is done. The reason speculated for the loss of viscosity when chocolate milk is homogenised could be associated with the gum. When the sample containing gellan gum is forced at a high pressure through a narrow gap in the homogenizer, gellan polymer breaks down (CP Kelco U S Inc, 1998). Homogenisation thus destroys the gellan gum leading to reduction in the viscosity.

Stability Test:

Figure 34 shows the height of sediment of cocoa particles obtained after centrifugation of chocolate milk containing 0.12% of gellan with and without homogenisation.

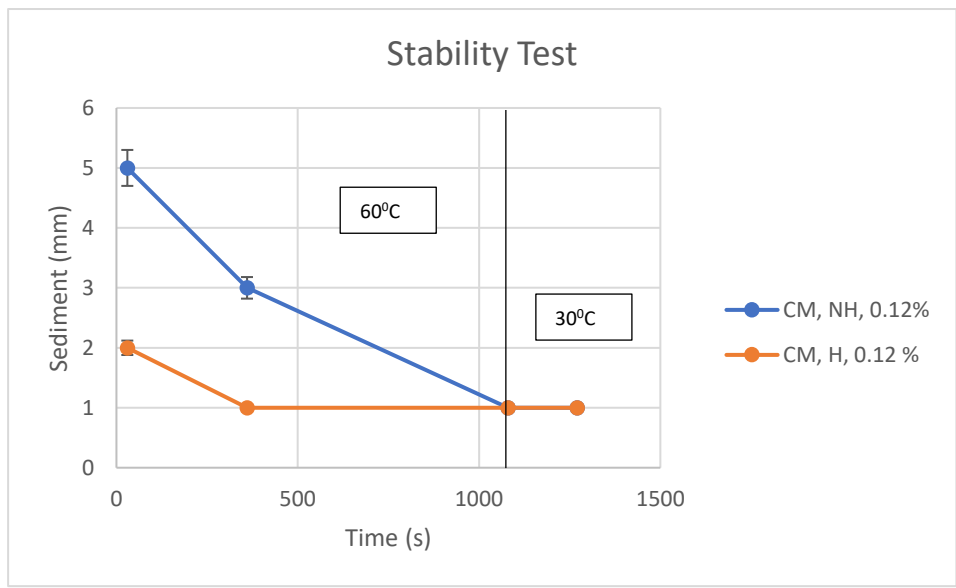


Figure 34: Height of the sediment obtained after centrifugation of chocolate milk as a function of time for a) CM, NH, 0.12% (Chocolate Milk, Non-homogenised, 0.12% gellan), b) CM, H, 0.12% (Chocolate Milk, Homogenised, 0.12% gellan). Error bars showing Standard deviation error with 95% confidence interval.

Stability of cocoa particle was tested with the help of centrifugation of the samples and measuring the amount of cocoa particle that were sedimented. Figure 34 shows the height of sediment after centrifugation of the samples at different time of mixing in the High Shear Mixer. For non-homogenised samples, the height of the sediment particles was comparatively higher for mixing time less than 200 seconds with respect to homogenised samples. Though, after the mixing was done for about 1000 seconds, both homogenised and non-homogenised samples have the same amount of sediment. For homogenised samples, the amount of sediment after 3 minutes of mixing was constant all through the process of heating and cooling.

Table A17 shows the height of sediment after centrifugation of the samples of chocolate milk with and without gellan. Non-Homogenised chocolate milk containing no gum had the highest deposition of the cocoa particles in the centrifuge tubes, while after homogenisation there was a little reduction in the deposition from 9 mm to 8 mm respectively. While, when 0.12% of gellan was added in the chocolate milk the

deposition of sediment reduced to 1 mm. There was not much difference seen in the homogenised and non-homogenised samples of chocolate milk containing gum. With the help of gums in a concentration of as less as 0.12% sedimentation of cocoa particles can be avoided as it forms networks in the chocolate milk which entraps the cocoa particles and does not let it sediment.

In conclusion, at lower concentration in water matrix, gellan does not show any effect of mixing speed but increase in temperature reduces the apparent viscosity of gellan solution. For both low and high concentration of gellan, at initial time of mixing the viscosity is constant and then there is a decrease in viscosity observed as the mixing time increases. While for higher concentration, at higher temperature observed viscosity is higher due to the fast solubilisation of gellan molecules. Also, the elastic component for 1% gellan solution is higher than the viscous component. Gellan is used in neutral pH beverages for its stabilizing properties. In the food matrix (chocolate milk) an increase of viscosity during the first 180 seconds of mixing, which was not observed in the water matrix. Further, when the gellan molecule were hydrated in chocolate milk, the viscosity continued to increase, unlike the water matrix. Finally, homogenisation of the chocolate milk lowers the viscosity due to the breaking of gellan molecules in the homogenizer. Mixing longer than 360 seconds in the mixer when including homogenisation would not improve the sedimentation. Not having a homogeniser requires mixing for 1080 seconds, which is a very long time especially when scaling up the tanks. Hence, homogenisation is required. Thus, presence of gellan in the chocolate milk slows down the process of sedimentation of cacao particles and makes the chocolate milk stable.

4.5. Carrageenan

4.5.1. Water matrix

Table 18 displays the test conditions for different concentrations (0.02%, 0.5% and 1%) of carrageenan in water. Carrageenan was dispersed in water at 20 °C for 120 seconds and the dispersion was heated up to 70 °C to be hydrated. Then the system was cooled down to 30 °C. The system was continuously mixed in 70% speed during the preparation time. Samples were taken at different times of mixing for different temperatures.

Table 18. Test conditions for Carrageenan solutions

Concentration (%)	Mixing speed (%)	Temperature (C)	Time (s)
0.02	70	20	120
		70	250-310-490-1570
		50	1810
		30	1930
0.5	70	20	120
		70	250-310-490-1570
		50	1810
		30	1930
1	70	20	120
		70	250-310-490-1570
		50	1810
		30	1930

4.5.1.1. Viscosity

The effect of mixing time and temperature: To understand the effect of temperature on the viscosity of Carrageenan solutions, the plot was made based on apparent viscosity at 100 s^{-1} versus mixing time during the preparation. Figure 35 illustrates the apparent viscosity of different concentrations of carrageenan solution during the mixing time at different temperatures ($20 \text{ }^\circ\text{C}$, $70 \text{ }^\circ\text{C}$, $50 \text{ }^\circ\text{C}$, $30 \text{ }^\circ\text{C}$) at 70% mixing speed.

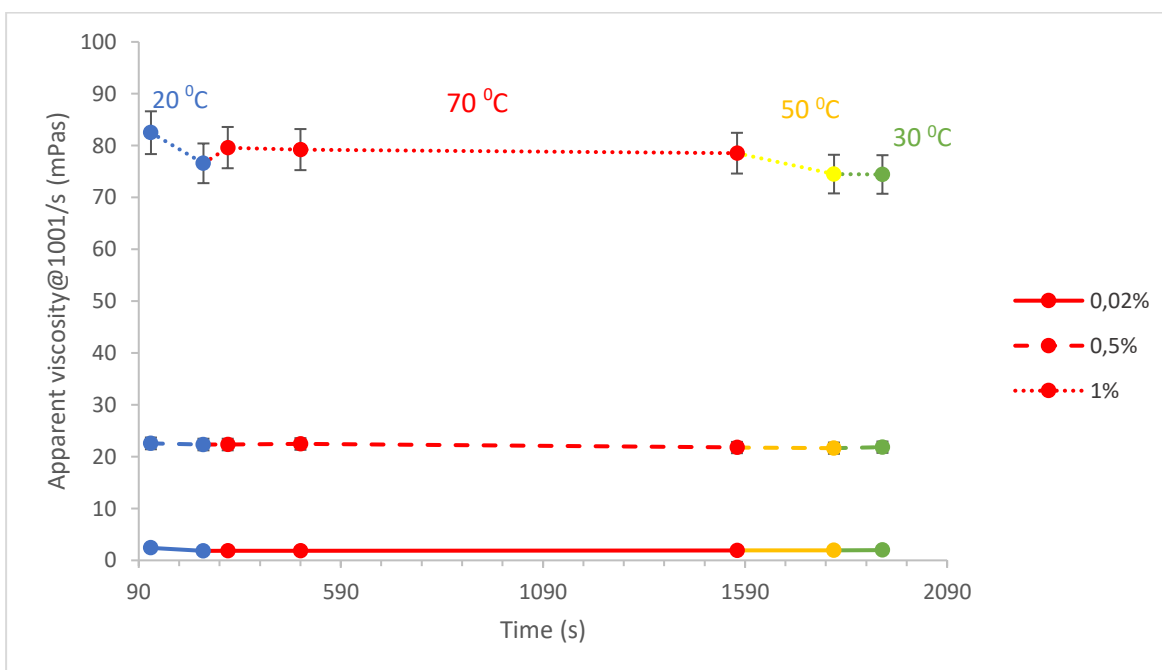


Figure 35. Apparent Viscosity of 1% (dotted line), 0,02% (simple line) and 0.5% (dashed line) carrageenan solutions during the mixing time at 20,70, 50, 30 °C at 70% speed. Error bar is showing standard deviation error with 95% confidence interval

According to *Figure 35*, different concentrations of carrageenan solutions (0.02%, 0.5% and 1%), is not affected by mixing time and temperature during the mixing in High Shear Mixer.

It is expected that temperature plays an important role in the functionality of kappa carrageenan. Heating the solution up to 70 °C and cooling down to 40 °C is of importance for the coil-helix confirmation transition in carrageenan molecules (Phillips and Williams, 2014). However, based on the result above, there is no effect of temperature observed on the viscosity of 1% carrageenan solution. The reason can be that when the system is heated, the dissolution and hydration of carrageenan molecules happens rapidly in water and thus, no viscosity change is observed due to heating (70 °C). Also, there was no effect of cooling observed.

4.5.1.2. Yield Stress

Yield Stress of 1% carrageenan solution tested by amplitude oscillatory method in rheometer. For this purpose, the final sample which was cooled down to 30 °C in high shear mixer was chosen. The result is illustrated in *Figure 36*.

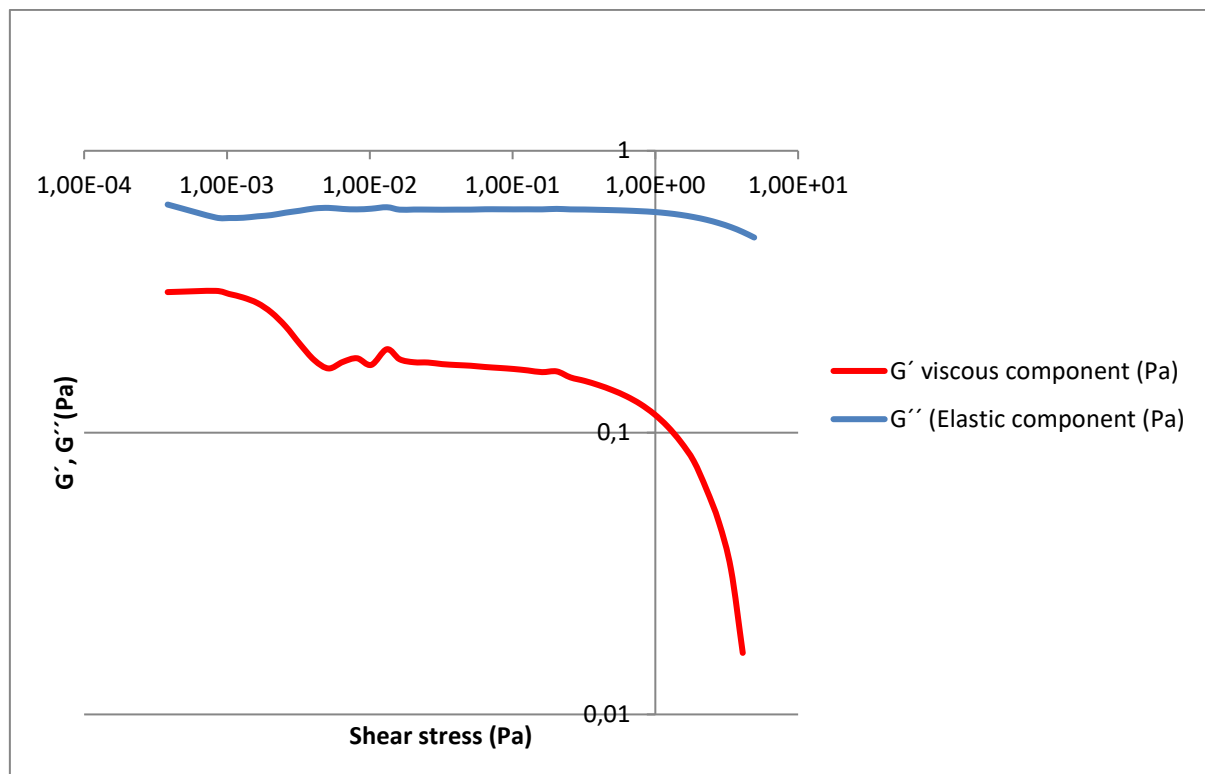


Figure 36. Amplitude Oscillatory sweep strain controlled of 1% Carrageenan solution prepared at mixing speed of 70%

From *Figure 36*, it is observed that the viscous component is dominating the elastic component in carrageenan depicting that, there is no elastic network formation in the 1% carrageenan solution, thus no yield stress.

4.5.1.3. Texture

Figure 38 illustrates the Stevens value of 1% carrageenan as a function of mixing time at different temperatures (20 °C, 70 °C, 50 °C ,30 °C) at 70% mixing speed.

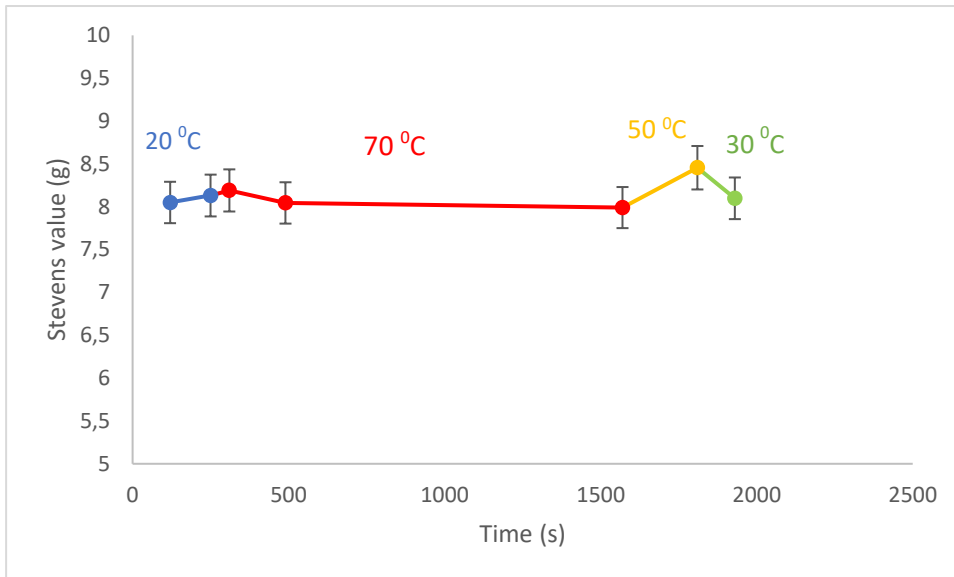


Figure 38: Stevens value of 1% carrageenan solutions during the preparation at 70% speed. Error bar is showing standard deviation error with 95% confidence interval.

According to Figure 38, there is no effect of temperature and mixing time on the Stevens value of 1% carrageenan solution observed as all the values lie in the measurement error range. This result follows the viscosity result and the reason is explained above.

4.5.2. Food-Matrix

Carrageenan has a wide application in neutral dairy beverages and thus, it is used in Chocolate milk as a stabilising agent. κ -Carrageenan interacts with the proteins that are present in milk (κ -casein). The amount of carrageenan used in chocolate milk is very low, i.e. 0.02%. The network formation that occurs in the milk is due to the interaction of carrageenan molecules with the κ -casein that aggregate into a three-dimensional network (Drohan et al., 1997).

To understand the behaviour of carrageenan in food matrix and water matrix, plots were made based on the apparent viscosity versus time for carrageenan in water matrix (0.02%) and carrageenan in chocolate milk (0.02%) as shown in Figure 39. Also, both homogeneous and non-homogeneous chocolate milk containing carrageenan were plotted to observe the effect of homogenization on carrageenan molecules.

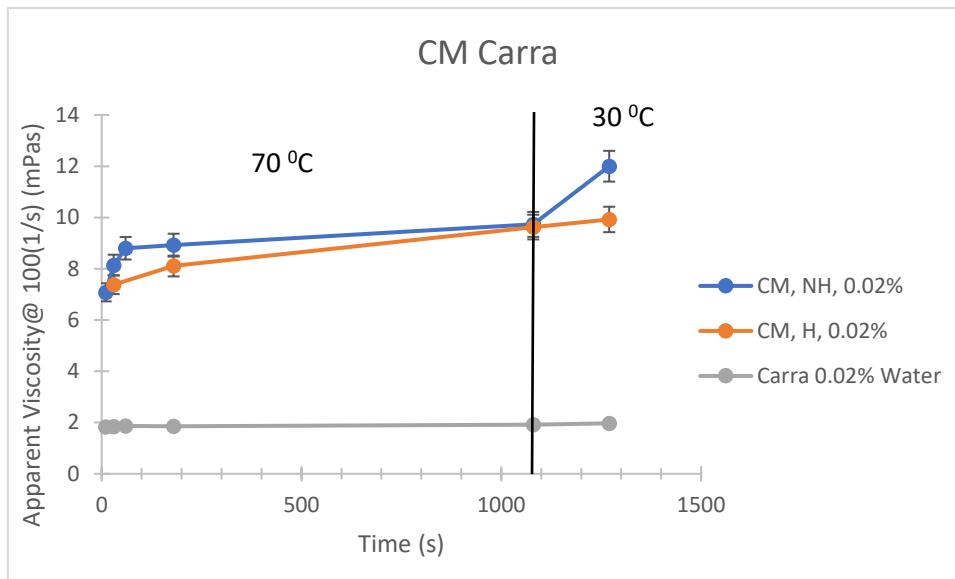


Figure 39. Apparent Viscosity @100 s(1/s) as a function of time for a) CM, NH, 0.02% (Chocolate Milk, Non-homogenised, 0.02% carrageenan), b) CM, H, 0.02% (Chocolate Milk, Homogenised, 0.12% carrageenan), c) Carrageenan 0.02% in water. Error bar is showing the Standard deviation error with 95% confidence interval.

Initial Dispersion: Carrageenan is easily dispersed in the milk matrix in less than 100 seconds. The viscosity of the solution increases with the increase in the time of mixing of chocolate milk in the High Shear Mixer. While in the water matrix, no change in the viscosity is observed at all, as the concentration of carrageenan is extremely low to form any viscosity in the water-matrix. But due to the presence of κ -casein in the chocolate milk, carrageenan molecules form a network even at a low concentration.

Hydration: In Figure 39, after 100 seconds, the viscosity of carrageenan (0.02%) in the milk matrix for non-homogenized sample increases while there is no change noticed in viscosity of carrageenan (0.02%) in the water matrix. As the mixing time increases the gum starts getting hydrated and they become more swollen which in turn, increases the viscosity of the system, as the carrageenan molecules starts resisting the flow (Imeson, 2010).

Cooling: When the chocolate milk is cooled from a temperature of 70 °C to 30 °C in the high shear mixer, carrageenan molecule is completely hydrated and a sudden increase in viscosity is observed in the chocolate milk. Carrageenan has a setting temperature of below 40 °C. Even though the cooling is done on the mixer and the chocolate milk undergoes continuous shear, the viscosity is not lost due to the network formed with the interaction of carrageenan molecule with the κ -casein present in the milk (Drohan et al., 1997).

Homogenisation: In the above graphs, there is a difference in the viscosity of homogenized and non-homogenized samples of chocolate milk containing 0.02% of carrageenan. Carrageenan shows the same behaviour as gellan mentioned in Section 4.4.2., i.e. lower viscosity when homogenized than without homogenisation.

Stability Test:

Figure 40 shows the height of sediment of cocoa particles obtained after centrifugation of chocolate milk containing 0.02% of carrageenan with and without homogenisation.

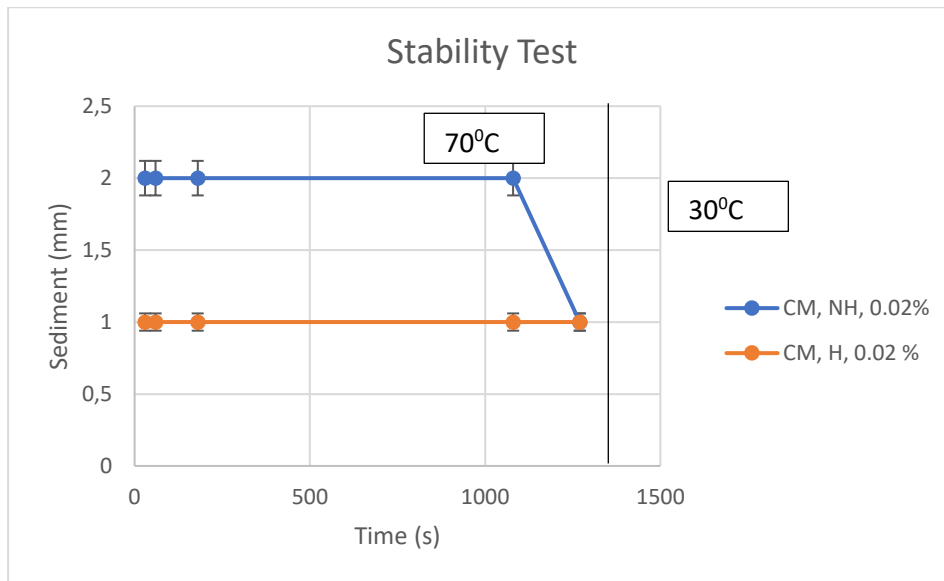


Figure 40: Height of the sediment obtained after centrifugation of chocolate milk as a function of time for a) CM, NH, 0.02% (Chocolate Milk, Non-homogenised, 0.12% carrageenan), b) CM, H, 0.02% (Chocolate Milk, Homogenised, 0.12% carrageenan). Error bar is showing the Standard deviation error with 95% confidence interval.

Stability of cocoa particle was tested with the help of centrifugation of the samples and measuring the amount of cocoa particle that were sedimented. Figure 40 shows the height of sediment after centrifugation of the samples at different time of mixing in the High Shear Mixer. For non-homogenised samples, the height of the sediment particles was comparatively higher for mixing time less than 1000 seconds with respect to homogenised samples. Though, after the cooling was done, both homogenised and non-homogenised samples have the same amount of sediment. For homogenised samples, the amount of sediment was constant all through the process of heating and cooling.

4.5.3. Cooling Effect

To understand the effect of cooling on carrageenan solution prepared in High Shear Mixer, different methods of cooling were performed, and the effect of viscosity was observed.

Table 19: Viscosity of 1% carrageenan solution after 18 minutes of mixing cooled at different conditions (High shear Mixer, Water bath, Room Temperature)

	K	N	Shear Stress (Pa)	Viscosity (mPas)
HSM	0,28	0,71	7,48	74,81
Water Bath	0,28	0,71	7,51	75,12
Room Temperature	0,31	0,70	7,62	76,23

Table 19 shows viscosity of 2% carrageenan solutions that were cooled down to 30 °C by different methods that include cooling of the samples while mixing in High

Shear Mixer, water bath and leaving the samples at room temperature. The viscosity values obtained while cooling the system in High Shear Mixer were almost the same and no difference of different cooling method is observed.

Table 20: Viscosity of chocolate milk containing 0.02% carrageenan after different time of mixing, cooled at different conditions (High shear Mixer, Water bath, Room Temperature).

	Time (minutes)	K	N	Shear Stress (Pa)	Viscosity (mPas)
Water Bath	3	0,0047	1,0948	0,73	7,27
Room Temperature	3	0,0080	1,0237	0,89	8,92
HSM	3	0,0137	0,9537	1,11	11,07
Room Temperature	18	0,0101	0,9920	0,97	9,73
HSM	18	0,0160	0,9376	1,20	12,00

Table 20 shows viscosity of chocolate milk containing 0.02% of carrageenan, that were cooled down to 30 °C by different methods that include cooling of the samples while mixing in High Shear Mixer, water bath and leaving the samples at room temperature. The viscosity values obtained while cooling the system in High Shear Mixer was higher when compared to the viscosity of chocolate milk cooled at either room temperature or water bath. It this has to do with the setting temperature of carrageenan. If the cooling is performed while exposed to mixing, then we get a more viscous product as the mixing is efficient.

From Table 19 and 20, it is observed that carrageenan does not show any changes in viscosity for different cooling conditions in water. However, when carrageenan is dispersed in milk system in a concentration of as low as 0.02% a difference in viscosity is observed when the system is cooled in High Shear Mixer due to presence of milk protein (casein) in milk and its interaction with kappa-carrageenan.

In conclusion, the viscosity of 0.02%, 0.5% and 1% kappa carrageenan in water was not affected by time and temperature of mixing. Also, 1% of kappa carrageenan solution did not form any elastic component and showed no yield stress. Viscosity increased in chocolate milk when compared to water. In chocolate milk, the viscosity continued to increase slightly during hydration of carrageenan while no change is observed in the water matrix. In chocolate milk containing 0.02% kappa carrageenan, the viscosity was affected by temperature and there was a drastic increase in viscosity by cooling the chocolate milk from 70 °C to 30 °C in High Shear Mixer. Kappa carrageenan has a property to bind with the milk proteins (k-casein) present in the milk which helps in the formation of network, entrapping the cacao particle and stabilizing the chocolate milk. It was also observed that the viscosity of carrageenan in chocolate milk was higher without homogenization. For network formation cooling step while mixing is important and as the stability result showed, the sedimentation of cocoa particles reduced considerably after cooling to 30 °C. There was no large difference between the stability of homogenised and non-homogenised samples.

5. Conclusion

From the above study we can conclude that there is an effect of High Shear Mixer on rheological behaviour of non-starch-based hydrocolloids. Altering parameters like mixing temperature and time, influences the functionality of the hydrocolloid solution. However, there was no effect of mixing speed observed on the viscosity of hydrocolloid solutions. It was also observed that there is an effect of mixing time which is dependent on the type of the hydrocolloids used and its structure. The molecular structure of hydrocolloids plays an important role on the functionality, as it affects the mechanism with which hydrocolloids disperses and hydrates in the system.

In general, at low concentration of hydrocolloids, no effect of time, temperature and speed of mixing was observed, after 180 seconds of mixing. While at higher concentration, samples were collected after 10 seconds of mixing and it was observed that there was a significant difference in rheology behaviour in the initial mixing time (i.e. 10 seconds to 60 seconds) and after about 200 seconds of mixing the system was stable. Thus, it is concluded that at higher concentration the mixing time was an important parameter affecting the rheology of the hydrocolloid solution. Also, temperature of the system influences the functionality of the gum and is highly dependent on the concentration and type of hydrocolloid used.

Food matrix is a complex system, consisting of multi-components like sugar, fat, etc. It was observed that the behaviour of hydrocolloids in aqueous system was different from their behaviour when dispersed in the food matrix. Hydrocolloids can be affected by different factors like pH, ions, sugar, proteins, etc. which are present in the food system, influencing their functionality. For apple juice, loss in viscosity of hydrocolloids was observed when compared to the water matrix due to the low pH in apple juice which degrades the hydrocolloids. However, for chocolate milk, it was noticed that with the addition of hydrocolloids, there was an increase in viscosity and stability of the chocolate milk was improved.

6. Reference

- Alderman, N. (1997). Non-Newtonian Fluids: Guide to Classification and Characteristics. Project: Slurry, Paste and Polymer Rheology, [online] Report number: ESDU Data Item 97034.
- AlsaidYagoub, N. and Osman M. Nur, A. (2013). The influence of thermal treatment on physical properties of guar gum. *International Journal of Innovations in Pharmaceutical Sciences*.
- Barnes, H. (1997). Thixotropy—a review. *Journal of Non-Newtonian Fluid Mechanics*, 70(1-2), pp.1-33.
- Beaudoin, J. (2001). *Handbook of Analytical Techniques in Concrete Science and Technology*. William Andrew, pp.333-367.
- Beckett, S. (1996). *Physico-Chemical Aspects of Food Processing*. 2nd ed. Boston: Springer US, pp.49-64.
- Bemiller, J. (1986). ChemInform Abstract: An Introduction to Pectins: Structure and Properties. *Chemischer Informationsdienst*, 17(48), pp.3-10.
- Bevington, Phillip and Robinson, D. *Data Reduction and Error Analysis for the Physical Sciences*, 2nd. ed. McGraw-Hill: New York, 1991.
- Cedergårdh, F., (2014) Characterization of commercial mayonnaise products – texture, viscosity and droplet size. Lund University. Master thesis.
- Clark, S., Costello, M., Drake, M. and Bodyfelt, F. (2009). *The Sensory Evaluation of Dairy Products*. 2nd ed. New York: Springer, New York, NY, p.120.
- CP Kelco U S Inc (1998). Reduced molecular weight native gellan gum. US6242035B1.
- Drohan, D., Tziboula, A., McNulty, D. and Horne, D. (1997). Milk protein-carrageenan interactions. *Food Hydrocolloids*, 11(1), pp.101-107.
- Heldman, D., Lund, D. and Sabliov, C. (2007). *Handbook of food engineering*. 2nd ed. Boca Raton: CRC Press.
- Hoefler, A. (2004). *Hydrocolloids*. 1st ed. St. Paul, Minnesota: Eagan Press.
- Imeson, A. (2010). *Food stabilisers, thickeners and gelling agents*. 1st ed. Chichester, U.K.: Wiley-Blackwell Pub.
- Lai, V., Wong, P. and Lii, C. (2000). Effects of Cation Properties on Sol-gel Transition and Gel Properties of κ -carrageenan. *Journal of Food Science*, 65(8), pp.1332-1337.
- Lopes da Silva, J., Gonçalves, M. and Rao, M. (1994). Influence of temperature on the dynamic and steady-shear rheology of pectin dispersions. *Carbohydrate Polymers*, 23(2), pp.77-87.

Metodiev Zhivkov, A. (2013). Cellulose, Fundamental aspects. 1st ed. Montreal: Intecopen, pp.197-222.

Muhidinov, Z., Fishman, M., Avloev, K., Norova, M., Nasriddinov, A. and Khalikov, D. (2010). Effect of temperature on the intrinsic viscosity and conformation of different pectins. Polymer Science Series A, 52(12), pp.1257-1263.

Phillips, G. and Williams, P. (2014). Handbook of hydrocolloids. 2nd ed. Cambridge: Woodhead Publishing Limited.

Steffe, J. and Morgan, R. (1987). On-line measurement of dynamic rheological properties during food extrusion. Journal of Food Process Engineering, 10(1), pp.21-26.

Sworn, G., Sanderson, G. and Gibson, W. (1995). Gellan gum fluid gels. Food Hydrocolloids, [online] 9(4), pp.265-271.

Tetrapak.com. (2019). *Tetra Pak High Shear Mixer, a complete mixing solution*. [online] Available at: <https://www.tetrapak.com/processing/mixing/tetra-pak-high-shear-mixer> [Accessed 20 May 2019].

7. Appendix

7.1. Material and Methods

7.1.1. Rheometer, Kinexus Pro+

Rheometer used for the viscosity and yield stress measurement is shown in Figure A1.

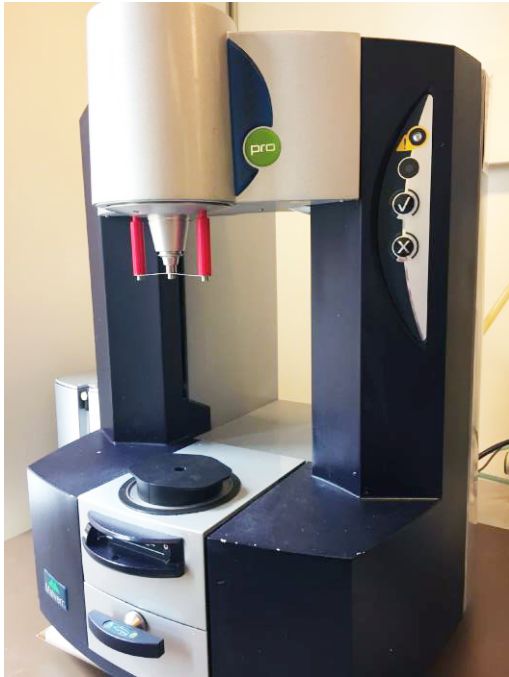


Figure A1: Kinexus Pro+, Rheometer.

7.1.2. Texture Analyser

Settings applied on the Texture analyser for measuring the Stevens value are given in table A1.

Table A1: Settings applied on the Texture Analyser while measuring Stevens value.

Parameter	Setting
Test Mode	Compression
Pre-Test speed	2 mm/sec
Test Speed	2 mm/sec
Post-Test Speed	2 mm/sec
Target Mode	Distance
Distance	25 mm
Trigger Type	Auto (Force)
Trigger Force	0.5 g
Probe Type	Disc plunger
Sample Shape	Cylindrical
Sample volume	100 ml

7.1.3. Homogeniser and Centrifuge

Homogeniser and the centrifuge used for chocolate milk are shown below in Figure A3 a) and b) respectively.



Figure A3: a) High pressure Panda PLUS homogenizer, b) Centrifuge Allegra X-15R

7.1.4. Stability Test

For stability test, the samples to be tested was chocolate milk. Chocolate milk was prepared in the High Shear Mixer without any addition of gums and without homogenising it.

To develop this method, the samples were filled up to 10 ml in the centrifuge tubes and were centrifuged at different speeds for different times. Different speeds used were 500, 1000, 1500 and 2000 rpm and times used were 5, 10, 15 and 20 minutes

in different combinations as shown in Table A2. After the centrifugation, the tubes were taken out and with the help of a scale, the height of the sediment deposited was noted.

For 500 rpm no clear border was observed. While in the case of 1000 rpm for lower time the sediment of cacao particles was not clear to be measured, but at higher time the sedimentation was incomplete. Thus, the samples that were centrifuged for speed less than 1500 rpm, showed incomplete sedimentation of cocoa particles.

After centrifugation at 2000 rpm for 20 minutes the sediment obtained was 9mm which was the maximum sediment that can be obtained. Thus, at 1500 rpm after 15 minutes of centrifugation the same amount of sediment was seen. Therefore, for all the trials done further for testing the stability of chocolate milk 1500 rpm for 15 minutes was used.

Table A2: Test conditions for stability test.

RPM	Time (mins)	sediment (mm)
500	5	no sediment
	10	no sediment
	15	no clear border
	20	no clear border
1000	5	no clear border
	10	no clear border
	15	5
	20	7
1500	5	no clear border
	10	6
	15	9
	20	9
2000	5	7
	10	9
	15	9
	20	9

7.2. Sources of Error

7.2.1. Batch to batch error:

To calculate the batch to batch error, five batches were prepared with the same conditions and concentration of xanthan gum in the High Shear Mixer. The conditions are mentioned in *Table A3*.

Table A3: Conditions for samples prepared with Xanthan.

Concentration (%)	Temperature (°C)	Speed (%)	Time (mins)
0.05	60	70	3

For all the 5 samples, rheometer measurement for viscosity was performed and the results obtained are as below in *Table A4*:

Table A4: Shear stress value for 5 batches of samples obtained from the Rheometer measurements.

Shear rate(s-Å ¹)	Shear stress(Pa) (1)	Shear stress(Pa) (2)	Shear stress(Pa) (3)	Shear stress(Pa) (4)	Shear stress(Pa) (5)
20,00	0,30	0,25	0,27	0,27	0,29
31,70	0,37	0,32	0,34	0,34	0,36
50,24	0,46	0,40	0,42	0,43	0,45
79,63	0,58	0,52	0,54	0,54	0,57
126,20	0,76	0,68	0,71	0,71	0,74
200,00	1,02	0,93	0,97	0,98	1,01
317,00	1,45	1,40	1,41	1,43	1,45
502,40	3,03	2,94	2,99	2,99	3,01
796,30	5,02	4,85	4,92	4,98	5,06
1000,00	6,79	6,78	6,70	6,80	6,93

From all the shear stress values obtained from *Table A4*, average and standard deviation of the values is calculated. Double of standard deviation is divided by the average and the percentage of the obtained value is calculated as shown in *Table A5*.

Table A5: Average (Avg), standard deviation (SD) and Percentage values.

Avg (Pa)	SD (Pa)	SD*2 (Pa)	(SD*2)/avg	% ((SD*2)/avg)
0,27	0,02	0,04	0,07	6,57
0,34	0,02	0,04	0,06	5,99
0,43	0,02	0,05	0,05	5,27
0,55	0,03	0,05	0,05	4,77
0,72	0,03	0,06	0,04	4,17
0,98	0,04	0,07	0,04	3,60
1,43	0,02	0,05	0,02	1,67
2,99	0,04	0,07	0,01	1,19
4,96	0,08	0,16	0,02	1,66
6,80	0,08	0,16	0,01	1,20

The highest value obtained of the error from *Table A5* is 6.5. Thus, the error considered for batch to batch was 6.5%. It is observed from *Table A5* that error decreases with an increase in shear rate.

7.2.1. Measurement Error

To calculate the measurement error, samples of xanthan gum made in the High Shear Mixer were tested thrice in Rheometer. The conditions are mentioned in *Table A6*.

Table A6: Conditions for samples prepared with Xanthan for Measurement Error.

Concentration (%)	Temperature (°C)	Speed (%)	Time (mins)
0.05	60	70	3

For 3 times, rheometer measurement for viscosity was performed and the results obtained are as below in *Table A7*:

Table A7: Shear stress value for samples tested thrice, obtained from the Rheometer measurements.

Shear rate(s-Å¹)	Shear stress(Pa) (1)	Shear stress(Pa) (2)	Shear stress(Pa) (3)
20,00	0,30	0,29	0,29
31,70	0,37	0,36	0,36
50,24	0,46	0,46	0,45
79,63	0,58	0,58	0,57
126,20	0,76	0,75	0,74
200,00	1,02	1,01	1,01
317,00	1,45	1,45	1,44
502,40	3,03	3,05	3,04
796,30	5,02	5,04	5,04
1000,00	6,79	6,78	6,70

From all the shear stress values obtained from *Table A7*, average and standard deviation of the values is calculated. Double of standard deviation is divided by the average and the percentage of the obtained value is calculated as shown in *Table A8*.

Table A8: Average (Avg), standard deviation (SD) and Percentage values.

Avg	SD	SD*2	(SD*2)/avg	% ((SD*2)/avg)
0,293	0,005	0,010	0,018	1,775
0,365	0,006	0,012	0,016	1,630
0,455	0,006	0,013	0,014	1,386
0,576	0,007	0,014	0,013	1,252
0,749	0,008	0,016	0,011	1,064
1,013	0,009	0,018	0,009	0,895
1,447	0,007	0,013	0,005	0,460
3,037	0,008	0,016	0,003	0,263
5,033	0,012	0,024	0,002	0,235
6,787	0,004	0,008	0,001	0,060

The highest value obtained of the error from *Table A8* is 1.8. Thus, the measurement error considered was 6.5%.

7.2.2. Texture Analysis error

To calculate the texture analysis error, same samples of CMC gum prepared in the High Shear Mixer were tested thrice. The conditions are mentioned in *Table A9*.

Table A9: Conditions for samples prepared with CMC for Measurement Error of Texture Analyser.

Concentration (%)	Temperature (°C)	Speed (%)	Time (mins)
1	60	70	3

For 3 times, texture analysis for Stevens value was performed and the results obtained are 11.16, 11.38 and 11.49 grams.

From all the values obtained from, average and standard deviation of the values is calculated. Double of standard deviation is divided by the average and the percentage of the obtained value is calculated as shown in *Table A10*.

Table A10: Average (Avg), standard deviation (SD) and Percentage values.

Avg	SD	SD*2	(SD*2)/avg	% ((SD*2)/avg)
11,34	0,17	0,34	0,03	2,97

The highest value obtained of the error from *Table A10* is 6.5. Thus, the texture analysis error considered was 2.97%.

7.3. K and n values

Table A11: K and n values obtained for tap water in the Rheometer.

	K	n	Shear stress [Pa]	Viscosity[mPas]
Water	0,0001	1,5688	0,14	1

7.3.1. Xanthan

Table A12: K and n values obtained for Xanthan in water matrix for different parameters in the Rheometer.

Time [s]	Conc. (%)	Temp. (°C)	Speed (%)	K	n	Shear Stress [Pa]	Viscosity [mPas]
180	0,05	60	70	0,0209	0,7912	0,798999	7,9899909
600	0,05	60	70	0,0186	0,8075	0,766501	7,6650139
1200	0,05	60	70	0,0176	0,8186	0,763331	7,6333059
180	0,05	20	90	0,0113	0,8892	0,678388	6,7838845
600	0,05	20	90	0,0156	0,8385	0,741523	7,4152295
1200	0,05	20	90	0,0129	0,8678	0,701762	7,0176178

180	0,05	60	90	0,0166	0,8253	0,74252	7,4251988
600	0,05	60	90	0,0148	0,8253	0,662006	6,6200568
1200	0,05	60	90	0,0129	0,8639	0,689271	6,8927053
180	0,05	20	70	0,0159	0,8347	0,742672	7,4267213
600	0,05	20	70	0,0138	0,856	0,711016	7,1101553
1200	0,05	20	70	0,0123	0,8755	0,693274	6,9327432
180	0,1	60	70	0,0747	0,6401	1,424035	14,240348
600	0,1	60	70	0,079	0,626	1,411325	14,113252
1200	0,1	60	70	0,0765	0,6288	1,3844	13,843995
180	0,1	20	90	0,0897	0,6189	1,550931	15,509309
600	0,1	20	90	0,0868	0,6203	1,510497	15,104965
1200	0,1	20	90	0,0828	0,6265	1,482622	14,826216
180	0,1	60	90	0,086	0,6203	1,496575	14,965749
600	0,1	60	90	0,0888	0,6101	1,474392	14,74392
1200	0,1	60	90	0,0836	0,6165	1,429573	14,295728
180	0,1	20	70	0,0945	0,6165	1,615964	16,159645
600	0,1	20	70	0,0917	0,6163	1,56664	15,666405
1200	0,1	20	70	0,0914	0,6143	1,547199	15,471991
180	0,5	20	70	2,3568	0,31	9,824778	98,247776
600	0,5	20	70	2,3568	0,31	9,824778	98,247776
1200	0,5	20	70	2,0889	0,3108	8,740125	87,401251
10	1	20	70	3,5951	0,3875	21,41	214
30	1	20	70	3,6291	0,3753	20,44	204
60	1	20	70	3,6453	0,3594	19,08	191
180	1	20	70	3,5348	0,3587	18,44	184
1080	1	20	70	3,4535	0,3511	17,40	174
10	1	60	70	3,4425	0,3517	17,39	174
30	1	60	70	3,4824	0,3447	17,03	170
60	1	60	70	3,1996	0,3447	15,65	156
180	1	60	70	3,0367	0,3502	15,23	152
1080	1	60	70	2,6689	0,353	13,56	136

7.3.2. CMC

Table A13: *K* and *n* values obtained for CMC in water matrix for different parameters in the Rheometer.

Time [s]	Conc. (%)	Temp. (°C)	Speed (%)	K	n	Shear Stress [Pa]	Viscosity [mPas]
180	0,05	20	90	0,0035	1,1065	0,571568	5,715682
360	0,05	20	90	0,0029	1,1372	0,545504	5,455041
1080	0,05	20	90	0,0021	1,1807	0,482636	4,826355
180	0,05	60	90	0,0032	1,1175	0,549731	5,497307
360	0,05	60	90	0,0027	1,1434	0,522593	5,225932
1080	0,05	60	90	0,0019	1,1955	0,46747	4,674698
180	0,05	60	70	0,004	1,086	0,594374	5,943743
360	0,05	60	70	0,0038	1,0966	0,592903	5,929029
1080	0,05	60	70	0,0028	1,1405	0,534759	5,347589
180	0,05	20	70	0,0039	1,0886	0,586495	5,864954
360	0,05	20	70	0,0033	1,1156	0,561971	5,61971
1080	0,05	20	70	0,0027	1,1451	0,526701	5,267005
180	0,1	20	90	0,0187	0,9217	1,303892	13,03892
360	0,1	20	90	0,0167	0,9386	1,258683	12,58683
1080	0,1	20	90	0,0128	0,9761	1,146595	11,46595
180	0,1	60	90	0,0174	0,9272	1,244369	12,44369
360	0,1	60	90	0,0156	0,9451	1,211503	12,11503
1080	0,1	60	90	0,0118	0,983	1,091144	10,91144
180	0,1	60	70	0,0187	0,9166	1,273625	12,73625
360	0,1	60	70	0,0176	0,9269	1,256935	12,56935
1080	0,1	60	70	0,0138	0,9586	1,140457	11,40457
180	0,1	20	70	0,0206	0,9039	1,323327	13,23327
360	0,1	20	70	0,0201	0,9114	1,336583	13,36583
540	0,1	20	70	0,0189	0,9211	1,314201	13,14201
1080	0,1	20	70	0,0163	0,9427	1,251952	12,51952
180	0,5	20	90	0,8019	0,5913	12,21013	122,1013

1080	0,5	20	90	0,7768	0,5982	12,20982	122,0982
180	0,5	60	90	0,6257	0,6107	10,41756	104,1756
1080	0,5	60	90	0,5278	0,6237	9,329737	93,29737
180	0,5	60	70	0,6638	0,6064	10,83521	108,3521
1080	0,5	60	70	0,6342	0,6129	10,66661	106,6661
180	0,5	20	70	1,0222	0,5449	12,57002	125,7002
360	0,5	20	70	1,0324	0,5435	12,61386	126,1386
540	0,5	20	70	1,0136	0,546	12,52756	125,2756
1080	0,5	20	70	1,0115	0,5473	12,57668	125,7668
10	1	20	70	24,07	0,3289	109,47	746
30	1	20	70	14,368	0,3577	74,61	551
60	1	20	70	9,3513	0,3853	55,14	447
180	1	20	70	6,8175	0,4083	44,69	446
1080	1	20	70	6,8149	0,4082	44,65	444
10	1	60	70	8,687	0,3891	52,13	544
30	1	60	70	9,2576	0,3846	54,41	477
180	1	60	70	7,6504	0,3972	47,65	457
1080	1	60	70	7,0861	0,4047	45,69	457

7.3.3. Gellan

Table A14: K and n values obtained for Gellan in water matrix for different parameters in the Rheometer.

Time [s]	Conc. (%)	Temp. (°C)	Speed (%)	K	n	Shear Stress [Pa]	Viscosity [mPas]
10	0,1	60	70	0,0136	0,8756	0,7669	7,669003
30	0,1	60	70	0,0145	0,8637	0,774048	7,740482
180	0,1	60	70	0,0152	0,8568	0,786038	7,860381
360	0,1	60	70	0,0138	0,8694	0,756274	7,562739
1080	0,1	60	70	0,0097	0,9179	0,664617	6,646174
180	0,1	80	70	0,0104	0,9089	0,683649	6,836492
360	0,1	80	70	0,0084	0,94	0,637205	6,372052
1080	0,1	80	70	0,0046	1,0241	0,513994	5,139937
180	0,1	60	90	0,0132	0,8772	0,749849	7,498492

360	0,1	60	90	0,0118	0,892	0,717599	7,175993
1080	0,1	60	90	0,0069	0,9667	0,591901	5,91901
180	0,1	80	90	0,0126	0,8861	0,745711	7,45711
360	0,1	80	90	0,011	0,9043	0,707934	7,079339
1080	0,1	80	90	0,0058	0,9911	0,556709	5,567087
10	0,2	60	70	0,1394	0,6026	2,235954	22,35954
180	0,2	60	70	0,1404	0,5989	2,213946	22,13946
360	0,2	60	70	0,1563	0,5834	2,294875	22,94875
1080	0,2	60	70	0,1328	0,6012	2,116402	21,16402
180	0,2	80	70	0,1022	0,6394	1,942007	19,42007
360	0,2	80	70	0,0832	0,6644	1,773876	17,73876
1080	0,2	80	70	0,0732	0,6714	1,6118	16,118
180	0,2	60	90	0,1422	0,5946	2,198364	21,98364
360	0,2	60	90	0,1232	0,6116	2,059732	20,59732
1080	0,2	60	90	0,1023	0,6352	1,90667	19,0667
180	0,2	80	90	0,1078	0,6301	1,962541	19,62541
360	0,2	80	90	0,0924	0,6475	1,822519	18,22519
1080	0,2	80	90	0,0613	0,6927	1,488883	14,88883
10	0,15	60	70	0,0539	0,7136	1,441414	14,41414
30	0,15	60	70	0,0549	0,7136	1,468156	14,68156
180	0,15	60	70	0,0515	0,7152	1,387418	13,87418
360	0,15	60	70	0,0499	0,7164	1,351763	13,51763
1080	0,15	60	70	0,0421	0,7368	1,252801	12,52801
180	0,15	80	70	0,0386	0,7507	1,22458	12,2458
360	0,15	80	70	0,03	0,7853	1,116147	11,16147
1080	0,15	80	70	0,0226	0,8126	0,953473	9,534731
180	0,15	60	90	0,0537	0,7076	1,396929	13,96929
360	0,15	60	90	0,0483	0,7207	1,334588	13,34588
1080	0,15	60	90	0,0322	0,7752	1,143552	11,43552
180	0,15	80	90	0,0423	0,7408	1,282155	12,82155
360	0,15	80	90	0,0374	0,7537	1,203017	12,03017

1080	0,15	80	90	0,0162	0,8576	0,840843	8,408432
10	1	60	70	12,336	0,2442	37,9817	379,817
30	1	60	70	13,493	0,2368	40,15212	401,5212
60	1	60	70	13,868	0,234	40,73933	407,3933
180	1	60	70	11,308	0,246	35,10636	351,0636
1080	1	60	70	13,681	0,2117	36,26752	362,6752
10	1	80	70	18,626	0,2001	46,80795	468,0795
30	1	80	70	19,455	0,1939	47,51505	475,1505
60	1	80	70	19,142	0,1877	45,43466	454,3466
180	1	80	70	17,08	0,191	41,16118	411,6118
1080	1	80	70	18,495	0,1582	38,32254	383,2254

7.3.4. Carrageenan

Table A15: K and n values obtained for Carrageenan in water matrix for different parameters in the Rheometer.

Time[s]	Conc. [%]	Temp. [°C]	K	n	Shear stress [Pa]	Viscosity [mPas]
120	0,02	20	0,0003	1,452	0,240503	2,41
250	0,02	70	0,0002	1,481	0,183244	1,83
310	0,02	70	0,0002	1,4848	0,186479	1,86
490	0,02	70	0,0002	1,4827	0,184684	1,85
1570	0,02	70	0,0002	1,4916	0,192411	1,92
1810	0,02	50	0,0002	1,4919	0,192677	1,93
1930	0,02	30	0,0002	1,4971	0,197347	1,97
120	0,5	20	0,0401	0,8751	2,256027	22,56
250	0,5	70	0,0377	0,8864	2,234299	22,34
310	0,5	70	0,0377	0,8864	2,234299	22,34
490	0,5	70	0,038	0,8857	2,244831	22,45
1570	0,5	70	0,0356	0,8931	2,175955	21,76
1810	0,5	50	0,035	0,8956	2,164054	21,64
1930	0,5	30	0,0361	0,8908	2,183269	21,83
120	1	20	0,3844	0,6658	8,248659547	82,49

250	1	70	0,2917	0,7096	7,658371	76,58
310	1	70	0,3152	0,7012	7,961341	79,61
490	1	70	0,3144	0,7007	7,92287	79,23
1570	1	70	0,3047	0,7056	7,853666	78,54
1810	1	50	0,2772	0,7147	7,450634	74,51
1930	1	30	0,2796	0,7126	7,442814	74,43

Carrageenan with calcium

Time [s]	Conc. [%]	Temp. [°C]	K	n	Shear stress [Pa]	Viscosity
120	1	20	0,2566	0,6686	5,577719	55,78
250	1	70	0,3418	0,6701	7,481214	74,81
310	1	70	0,3455	0,6719	7,625144	76,25
490	1	70	0,352	0,6696	7,686748	76,87
1570	1	70	0,3389	0,6741	7,555645	75,56
1810	1	50	0,3216	0,6792	7,340338	73,40
1930	1	30	0,3291	0,6744	7,347302	73,47

7.3.5. Apple juice

Table A16: K and n values obtained for Apple juice for CMC, Xanthan and Pectin in the Rheometer.

Hydrocolloid	Conc. (%)	Time (s)	Temp. (°C)	K	n	Shear Stress (Pa)	Viscosity (mPas)
CMC	0,05	10	20	0,0011	1,2631	0,36948	3,69
	0,05	120	20	0,0015	1,2206	0,41428	4,14
	0,05	250	80	0,0006	1,3583	0,31243	3,12
	0,05	270	80	0,0006	1,3587	0,31300	3,13
	0,05	300	80	0,0006	1,3609	0,31619	3,16
	0,05	360	80	0,0006	1,3632	0,31956	3,20
	0,05	420	80	0,0006	1,3626	0,31868	3,19
	0,05	600	40	0,0005	1,372	0,27731	2,77

Xanthan	0,05	10	20	0,0133	0,8886	0,79625	7,96
	0,05	120	20	0,0121	0,8994	0,76135	7,61
	0,05	250	80	0,0063	0,9931	0,61030	6,10
	0,05	270	80	0,0066	0,9879	0,62423	6,24
	0,05	300	80	0,0063	0,9931	0,61030	6,10
	0,05	360	80	0,0065	0,9903	0,62160	6,22
	0,05	420	80	0,0061	0,9985	0,60580	6,06
	0,05	600	40	0,0054	1,017	0,58397	5,84
Pectin	0,2	10	20	0,0006	1,3649	0,32207	3,22
	0,2	120	20	0,0005	1,3732	0,27885	2,79
	0,2	250	80	0,0005	1,3911	0,30281	3,03
	0,2	270	80	0,0005	1,377	0,28377	2,84
	0,2	300	80	0,0005	1,3878	0,29824	2,98
	0,2	360	80	0,0005	1,3937	0,30646	3,06
	0,2	420	80	0,0005	1,3921	0,30421	3,04
	0,2	600	40	0,0005	1,3737	0,27949	2,79
Pectin	2	10	20	0,1192	0,8968	7,41099	74,11
	2	120	20	0,1435	0,8866	8,51240	85,12
	2	250	80	0,0843	0,9312	6,14084	61,41
	2	270	80	0,0844	0,9296	6,10299	61,03
	2	300	80	0,0845	0,9305	6,13560	61,36
	2	360	80	0,0781	0,9387	5,88913	58,89
	2	420	80	0,0787	0,938	5,91527	59,15
	2	600	40	0,0635	0,9524	5,10004	51,00

7.3.6. Chocolate milk

Table A16: K and n values obtained for Chocolate Milk for Carrageenan and Gellan at different times in the Rheometer.

Gums	Conc. [%]	Temp. [C]	Time [s]	Homogenization	K	n	Shear Stress [Pa]	Viscosity [mPas]
Carrageenan	0,02	70	10	no	0,0046	1,0937	0,71	7,08
Carrageenan	0,02	70	30	no	0,0067	1,0423	0,81	8,14
Carrageenan	0,02	70	30	yes	0,005	1,0845	0,74	7,38
Carrageenan	0,02	70	60	no	0,0078	1,0263	0,88	8,80
Carrageenan	0,02	70	180	no	0,008	1,0237	0,89	8,92
Carrageenan	0,02	70	180	yes	0,0063	1,0547	0,81	8,10
Carrageenan	0,02	70	1080	no	0,0101	0,992	0,97	9,73
Carrageenan	0,02	70	1080	yes	0,0092	1,0098	0,96	9,62
Carrageenan	0,02	30	1270	no	0,016	0,9376	1,20	12,00
Carrageenan	0,02	30	1270	yes	0,0099	1,0005	0,99	9,92
Gellan	0,12	60	10	no	0,001	1,3073	0,41	4,12
Gellan	0,12	60	30	no	0,0014	1,2603	0,46	4,64
Gellan	0,12	60	30	yes	0,0021	1,1997	0,53	5,27
Gellan	0,12	60	60	no	0,0023	1,1892	0,55	5,50
Gellan	0,12	60	180	no	0,0051	1,084	0,75	7,51
Gellan	0,12	60	180	yes	0,006	1,0624	0,80	8,00
Gellan	0,12	60	1080	no	0,0101	0,995	0,99	9,87
Gellan	0,12	60	1080	yes	0,0055	1,0795	0,79	7,93
Gellan	0,12	30	1270	no	0,0082	1,0232	0,91	9,12
Gellan	0,12	30	1270	yes	0,0047	1,0989	0,74	7,41

7.4. Stability Test Result

7.4.1. Gellan

Results for sedimentation measured after centrifugation of chocolate milk containing gellan and without gellan.

Table A17: Results of the sediment measured after centrifugation of chocolate milk (final product).

Sample	Sediment (mm)
Chocolate milk (no gums, non-homogenised)	9
Chocolate milk (no gums, homogenised)	8
Chocolate milk (0.12% gellan, non-homogenised)	1
Chocolate milk (0.12% gellan, homogenised)	1

7.4.2. Carrageenan

Results for sedimentation measured after centrifugation of chocolate milk containing carrageenan and without carrageenan.

Table A18: Results of the sediment measured after centrifugation of chocolate milk (final product).

Sample	Sediment (mm)
Chocolate milk (no gums, non-homogenised)	9
Chocolate milk (no gums, homogenised)	8
Chocolate milk (0.02% carrageenan, non-homogenised)	1
Chocolate milk (0.02% carrageenan, homogenised)	1

7.5. Effect of Calcium on carrageenan

One of the main purposes of this project was to investigate the functionality of gums in both water and food products. Chocolate milk was chosen as the food matrix for kappa carrageenan since it is a popular stabilizing agent in chocolate milk. The reason is that the interaction of kappa carrageenan with kappa-Casein present in milk, which leads to the formation of network and stabilize the cocoa suspension (Phillips and Williams, 2014). Also, it is expected that calcium ions present in milk have a role to affect the junction zones in the network and improve the strength of formed network (Lai, Wong and Lii, 2000). Therefore, it was decided to experiment the influence of calcium ions on the carrageenan gel formation in water matrix. As the calcium content of milk is 125 mg per 100 g, the same amount of calcium chloride was added in 1% carrageenan solution.

Figure A4 depicts the viscosity change of carrageenan solutions with and without calcium chloride versus time.

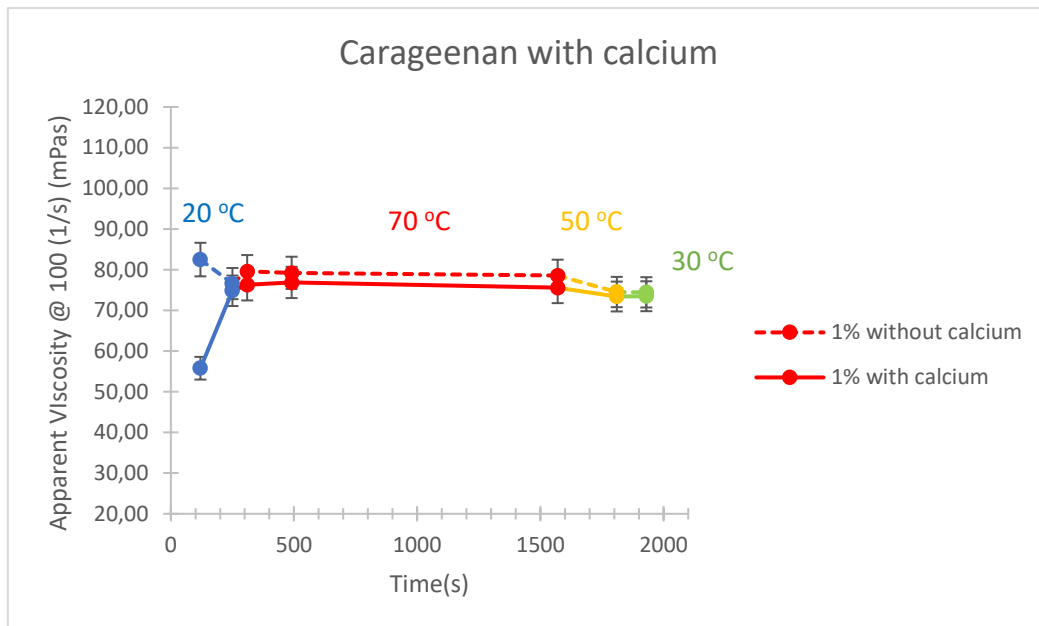


Figure A4: Apparent Viscosity of 1% carrageenan solutions and 125 mg of calcium chloride during the preparation at 70% speed. Error bar is showing standard deviation error with 95% confidence interval.

According to the Figure A4, it is observed that in the solution containing calcium chloride at 20 °C, there is a drastic increase in viscosity from 10 to 250 seconds in viscosity and post that the viscosity remains stable over the mixing time. The reason for the increase could be due to the dispersion of powder after 250 seconds of mixing. By increasing the temperature due to the complete dissolution of the dispersed particles in the system, there is no viscosity change observed during the preparation.

Furthermore, apart from the dispersion point there is no significant differences between the viscosity of carrageenan solution and carrageenan solution containing calcium in Figure A4. Both the curves lie in the measurement error range depicting that no effect on viscosity is observed.

Texture

Figure A5 illustrates the Stevens value of 1% carrageenan as a function of mixing time at different temperatures (20 °C, 70 °C, 50 °C, 30 °C) at 70% mixing speed.

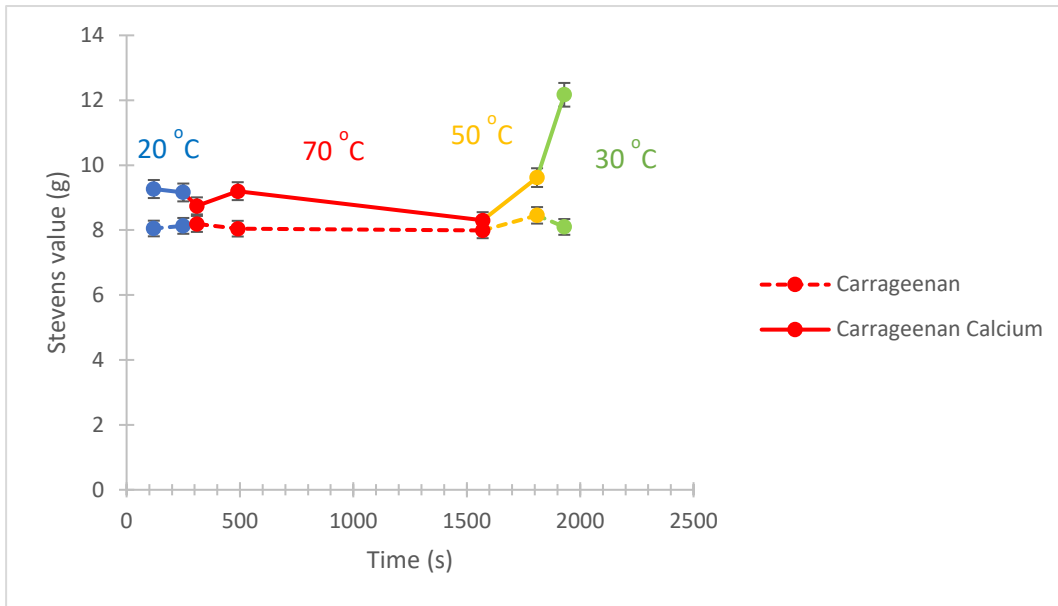


Figure A5: Stevens value of 1% carrageenan solution and 1% carrageenan solution containing 125 mg of calcium chloride during the preparation at 70% speed. Error bar is showing standard deviation error with 95% confidence interval.

Both the solutions show almost stable Stevens value during the dispersion and heating stage up to 1570 seconds as shown in Figure A5. In the case of solution containing calcium chloride, there is a drastic increase in Stevens value at cooling which is not observed in carrageenan solution without calcium.

The result in texture shows there is an impact of cooling on the Stevens value. This can depict the role of the salt to improve the gelling strength in carrageenan solution by improving the junction zones in the formed network (Lai, Wong and Lii, 2000).

The possible reason why there is no difference observed in viscosity result for calcium solution might be due to the method differences to characterise the gelling strength. In viscosity measurement in rheometer, the solution is being sheared continuously and the viscosity is taken at 100 (1/s) while the texture result is done when the solution was at rest and then the compression force was applied. Therefore, it can be concluded that 125 mg of calcium chloride can facilitate the network formation by 1% of kappa carrageenan in water.

7.6. Hydrocolloid Table

In Table A19, general information regarding the concentration, temperature, pH, etc. are mentioned for each of the hydrocolloids used in this project.

Table A19: Properties of different hydrocolloids.

Gum	pH	Dissolving temp (°C)	Hydration temp (°C)	Setting temp (°C)	Concentration	Sugar	Presence of ions	Other effects
Xanthan	3-7	Cold and hot water	Cold and hot water	-	0.05-0.3%	Facilitates the dispersion in a low amount	Below 0.25% gum monovalent salts cause a slight decrease in viscosity	
Gellan (HA)	3-7	Hot water (above 70)	80	Below 40	0.1-0.12%	HA gellan gum gels are more elastic above 60% total soluble solids content	the gel structure increases sharply with increasing cation levels	Addition of a sequestrant such as sodium citrate binds calcium and helps hydration.
CMC	4-7	Cold and Hot water	Cold and hot water	-	0.03-0.5	Independent	Monovalent ions will form soluble salts with CMC	
Carrageenan (Kappa)	5-7	Hot water (above 70)	70	40	0.01-0.03%	it is not possible to use kappa carrageenan in foods with a sugar content higher than 60%.	Gels most strongly especially potassium ions.	k Carrageenan Interacts with k casein in milk Better to be dispersed before being heated to ease the hydration
High Methyl Ester Pectin	3-5	50-80	80	Below 40	0.2%-0.3%	Presence required	Presence required	Pectin is very sensitive to pH, sugar content and cations.