The effect of mechanical shear in ambient yoghurt

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Abbreviations and symbols

CCP : Coloidal calcium phosphate

DM: Dry matter content

EPS: Expopolysaccharides

FAO: Food and Agriculture Organization of the United Nations

F1C: Formula 1 control

F1: Formula 1

F2C: Formula 2 control

F2: Formula 2

HA gellan gum: High Acyl gellan gum

HPDSP: Hydroxypropyl distarch phosphate modified starch

HP pectin: High Methoxyl pectin

LAB: Lactic Acid Bacteria

LA gellan gum: Low Acyl gellan gum

LM pectin: Low Methoxyl pectin

LSR: Low shear rate

MSR: Medium shear rate

HSR: High shear rate

PHE: Plate heat exchanger

SPM: Skim milk powder

TSC: Texturing starter culture

TS: Total solids

WHO: World Health Organization

%SV: percentage of study variation

%WR: percentage of water retention

Symbols

η: viscosity

σ: shear stress

$\dot{\gamma}$: shear rate

u: mean velocity

D: diameter of the pipe

Q : flow rate

A : area of the hose

V: velocity

d : distance

t: time

$s2$: Weight of the sediment

$s1$: Weight of the sample

τ: shear stress

k: fluid consistency unit

n: power in law (flow index

Acknowledgments

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Abstract

Yoghurt is a fermented product form the milk, well known in the world. The fermentation is carried out by Lactic acid bacteria (LAB). The most common are *Streptococcus thermophilus* and *lactobacillus bulgaricus*, these bacteria make the milk acid, generating a disturbance in the casein micelles making a solid mass called coagulum. This specific LAB also produces expopolysaccharides (EPS) that form the characteristic ropy in the yoghurt. There are many types of yoghurt based in its consistency; the most popular are the set and the stirred type. Set yogurt is the solid coagulum where the yoghurt is incubated, cooled and retailed to the consumer in the same container. The stirred yoghurt is fermented in a big container and after fermentation the coagulum is destroy gentle, cooled and pumping to the final package to the consumer.

Ambient yoghurt production is the same as stirred type whit the difference of the ambient yoghurt is heat-treated after fermentation in order to inactivate the LAB and so therefore prolong the shelf life of the product; due to this, ambient yoghurt doesn’t needs to be cooled. Heat-treatment and pumping have an impact in the final viscosity and stability as water retention in the product since the yoghurt gel is shear sensitive. The use of different stabilizers is needed to maintain the quality parameters of the yoghurt as the viscosity perceived as smoothness and avoid the syneresis (separation of the whey). The correct mix in the stabilizers added to the yoghurt and to know how yoghurt behaves during shearing in the process presents new challenges in the dairy industry with the finality to give the consumer a product with long shelf life that keeps the smoothness and stability characteristic in the yoghurt.

Taking into account these considerations, the purpose of this study was to evaluate the rheological behaviour of the yoghurt after applying mechanical shear to the stirred yoghurt. The procedure of the experiment consist in 3 parts: 1) the setting of the rig to simulate the shear rates in the industry (called as low, medium and high shear rate), from low shear rates, medium and high 2) Yoghurt production based in two different standard ambient yoghurt formulations with stabilizers: a) Formula 1 (F1) and its control (F1C) and Formula 2 (F2) and its control (F2C). F1 and F2 had modified starch and pectin, with the difference that F1 had gellan gum and F2 gelatin 3) applying the shear rates in different times (30 and 60 seconds) to the yoghurt in the rig and in the rheometer. Before and after the shearing of the product in the rig and in the rheometer, it was measured the percentage of water retention (%WR) and the viscosity in a rheometer to see how were the changes of F1 and F2 after shearing in rig and rheometer.

The results showed that both formulas and controls were sensitive to shear but behave different due to the stabilizers used. F1 viscosity after shearing shows better stability, it can be attributed to the gellan gum, on the other hand it doesn´t have good %WR even though with the addition of stabilizers and had a continuous decreasing after medium and high shearing. F2 has higher viscosity and better higher retention than its own control but does not have a good viscosity stability having a constant decreasing when shear was applied, however the %WR was higher and more stable at high mechanical shear; gelatin could be the answer for this behaviour. The different times that the same shear rates were applied to the samples (30 and 60 sec) doesn´t show a significative difference in the viscosity and the %WR. Finally the viscosity results of the yoghurt which run in the rig are not the same as the ones that run in the rheometer, even though the formulas used in the development of the rig were correct, the prove is the %WR in both samples which run in different systems (rig and rheometer) has not significative difference.

1 Introduction

Yogurt is an ancient fermented milk product dating more than 10,000 years (Tamime and Robinson 2007). Over time, the use of fermented milk products for the preservation of milk and its nutrients has been popularized. Today, yogurt is one of the most famous fermented milk products around the world (Tetra Pak 2015).

To preserve the yoghurt longer time and without any necessity to maintain the cool-chain, the dairy industry has develop different types of process, this is the case of a ambient yogurt in which the cultures have been killed by heat treatment after the product has been made, to prevent any post-packaging fermentation, effectively extending the shelf life of the product to months (Robertson, G. 2010).

The most rising market for drinkable yogurts in ambient temperature is in Asia (Adf.farmonline.com.au, 2015). Yogurt, which is a delicate product with a pleasant texture require great care on the production process (Robertson G. 2010). Where, if the product is not well made the textural characteristics such as firmness, viscosity, and water retention can be lost (Tetra Pak 2015).

The post treatment process of the yoghurt as the heat treatment and pumping affects the gel structure causing the syneresis of the product and loss in the viscosity. This presents new challenges to the dairy industry due to the need of a smooth, stable, and long shelf living yogurt product without any type of refrigeration. To avoid syneresis, the separation of the whey, is used the addition of stabilizers in order to maintain the desired texture in the ambient yogurt. The most common stabilizers used in yogurt are gelatine, pectin, modified starch and agar-agar (Tetra Pak 2015).

The aim of this work was to investigate how the quality of the stirred ambient yoghurt is affected by the post fermentation process.

2 Hypothesis

Two different ambient yoghurt formulations with different stabilizers will not be present a significant mechanical shear sensitiveness that reflects any change in their viscosity and water retention after applying specific mechanical shear used in the industry at different times.

3 General Objective

Provide advice in how the viscosity and water retention in ambient yoghurt, which has been treated with different stabilizers, changes with a mechanical shear condition applied after the fermentation.

4 Scope

- Identify quality methodology to see the changes of the yoghurt before and after the shear.
- Develop a method that resembles the mechanical shear experienced after fermentation in ambient yoghurt.
- Record the changes generated in the ambient yoghurt with different stabilizers before and after the mechanical treatment in the rheological behaviour and water retention.
- Compare the different results obtained with different stabilizers in ambient yogurt before and after mechanical shear treatment.

5 Limitations

The focus in this work was the application of the mechanical shear in yoghurt after fermentations and stirring. There was no applied post heat treatment in the yoghurt as is done in the industry. There was not equipment availability as the texturometer. The repeatability of the yoghurt production couldn't be calculated due to the lack of enough data to make a proper sadistic analysis.

6 Back ground

6.1 Fermented milks

Fermented foods are very important in human history; the fermentation process was used to extend the shelf-life of different food products. The food that undergoes fermentation is invaded or overgrown by edible microorganism, which hydrolyze the macromolecules in the food as polysaccharides, proteins and lipids, to products with flavours, aromas and textures attractive and pleasant to the human (Steinkraus K. 1997).

The origin of making fermented milks is very old; it could be from 10-15000 years ago (Tamime and Robinson, 2007). By fermenting the milk, important nutrients are conserved or could metabolized better by the body from the milk (Chandan and Kilara, 2013). The most ancient lactic fermentation is probably fermented/sour milk. Milk is a normal habitat of a number of lactic acid bacteria. Raw unpasteurized milk will rapidly become sour because the lactic acid bacteria present ferment milk sugar, lactose to lactic acid (Steinkraus 1997). The process allowed the consumption of milk constituents for a longer period compared with the raw milk (Chandan and Kilara, 2013).

There are around 400 diverse products derived from fermentation of milk. Milk of various ruminants differs in composition and produces fermented milk with different characteristics in texture and flavour (Chandan and Kilara, 2013, Robinson and Tamime, 2007). Considering the type of microorganism present in the fermentation of the milk there are different classifications described by Robinson and Tamime (2007).

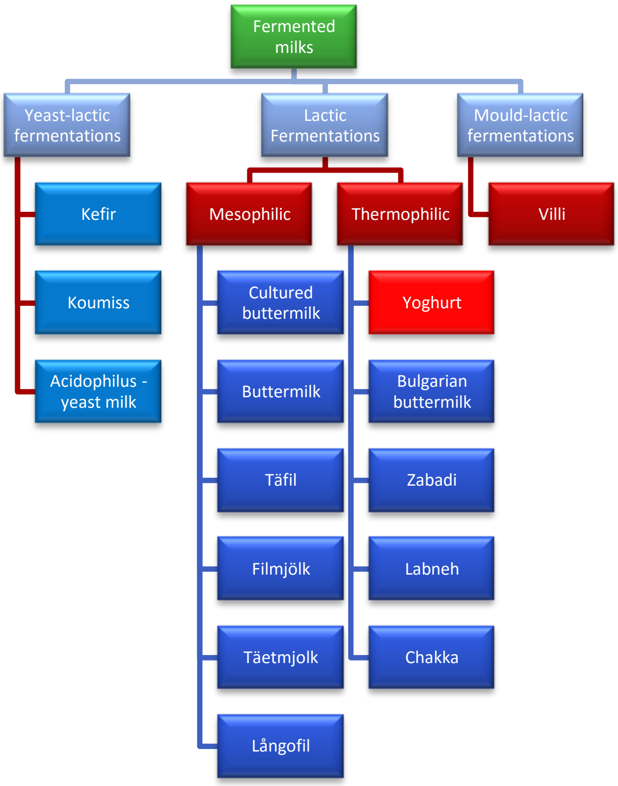


Figure 1 Scheme for the classification of fermented milks. Adapted from Robinson and Tamime (2007)

Cow´s milk is the predominant product that is used for the production of fermented milks, including yoghurt (Chandan and Kilara, 2013).

6.2 Yoghurt

The etymology of the yoghurt name comes from the Turkish yoğurt and is associated to yoğurmak and yoğun that means “to knead” and “dense” or “thick” respectively (Yildiz, 2010).

Yoghurt is made by inoculation of specific bacteria strains into milk, called Lactic Acid Bacteria (LAB) the most common are *Streptococcus thermophilus* and *Lactobacillus delbrueckii s*ubsp*. bulgaricus.* These bacteria like to ferment under controlled temperatures between 42 and 43°C. In the first stage of the fermentation the *S. thermophilus* have much activity and ingest the natural milk sugar called Lactose and release lactic acid as a waste product and diacetyle as a characteristic flavour component in the yoghurt. The lactic acid lows the acidity of the yoghurt between pH 4.5 and 3.7, *S. thermophilus* decrease its activity in this acid media and *L. bulgaricus* starts to have more activity, this bacteria produce acetaldehyde which gives yoghurt typical taste and sour aroma. The low acidity in the yoghurt also does not allow the growing of pathogenic bacteria (Alakali et al., 2009).

The acidification in the milk causes a disturbance in the structural properties of casein micelles. The caseins approach to their isoelectric point (pH 4.6) this, cause electrostatic and protein attraction between hydrophobic interactions in the system, forming a solid mass called curd or coagulum (Lee and Lucey, 2010; Lobato-Calleros et al., 2014). The curd has semisolid texture; it is produced by lactic acid coagulation of the milk without drainage. It is a composite gel in which the casein micelles, made a casein matrix that includes milk fat globules in their final structure (Lobato-Calleros et al., 2014; Sodini et al., 2004; Yildiz 2010). *S. thermophilus* and *L. bulgaricus* produce also expolyssacharides (EPS), to form the characteristic sliminess/ropyness called ropy. The EPS are formed by glucose and galactose. However this formation can have an effect of the final product, the ropiness generation help to increase the viscosity of yoghurt and reduce the whey separation (Robinson and Tamime, 2007). It depends in with on the type of culture, and how is the interaction with the bacterial cell surface and the protein in the yoghurt matrix, and affect the quality of the product (Schonbrun, 2002; Lee and Lucey, 2010; Robinson and Tamime, 2007; Yildiz, 2010).

The structural characteristics of the yoghurt depends in many physicochemical factors, the first and most important factor is the milk, from the origin to the treatment that it has during the process have an influence to the final product. Then comes the starter cultures, followed by the additives and even the process design in the yoghurt give a different physical results in the yoghurt (Lyck and Tamime, 2006; Schonbrun, 2002).

The dimensions, the design of the equipment and process conditions as temperatures and flow rates affect directly to the structure of the yoghurt. The physical treatment that the yoghurt has in the line can be quantified by measuring the shear. Shearing is a crucial factor in stirred ambient yoghurt processing. (Benezech and Maingonnat, 1994; Nilsson, Lyck and Tamime, 2006).

To maintain or improve the characteristics of the texture of the yoghurt, it is common to use stabilizers; the most used in yoghurt industry are hydrocolloids as pectin or gelatin. This stabilizers are added in to the milk base to increase or keep the physical characteristics of the yoghurt as texture, viscosity and mouth feel (Lee and Lucey, 2010; Nilsson, Lyck and Tamime, 2006; Schonbrun, 2002).

Yoghurt is a product well known in the world. Yoghurt manufacturing processes differ minimum depending in the country or region that are developed, even though the flavour, consistency and flavour can be different. In some areas it is consumed in the form of a highly viscous product or in other countries prefers to eat it in the presentation of a soft or fluid gel. The core of the production of the yoghurt is the lactic acid fermentation that affects the stabilization of the protein system, generating the gelification of the milk (Sodini et al., 2004, Tetra Pak 2015)

6.2.1 Yoghurt a food gel

Yoghurt is a food gel, BeMiller and Whistler (1996) defines a gel as a “continuous three dimensional network of connected molecules or particles, entrapping a large volume of a continuous liquid phase”. In food products as yoghurt the gel network consist of polymer, mix of polysaccharides and or proteins joined by electrostatic attractions in polar groups as hydrophilic groups and hydrophobic associations, ionic cross bridges, enlargements and aqueous phase (BeMiller and Whisler, 1996).

The yoghurt formation starts with the fermentation of the lactose (around a 20-30%) in the milk by the culture *(*Sharma, 2013). Both bacteria in the culture have a synergy, *S. thermophilus* reproduce faster than *L. bulgaricus* producing lactic acid and carbon dioxide *is* the responsible for the initial drop in pH around 5.0. Now the pH is optimal for the growing of *L. bulgaricus*, which has a high proteolytic activity, broke the protein in to peptides. These peptides are used by the *S. thermophilus* to produce acetaldehyde (gives the characteristic flavour of the yoghurt) and acid dropping the pH below to 5.0 *(*Sharma, 2013; Lucey, 2004). LAB also contributes to the gel formation of the stirred yoghurt, through the formation of the EPS, which prevents the syneresis. Stirred yoghurts fermented with EPS producing cultures show higher mouth thickness, higher ropiness, higher creaminess but lower gel firmness (Béal and Helinck, 2015).

During acidification of milk, casein micelle has many changes in its integrity. When the pH falls around 5.5 to 5.0, the caseins start to dissociate. As the pH continues dropping and approaches in the region of 4.6-4.3, the colloidal calcium phosphate (CCP) solubilises and diffuses into the aqueous phase of the milk. With these changes the calcium reduces in the casein micelles resulting in the coagulation of the casein, the charge on the caseins is altered destabilizing its isoelectric point. This change reduce the negative charge of the casein micelles, which lows the electrostatic repulsion between casein charged groups and generates aggregates forming a three dimensional gel matrix, the casein micelles are linked in long chains immobilizing the liquid phase forming the yoghurt structure Figure 2 (Aguilera and Stanley, 1999; Béal and Helinck, 2015; Rawson and Marshall, 1997; Lucey and Singh 1998).

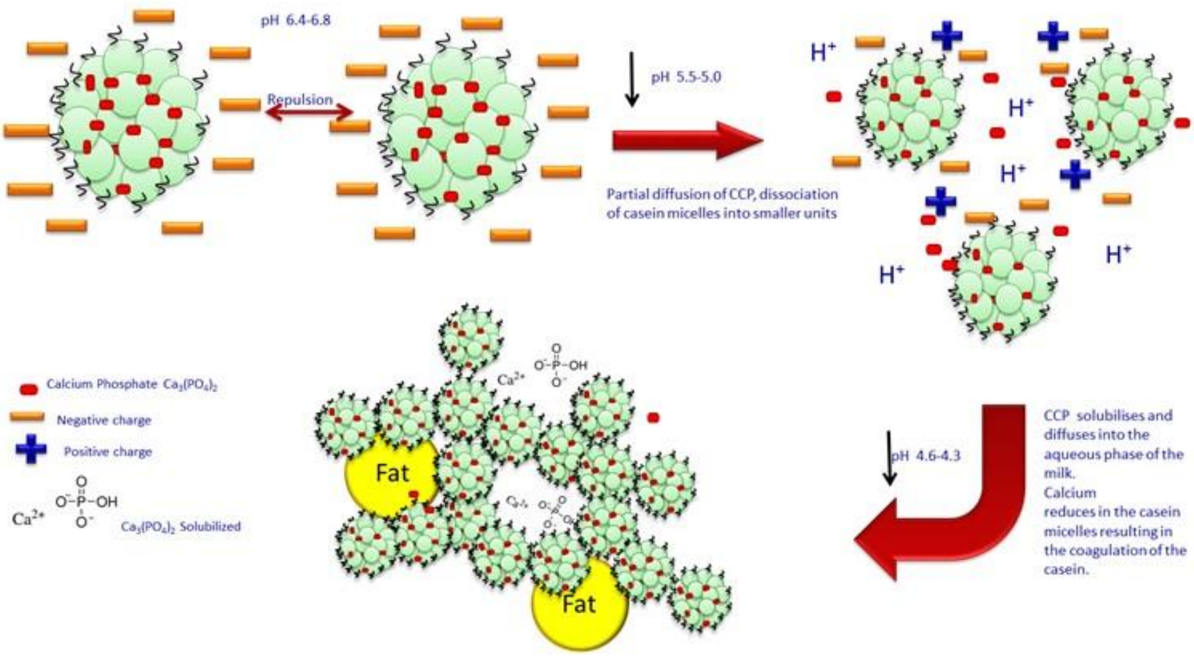


Figure 2 Yoghurt network formation, interactions with the casein micelles

6.2.2 Yoghurt types

There are different types of yoghurt presentations that can find on the market. In can be classified depending on how the product has been process. The different classification of the yoghurt depends more in how is the process after fermentation of this product.

- Set yoghurt: A solid set where the yoghurt is incubated and cooled in the consumer container (Tetra Pak 2015, Yildiz 2010).
- Stirred yoghurt: The yoghurt is fermented in a container, then the coagulum is gentle destroy then proceed to be cooled and package in packages to the consumer. The consistency of this yoghurt is less firm than the set type yoghurt (Tetra Pak 2015, Yildiz 2010).
- Drinking yoghurt: The process is like the stirred type yoghurt but the coagulum is broken to form a more liquid consistency (Tetra Pak 2015).
- Frozen Yoghurt: After the fermentation of the yoghurt it is frozen by batch or continuous freezers (Yildiz 2010).
- Concentrated yoghurt. It is concentrated by action of filtration to give a thicker consistency and then cooled before been packaged (Tetra Pak 2015, Yildiz 2010).

In the shelf life period of the yoghurt after the processing, it is important to have a global vision in the chain of distribution, market, and eventual consumption of the product. Yoghurt under refrigeration temperature (4- 6 °C) may achieve up to 16 to 21 days of shelf life, taking in to consideration at the cool chain was not disturbed, when and during the consumer acquire the product to its consumption. The packaging of the yoghurt helps also to maintain the integrity of the product. (Alakali et al., 2009; Chandan and O´Rell, 2013). Cold chain is necessary to maintain the quality and shelf life of the yoghurt, nevertheless the acidity of the product will increase 0.2% during this shelf life period. The increasing of the acidity of the yoghurt are a change relatively insignificant compared with other quality alterations that the product can experiment as loosing the texture or the whey separates from the system to mention two (Tamime, and Robinson, 1999).

In many countries consumers many countries involve more consumers that do not have the possibility to access to a refrigerator or don´t have the shop near their homes to maintain the cool chain. For this access issues to the product there are developing new strategies to maintain the quality to the yoghurt without the necessity for chill it. This is the case of the ambient yoghurt that is a growing market in Asia and is extending in other parts of the world.

6.3 Yoghurt production

There are many types of yoghurt in the market; two types dominate the market for fermented milks. One is the set-type yoghurt, has firm gel structure and the other type is stirred yoghurt, has a thick consistency and the flavour can modified by adding fruits or flavour (Tamime, 2006).

The manufacturing stages for the yoghurt in any of these presentations are similar in the treating of milk (Fig 3) (Tamime 2006). The milk first need to be standardize of fat content and dry matter content (DM), also homogenize milk and the heat treatment. To standardize DM content, milk powder is added, the additives are mixed in the milk before the homogenizing and heat treatment (Tetra Pak, 2015).

The pre-treated or standardized milk is cooled to reach the incubation temperature and pump it to incubation tanks, at the same time the culture is added into the milk. Commonly in the production of stirred yoghurt the incubation time is 4-5 hours at 42-43°C. When the yoghurt reaches the desired pH, it is cooled to ambient temperature 15-22°C, at the same time the coagulum is broken gently (Tetra Pak, 2015).

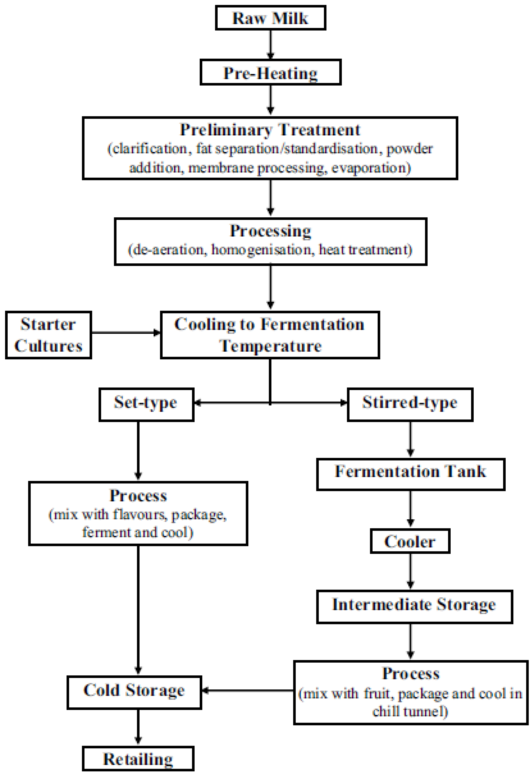


Figure 3 Flow diagram of the manufacturing process of set and stirred yoghurt. Image from Tamime, 2006. Fermented milks

The yoghurt gel when is broken maintains a structure in which the whey is retained. Stirred yoghurt maintains the viscous structure due the particles of fragmented acid-casein gel that reminds in contact with each other (fig 4), due to the EPS of the bacteria and the pre-process of the milk (Rawson and Marshall, 1997; Kiani et al., 2010).

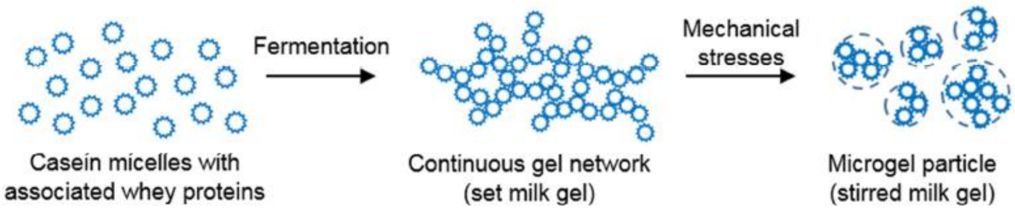


Figure 4 Structure of the gel network of the yoghurt before and after the stirring picture from (Mokoonlall, Nöbel and Hinrichs, 2016)

6.3.1 Process effects in the yoghurt

Yoghurt is manufactured in a very large scale and whatever the type of yoghurt is going to be produced, the industrial process of yoghurt it involves three main steps (Béal and Helinck, 2015; Lucey, 2004).

1. Milk homogenization
2. Milk heat-treatment
3. Milk inoculation

6.3.1.1 Milk homogenization

Fat improves the mouth feel of the product and provides the perception of creaminess assures a uniform distribution of the fat in the yoghurt and gives a good stability of the coagulum against whey separation (Lucey and Singh,1998; Chandan and O´Rell, 2013). Homogenization helps in the mixing of the stabilizers and other ingredients put it in the milk (Lucey, 2004).

Fat globules before homogenization don´t interact with casein particles, after the homogenization the fat membrane is replaced and have more interaction with the casein matrix and whey protein in the system; this effectively increases the number of structure-building component (Lucey, 2004). Homogenization reduces the fat globules from 1-10 µm to 1-2 µm with these the fat surface area increase. The new fat globules now are covered mainly by casein micelles and whey proteins (Figure 5). This prevents fat separation during incubation period, enhance the consistency, reduce whey separation from the coagulum and increase whiteness (Béal and Helinck, 2015; Chandan and O´Rell, 2013; Lucey and Singh, 1998).

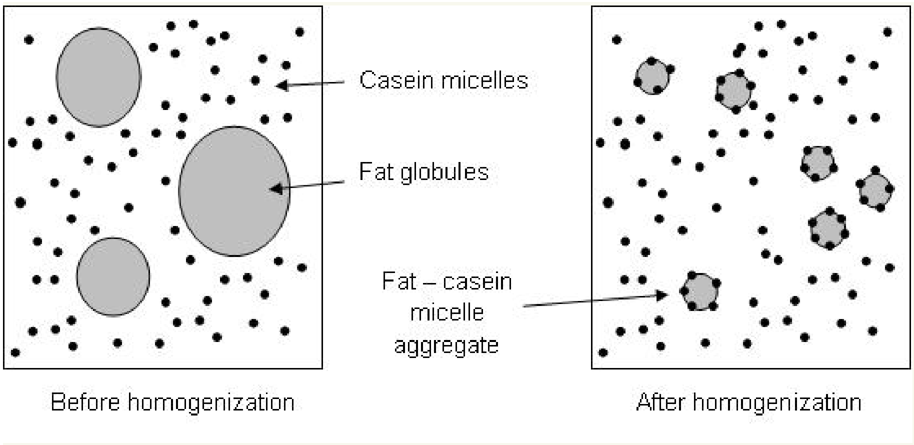


Figure 5 Effect of the homogenization in the fat globule and the caseins in the milk. Image from http://web.utk.edu/~fede/high%20pressure%20homogenization.html

Milk homogenization is done before heat treatment in order to avoid any recontamination of the milk (Béal and Helinck, 2015). The homogenization pressures for the milk are recommended in the range of 10-20 MPa (100-250 bar) at temperatures of 55-80°C. This is because when the fat phase is in a liquid state the homogenization is most efficient (Chandan and O´Rell, 2013; Lucey and Singh, 1998).

However Sodini et al. (2004) comments that homogenization conditions don´t have a high influence on the rheological properties of the yoghurt. The capacity of interaction of fat globules depends strongly on the proteic material that is attached on it membrane surface. Homogenizing fat with skim milk powder (SPM) gives an increase of the interaction of fat globules in the system (Sodini et al. 2004)

6.3.1.2 Milk heat treatment

Heat treatment of the milk has principally two uses. One is the destruction of the pathogens and spoilage micro flora that could be in the milk. When there are no other cultures to compete in the milk system the growth of the LAB is promoted. Additionally the heat treatment results in the expulsion of oxygen, generating sulfhydryl compound due to the reducing conditions without oxygen release compounds as peptides, free amino acids. That enhance the growth of *S. thermophilus* and *L. bulgaricus* (Béal and Helinck, 2015; Chandan and O´Rell, 2013).

The β-lactoglobulin, a whey protein, denaturate when the milk heats around 60-65 °C (Chandan and O´Rell 2013). This denaturation leads a hydrophobic interaction between β-lactoglobulin and κ-casein from the casein micelle forming a complex. Casein micelles with the β-lactoglobulin-κ-casein complex have a limited ability to aggregate forming short branched micelle chains that also coats the homogenized fat globules Figure 6. (Béal and Helinck, 2015; Chandan and O´Rell 2013; Kalab 2011).

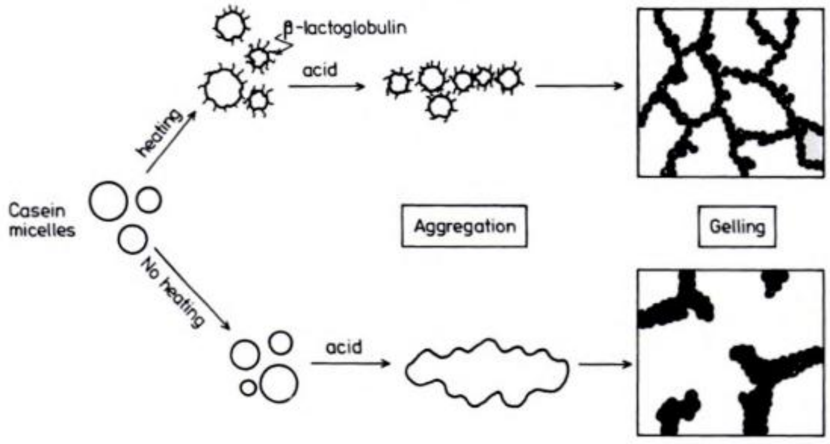


Figure 6 Effect of the milk heating and how affects in the casein micelles in the yoghurt formation image from Aguilera and Stanley (1999).

Due to the interactions of the β-lactoglobulin-κ-casein complex, the viscosity in the yoghurt improves when the milk has a heat treatment. The best result in the consistency of yoghurt is when the milk is heated 90-95°C with a holding time of 5-10 minutes. This enhances water retention capacity, creating a smooth consistency, high viscosity and stability from the whey separation in yoghurt (Aguilera and Stanley ,1999; Chandan and O´Rell, 2013).

6.3.1.3 Milk inoculation

After the homogenization and the heat treatment of the milk is time to inoculate the milk in order to form the yoghurt. As it was mentioned before starter culture normally conformed by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Incubation temperatures for the optimal fermentation of the milk by these bacteria are in the range of 40-45°C. However Sodini et al. (2004) writes that is recommended to lower incubation temperature from ~44°C to ≤38°C. Lower the incubation temperature in yoghurt makes the product more viscous, smoother and slimy. This is because at low temperatures aggregation of the casein micelles occurs more slowly. The fermentation time is around 4-6 hours depending on the amount of starter addition and the incubation temperature (Lucey and Singh, 1998; Sodini et al., 2004). When yoghurt has reached the pH desired values, usually ≤4.6, yoghurt is cooled to ambient temperature to stop the acid generation (Lucey and Singh 1998).

6.4 Ambient Yoghurt (Long-life yoghurt)

In ambient yoghurt called also long life yoghurt the product is heat-treated after fermentation to prolong the shelf life. The aim of heat treating the yoghurt is to inactivate the starter bacteria and their enzymes to minimize post acidification and also inactivation of yeast and moulds that gives negative characteristics to the product, if there is contamination of the product. In stirred ambient yoghurt the coagulum is heat treated at 75-110°C for some seconds (Tetra Pak, 2015). In this process the yoghurt experiments different mechanical shears. Shearing of the yogurt affects thickness, which is related to the viscosity, and smoothness, which is the absence of perceivable particles and also the reduction of water holding in the system (Weidendorfer et al., 2008).

6.4.1 Ambient yoghurt manufacturing process

Stirred ambient yoghurt has a difference in the last part of the fermentation as it was written before, the yoghurt bacteria needs to be inactivated to avoid the post fermentation applying heat treatment, as it shows in figure 7:

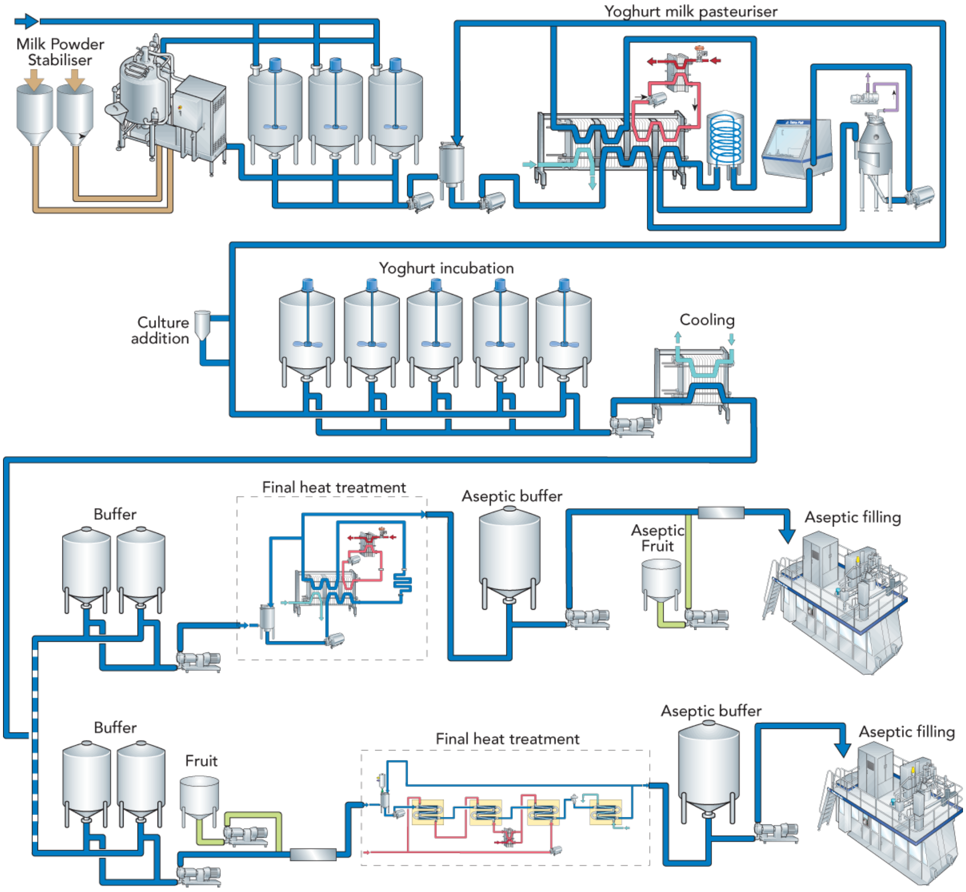


Figure 7 Process of Stirred ambient yoghurt, image from TetraPak Dairy process hand book (2005)

6.5 Quality characteristics in stirred yoghurt

Consumer acceptance is dependant of the sensory characteristics of the product. The sensory quality of the yoghurt is an important parameter in consumer choice including the lack of visual whey separation (syneresis), thickness (or viscosity), creaminess, sliminess (or ropiness) and smoothness (opposite to lumpiness, graininess or grittiness) (Bruzzone, et al 2013; Harte et al 2007; Sodini et al., 2004).

Yoghurt had two primary quality defects after processing and/or after certain time, variations in viscosity and syneresis, expulsion of the whey (Keogh and O´Kennedy, 1998). Syneresis is the expulsion of the serum (whey) from the yoghurt network, by the shrinkage or contraction of the gel due external forces or certain elapsed time without external forces "spontaneous syneresis" and is related to instability of the gel network due to the increasing of many rearrangements of the gel (Lee and Lucey 2010; Lucey and Sing, 1998). Syneresis had a negative input in consumer perception since the idea that is something microbiologically wrong (Lee and Lucey 2010).

After the visual exam that the consumer made to the yoghurt come the texture perception, when the stirred yoghurt goes in to the mouth. Texture affect the appearance of the yoghurt, mouth feel thus the overall acceptability (Ares G. et al 2006). The texture in stirred yoghurt can change due to:

- bad heat treatment conditions in the milk
- low fat content or bad homogenization conditions
- lack in consistency in the protein level
- not proper mixing of the stabilizers or usage levels,
- brake the coagulum before reaching the ideal pH (≤4.6),
- high temperature inoculation (<42 or >30 °C) and/or little inoculation time (less than 4 hours).
- mechanical effect like high shear rates (>447 s^{-1}) (vanMarle et al 1999)

To keep the uniform texture through the different steps in the processing line and the shelf life of the product is a continuous quality duty in yoghurt production. To know about the yoghurt texture the measuring of the viscosity is a routine standard method with this method is measured the rheological behaviour of the yoghurt (Ares et al 2006).

6.6 Rheology

Rheology is the study of the deformation and how the materials flow (Ritzoulis and Rhoades, 2013; Lee and Lucey 2010). In food technology is used to describe the consistency of a product (Nørregaard, 2012). Ritzoulis and Rhoades (2013), describes flow as "...the reorganization of the components of a system under the influence of an external force".

Materials have different flowing behaviour when an external force is applied to them depending on their physical properties, composition and structure (Afonso, 2003). Newtonian and non-Newtonian fluids are the classification of how the materials behave; a Newtonian fluid have a constant viscosity independent of the applied shear rate and follows the Newtonian law of viscosity (Tetra Pak, 2015):

$$\eta = \frac{\sigma}{\dot{\gamma}} \quad (1)$$

Where η is viscosity, σ shear stress, is the force that the flowing material applies on a surface expressed in Pascal (Pa) and $\dot{\gamma}$ the shear rate, is describe as the derivative of the velocity to the vertical distance ($\dot{\gamma} = \frac{dv}{dx}$), describe the flow by a velocity gradient, the units are s^{-1} (reciprocal seconds)(Ritzoulis and Rhoades, 2013; Nørregaard, 2012).

In the Newtonian fluid system the viscosity is defined as "the coefficient of proportionality between shear stress and shear rate"; viscosity is the resistance to flow. High viscosities associate with low shear rates fig 8 (Ritzoulis and Rhoades, 2013).

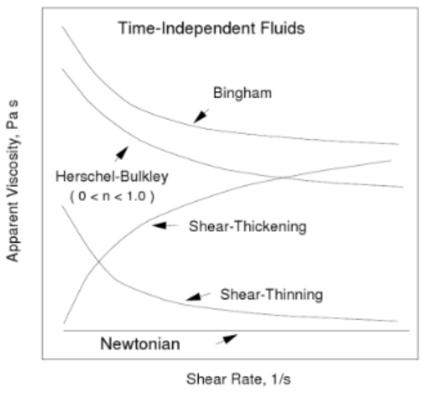


Figure 8 Viscosity in different type of fluids, Newtonian and non-Newtonian (Steffe, 1996)

Newtonian fluids have a directly connection between the shear stress and the shear rate, with zero intercept, the fluids that do not have this behaviour are called non-Newtonian (Steffe, 1996) fig 9.

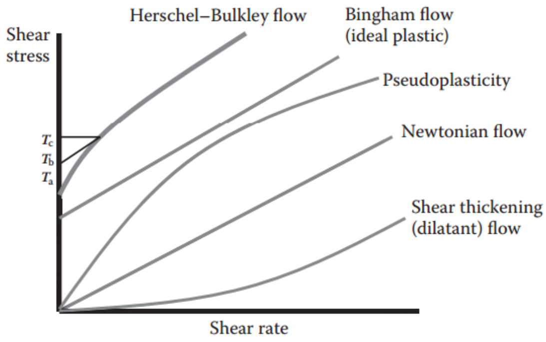


Figure 9 Different fluid behaviours Newtonian and non-Newtonian as a function of shear stress (Ritzoulis and Rhoades, 2013)

Viscosity values are used to measure the mouth feel of the food; human perception of thickness is approximately linked to the viscosity result when shear rate is 60 s^{-1}(Steffe, 1996).

Non-Newtonian fluids are shear-rate dependant, and its viscosity is also time-dependant, with these characteristics the viscosity of the product depends of the shear rate and the duration when the shear is applied (Tetra Pak, 2015).

Non-newtonian fluids have different classifications depending in their flow properties (Steffe 1996; Ritzoulis and Rhoades, 2013). Time dependant non-newtonian fluids are classified as thixotropic and rheopectic and non-time dependant non-Newtonian fluids are shear-thinning, shear thickening or plastic (Tetra Pak, 2015).

Yoghurt is a non-Newtonian fluid; with high time-dependant behaviour is classified as a thixotropic fluid but yoghurt is not a true thixotropic material since the breakdown of the structure due shear is not totally reversible when the application of shear stops (Lee and Lucey 2010).

Shear rate in pipes can be calculate as wall shear rate or average (representative) shear rate. The average shear rate include the variations of shear rates in all the cross-sectional tube area, with this the flow behaviour is average instead of considering only the wall shear rate that the fluid has (Bolmsted 1994). In this work the average shear rate formulas are going to be used in the design of the experiment.

6.7 Stabilizers

Stabilizers are hydrocolloids; hydrocolloids are high-molecular-weight water soluble macromolecules, which origin can be from plants or animals. It modifies the rheological properties of an aqueous continuous phase in the milk (Dickinson, 1992).

The goal to add stabilizers to yoghurt is to improve texture, consistency and maintain, build or increase the viscosity and mouth feel. Stabilizers in yoghurt are also used to reduce whey separation or syneresis in the yoghurt gel (Chandan and Kilara, 2013; Nauth, 2004). Yoghurt per se does not required addition of stabilizers to form its characteristic texture, the firm gel and high viscosity will occur naturally at fermentation. Nevertheless stabilizers are necessary and extensively used in stirred yoghurts due to maintain or enhance the yoghurt textural characteristics after the processing conditions and make the product more stable against the quality textural problems that was mentioned before (Béal and Helinck, 2015; Özer, 2009).

Stabilizers can form a three-dimensional (3D) gel network in which whey is trapped, limiting the movement of free water in the system and increasing water binding capacity of proteins in yoghurt. Stabilizers can interact with the protein molecules and therefore the gel network is more stable (Özer, 2009)

Yoghurt with lower milk solids has a mayor tendency to syneresis. Stabilizers as gelatin, agar, gums, pectin, carrageenen and pregelatinized starch is generally use combine and included in the formulation of the yoghurt (Clark and Plotka, 2004; Nauth 2004; Özer, 2009). However the quantity of stabilizers in the yoghurt mix depends in the total solids (TS) in the milk. As Özer (2009) write "the higher TS level in the milk, the lesser the amount of stabilizer added".

The denaturation of whey proteins increases the firmness and viscosity of the yoghurt (Lucey and Singh, 1997). The addition of SMP is common in the process of yoghurt. SMP increase TS in the milk, this result in a variation of proteins incrementing the casein ratio. High protein/TS ratio gives more surface area to make gels more tight and firmer reducing the syneresis and enhancing the viscosity of the yoghurt (Sodini et al., 2004).

The stabilizer used in yoghurt depends in their functional properties and usage levels also depend on how the interaction between stabilizers combinations affects the product. It is important to have in focus that some stabilizers are permitted by FAO and WHO, a resume description of the most used stabilizers in yoghurt are shown in table 1. Chandan and Kilara (2013) mention five points that is important to consider in the way to choose a stabilizer:

- Know what type of yoghurt will process: as set, stirred, drinkable etc
- Consider the fat content and total solids in the formulation
- Have decided the desired firmness and consistency of the yoghurt.
- To which market is directed, Kosher, organic, etc
- The equipment that is available in the line and can follow the instructions of usage of the stabilizer, as homogenizers, heating system, etc.

Table 1 Different stabilizer used in yoghurt approved by the FAO and WHO, their functions in the yoghurt and the recommended usage level.

Stabilizer	Description	Function in yoghurt	Recommended usage level %
Agar	A carbohydrate is derived from red algae.	Provides viscosity as a gelling agent.	0.25-0.7
Carboxy-methyl cellulose (CMC)	A carbohydrate derived from cellulose	Thickener, add viscosity, reduce syneresis. It develops a typical aroma and flavor in the yoghurt.	0.2-1,5
Carrageenan	Carbohydrates from red seaweed. There are three types. Kappa (k), iota (i) and lambda (ʎ).	Stabilizer and gelling agent. Stable at pH 3.5-4.0 K produces strong rigid gels I produce week gel but resist to syneresis ʎ have a strong interaction with proteins	0.2-1.5
Gelatin	Protein from animal collagen	More effective on the properties of the stirred yoghurt. Prevents syneresis. It has gelation properties. At concentrations ≥0.8%, viscosity decreases and sensory defects start to be noted.	0.3-2.0

Guar gum	Carbohydrate from guar seeds.	Thickener. Is stable in a big pH range, but not high temperatures.	0.2-1.5
Locust bean gum	Carbohydrate from carob seeds.	Thickener helps to gel properties and reduces syneresis. It contributes in to the formation of aroma and flavor compounds.	0.2-1.5
Maltodextrin	Carbohydrate.	Provides a custard-like reversible gel. It imitates the gel characteristics of gelatin.	1-5
Modified food starch from corn, potato or tapioca	Carbohydrate.	Thickener and gelation properties. Tapioca starch can replace 2% of solid non fat in milk (SNF) it is used in 0.6%. Modified starch is used to replace non fat dry milk with 40% modified starch. It doesn´t affects flavor, body and texture of the yoghurt.	0.1-0.7
Pectin	Two types: Low methoxy (LM) is used for set yoghurt, because requires calcium to gel, but not high solids and High methoxy (HM)for drinkable yoghurt It is a carbohydrate from citrus or apple.	Gelling agent and gives viscosity.	LM 0.08-0.12 HM 0.08-0.20
Xanthan gum	Carbohydrate synthesized from bacterial fermentation.	Thickener, stabilizer. Provides high viscosity, in occasions slimy. It does not affect bad sensoric properties in stirred yoghurt.	0.2-1.5

Source: Adapted from Clark and Plotka, 2004. *Yogurt and Sour Cream: Operational Procedures and Processing Equipment* and Özer, 2009. *Strategies for Yogurt Manufacturing*.

6.7.1 Ambient yoghurt stabilizers

Making stirred ambient yoghurt with mix stabilizers is necessary due to the processing and Özer (2009) advice for the using of locust bean gum, the mixture of agar–agar and Xanthan, or starch derivatives. Yogurt-mix preparations in general have a hydrocolloid combination carefully designed to give the proper and desire characteristic to the final product. However the stabilizers mentioned before are not the only ones used in the production of ambient yoghurt, there are other companies that suggest other combinations as CPKelko and Du Pont.

In this work it is going to be use different stabilizers mix made from Du Pont and Cp Kelko especially for the ambient yoghurt. Both formulas use modified starch

Grinsted®SB 264 is the mix that is provided by Du Pont; Grinsted® products includes hydrocolloids and emulsifiers, helps to improve the texture, appearance and shelf life of the product (Danisco.com, 2016).

Grinsted®SB 264 It is used in heat treated milk to make yoghurt and sour cream in long-shelf life products.

It increases in the viscosity and improves the consistency and texture, bind free moisture, prevents separation of serum (Yug-ingredient.ru, 2016). It can maintain stability in pH 4.3 and during the heat treatment. Maintains the viscosity and prevents the syneresis of the product in temperatures around 20-25°C (Danisco.com 2016). The ingredients that have this mix are: Modified starch, Gelatin and Pectin.

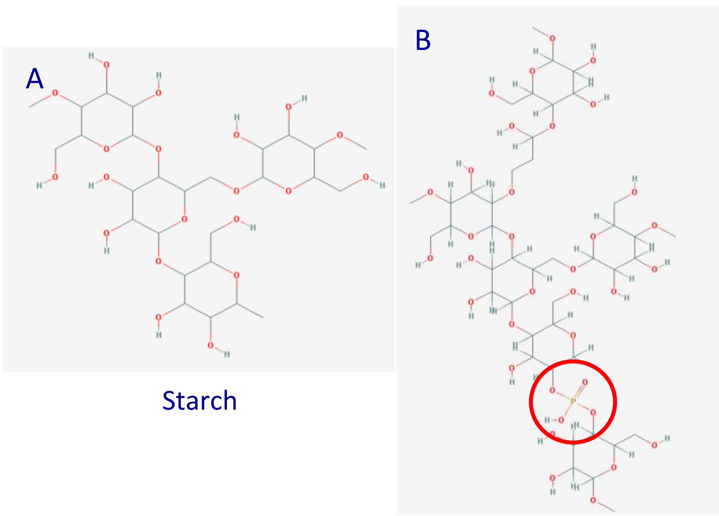
In Cp Kelko ambient yoghurt formulation it is used Kelkogel® YSS, is a special Gellan Gum focus in hold the free water in the system to avoid the syneresis and give structured in stirred yoghurt. It is used as replacement of gelatin, maintains the flavour profile (Cp Kelko data sheet 2013). Also in the formulation is used a combination of pectins by Cp Kelko called Genu® Pectin, is used as a high thickening agent in yoghurt formulations (Cp Kelko data sheet).

There are two thickeners that are use frequently in yoghurt processing. These are modified starch and gelatine (Ares et al., 2007). In both formulas of Cp Kelko and Du Pont have the modified starch and pectins in their mix but it differs in the gelatine, used by Du Pont and Cp Kelko has Gellan gum.

6.7.1.1 Modified starch

Starches are widely used in food industry to modify the texture of the product. The incorporation of starch increases the yoghurt viscosity. The ability to thicken, gel and retain water in native starch have some limitations that doesn´t give the ideal texture to the final product. For instance native starches give to the product excessive viscosity and if the milk has low solids content native starch give a deficient body and high susceptibility to retrogradation in the gel yoghurt. It gives in consequence a product with gel opacity and syneresis and it doesn’t harsh process tolerance. For this reasons most of the starch use in yoghurt needs to be modified chemically or physically in order to improve their suitability in the different process steps in the product (Huber and BeMiller, 2009).

Starch reacts due to its various hydroxyl groups, in modified starch only few hydroxyl groups are modified commonly with ester or ether groups. Modified starch reduces intermolecular associations with each other this lowering the tendency of the starch to agglutinate, thus the precipitation. To improve viscosity under acid media the hydroxypropylated starches are cross linked with phosphate groups, figure 10 (BeMiller and Whisler, 1996).



Starch

Hydroxypropyl distarch phosphate

Figure 10 (A) Starch without modification and (B) hydroxypropyl distarch phosphate. The chains have a phosphate in the molecule. Figures from https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=24836924

In ambient yoghurt it is necessary to have a starch that can resist severe shear processing conditions, low pH and high heat. The addition of starch, specifically the addition of hydroxypropyl distarch phosphate modified starch (HPDSP), which it was modified to have superiority against the normal starch as thickener and stabilizer (Chandan and Kilara, 2013).

To have the property to make a stronger gel and the decrease of the gelatinization temperature in the yoghurt, the hydroxypropyl groups will bond to the amylose making weaker the internal link structure of the starch granule by adding hydrogen groups into the amylose molecule (Cui et al., 2014; Singh et al,. 2007). The cross-linking treatment gives strength and stability to the starch granule, due the adding of intra and inter molecular bonds forms bigger casein flocs than the ones seen in yoghurt without stabilizers, also HPDSP remain hydrated, maintained clarity, had better high shear resistance, reduced syneresis and delay retrogradation during storage (Cui et al., 2014; Singh et al., 2007; Pang et al., 2015).

HPDSP don't retrogradate. Retrogradation is the reaction when starch forming molecules (amylose and amylopectin) realign themselves, causing the expulsion of the liquid in the gel (BeMiller and Whisler, 1996). HPDSP is frequently use as thickening agent in yoghurt production due to its easy processing and low cost compared with other hydrocolloid as pectin or gellan gum (Gonçalvez et al., 2005).

The HPDSP have different charges depending in the pH in the yoghurt system. It has a positive potential when the pH is around 6.2- 7, but when start decreasing between 4.5-3.8. Now the negative particle of HPDSP absorb the casein with that has positive charge. It occurs via electrostatic forces (Cui et al 2014). The stability of the yoghurt system with HPDSP is explain by electrostatic attraction theory, at the same time casein aggregates and starch micelles have positive charge and repulses each others. When the pH goes down some low affinity areas in the casein molecules, can stick in the surface in the starch micelles, this mechanism is called "steric stabilisation". In the yoghurt system with starch there is at the same time electrostatic repulsion and steric stabilisation, this is the attribute to the stability of the system (Cui et al., 2014).

To combine starches with sugars provide textural modification apart for their role to give sweeter the product. The gelatinization temperature of the starch is increased above the conventional gelling starches that is around 70°C. In the modified cross-linking starches the sugar stabilizes the amorphous region such as polyol-starch chain reaction. It delays the starch gelatinization and decrease the water activity by adding sugar (Abbas et al., 2010).

6.7.1.2 Gelatin

Gelatin is a protein from the animal white connective tissues and bones called collagen. The collagen is partially hydrolyzed to form the gelatin. The common sources to obtain the collagen gelatin are pig skins, bones and cattle hides (Nussinovitch and Hirashima, 2013).

In yoghurt manufacturing the gelatin used is one with high bloom. Bloom is a measurement of gel strength and rigidity and is measured in grades. The gelatin used in yoghurt has 225/250° Bloom (Chandan and Kilara, 2013; Nauth 2004; Nussinovitch and Hirashima 2013).

Gelatin is used widely as a stabilizer in the different types of refrigerated yoghurt. It improves the texture, giving firmer products with low tendencies to syneresis and is compatible with the milk system in a wide range of concentrations (Fiszman and Salvador, 1999). The interaction with the casein matrix and the gelatin gives a stronger three dimensional network in the product (Ares et al., 2007).

Yoghurt gel only stabilized with gelatine. Gelatine is weak by the rise of the temperature. Gelatin gels melts at temperature 25°C-40°C (Nussinovitch and Hirashima, 2013). It is clear that the role in gelatine in ambient yoghurt is not to maintain the structure of the gel. If only gelatine is used in ambient yoghurt, the product will have low viscosity and have a jelly-like body tending to have lumps (Chandan and Kilara, 2013; Nilsson et al., 2006). However gelatin is used because it has the unique property to melt in mouth temperature (36.5–37.5 °C) and give a pleasant sensation in the mouth feel (Chandan and Kilara, 2013; Fiszman and Salvador, 1999).

Gelatin cannot fulfil completely the quality characteristics of the ambient yoghurt, for this reason is mixed with modified starch, to produce body and a pleasant smoothness in the mouth (Chandan and Kilara, 2013). Other additives recommended and used with the gelatin or as potential alternatives to gelatin is the pectin and gellan gum in stirred yoghurt (Nilsson et al., 2006, Pang et al., 2015).

The utilization of gelatine in some food products can have limitations when the consumers choose the product, due to client religious beliefs or food life style as ovo-lacto vegetarian (egg, dairy). Impulses the search of new additives, the suggested mix of modified starch with pectin and gellan gum is suggested as an alternative for the ambient yoghurt (Chandan and Kilara, 2013, Pang et al., 2015). However gelatin has more effectiveness preventing syneresis than all this thickeners mentioned before (Gonçalvez et al., 2005, Ares et al. 2007).

6.7.1.3 Pectin

Native pectins are in the cell walls and intracellular layers of plants. Commercial pectins are obtained from citrus peel and apple and have a variety content of methyl ester groups Pectins have the ability to form gels in presence of sugar and acid or in presence of calcium ions (BeMiller and Whisler, 1996).

Commercial pectins have different preparations divided in two groups, the High methoxyl (HP) pectins in which half of the carboxyl groups are in methyl ester form (-$COOCH_3$) and the Low methoxyl (LM) pectins, in which structure have less than half of the carboxyl groups are in methyl ester form figure 11 (BeMiller and Whisler, 1996).

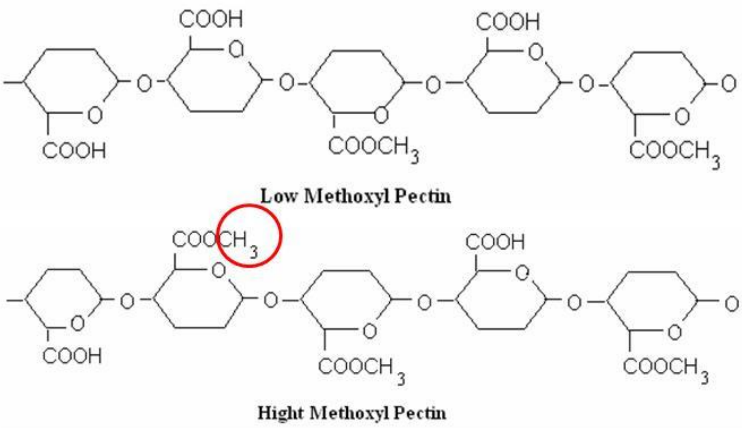


Figure 11 Structure of low and high methoxy Pectins (image from http://hoahocngaynay.com/vi/nghien-cuu-giang-day/bai-nghien-cuu/86-pectin-va-ung-dung.html)

HM pectin can gel only when sufficient acid and sugar (at least 55%) are present and the pH is low (Herbstreight and Fox, 2016; BeMiller and Whisler, 1996). Caseins have a negative charge in milk and pectins repelled when the milk system is pH neutral (6.5-6.7). During the acidification of the milk the casein change charge to positive due to the isoelectric point at pH 4.6 and that is when the pectin interacts with the casein. The use of high methylester pectins in yoghurt drinks, yoghurt fruit drinks and milk fruit drinks prevents the agglomeration of the proteins (Chandan and Kilara, 2013, Herbstreight and Fox, 2016).

LM pectin solutions make gel almost the same as HM pectin, but LM pectin has a better availability to form bonding in the presence of divalent cations such as Calcium (Ca^{+2}), which provide cross-bridges. LM pectin is relatively independent of the amount of TS in the milk, to gel. Increasing the concentration of divalent cations increases the gelling temperature and gel strength (Herbstreight and Fox, 2016, BeMiller and Whisler, 1996).

In the ambient yoghurt formulas it is used the low methyl ester pectin (LM pectin) since in the process of thermisation of the yoghurt makes the protein contracts and losses water, the consequence is the sandy structure of the proteins. The other type of pectins like the high methoxyl, not contain enough acid groups to make a gel with calcium ions, and need a high sugar concentration, around 80%. And the gelation mechanism is not completely reversible with the addition of heat producing a system with propensity to syneresis (May, 1990).

The structural influence that the LM pectin gives to the ambient yoghurt formulations is due the ionic linkage with calcium, making a stable "egg box" structure, figure 12. A section of two pectin chains must be free of ester groups to make the union with the calcium ions, which are formed by calcium cross-linking between free carboxyl groups. It is very important to the choosing of pectin and the pattern of methyl-esterification, due to it is also critical influence in the rheological properties of the yoghurt. This is the low concentration of calcium in the system with the pectin makes an elastic gel (May, 1990; Thakur et al., 1997; Willats W. et al 2006).

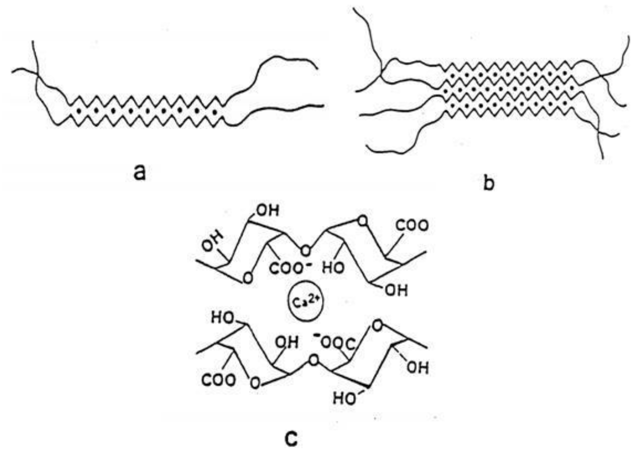


Figure 12 Calcium binding to pectin sequences (a) "Egg-box" dimer; (b) aggregation of dimers; (c) an "egg-box" cavity. Image addapted from Thakur et al. (1997)

To maintain the levels of calcium low and guarantee the forming and maintain the gel, in the ambient yoghurt, it is needed some calcium sequestrans, as citrates, fruit anions and sugar as fructose and dextrose. This reduction of the calcium levels helps the pectin to disperse in the system. In the other way a high concentration of sugar inhibit the formation of the gel, more than 50% (May et al., 1990; Thakur et al., 1997). In the formulations the percentage of sugar doesn't reach this percentage so it is assumed that the pectin was well disperse in the system.

Pectins are commonly used alone or in combination with other hydrocolloids to stabilized stirred yoghurt. LM pectin is the more used in stirred yoghurt due to the retention of the whey in the gel structure (Chandan and Kilara, 2013). LM pectin combined with gelatin prevents the syneresis of the system and give more thickness (Thakur et al., 1997).

As pectins are hydrocolloids are soluble in water and they form strong hydrated zones; resulting in a smooth mouth feel and prevent syneresis. The pectin dosage is important (0.20%). In the event of an overdose, the neutral milk may coagulate, resulting in a grainy-feeling product (Chandan and Kilara, 2013, Herbstreight and Fox, 2016).

6.7.1.4 Gellan Gum

Gellan gum is an anionic extra cellular polysaccharide secreted by the bacteria *Sphingomonas elodea*. It forms gels in the presence of cations when the hot milk is cooled. It has a linear tetrasaccharide repeating sequence, commercially is available in 2 presentations, depending in the substitutions, like Low Acyl (LA) and High Acyl (HA) gellan gum fig 12 (Kang et al., 2016; Kiani et al., 2010; Sworn, G. ,2009).

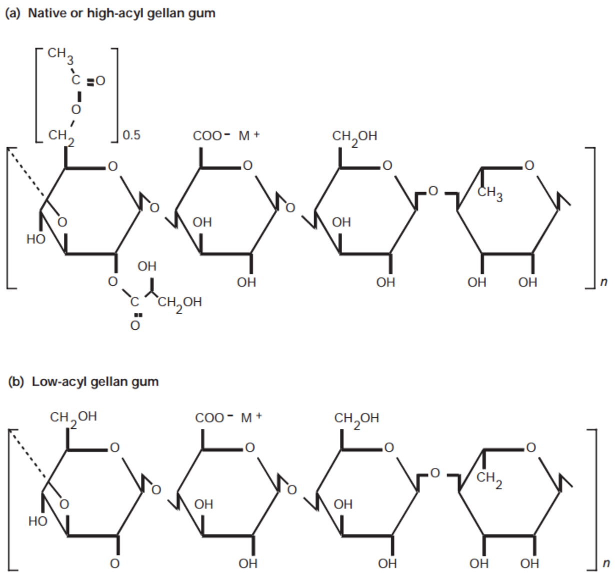


Figure 13 Primary structure of Gellan gum. Image from Valli and Clark 2010

LA gellan gum forms hard, non-elastic, brittle gels, due to the sensibility of the LA gellan gum to divalent cations. In the other side HA gellan gum gels are soft, elastic and non brittle, because HA gellan gum is less dependent on the concentrations of ions. (Sworn, 2009; Valli and Clark, 2010).

Both gellan gums can be mixed to give the desired texture in the final product and controlling the syneresis (Valli, R. and Clark, R. 2010). The way LA and HA gellan gums interacts interpenetrating each others, it could be said that the main gel structure is form in “gel within a gel” as Valli, R. and Clark, R. (2010) explains. This form a transparent gel with outstanding flavour release, the gel is resistant to shear and doesn´t depend on the pH in comparison with the pectin (Miyoshi, E. and Nishinari, K. 1999; Valli, R. and Clark, R. 2010).

For yoghurt, gellan gum is added to the milk before homogenization and pasteurization, due to these process help to hydrate the gum. Mix of two different acetylated gums has its limitations and the maximum percentage of LA gellan gum and HA gellan gum has their limitations in the quantity that can be added to the yoghurt formulation. LA gellan gum percentage of use is around 0.06% and HA gellan gum percentage usage is 0.1%. If it is added more than these percentages will cause excessive grainy texture in the yoghurt. It is recommended to combine with pectin or starch to form a heavier body in the yoghurt (Valli and Clark, 2010; Sworn 2009).

It is proposed to form stable aggregates to the yoghurt network it is needed the association of gellan helices, and Na^{+} cations which promote the aggregation process binding the helices as a result to minimize or suppress the electrostatic repulsion between them. Gellan gum is a negative charge hydrocolloid it interacts with the positive charge proteins (Bajaj et al., 2007). The divalent cations as Ca^{2+} concentration promotes the aggregation between the carboxyl groups in the helices, giving “egg-box” structures fig 14 (Kiani et al., 2010, Miyoshi and Nishinari, 1996; Sworn, 2009).

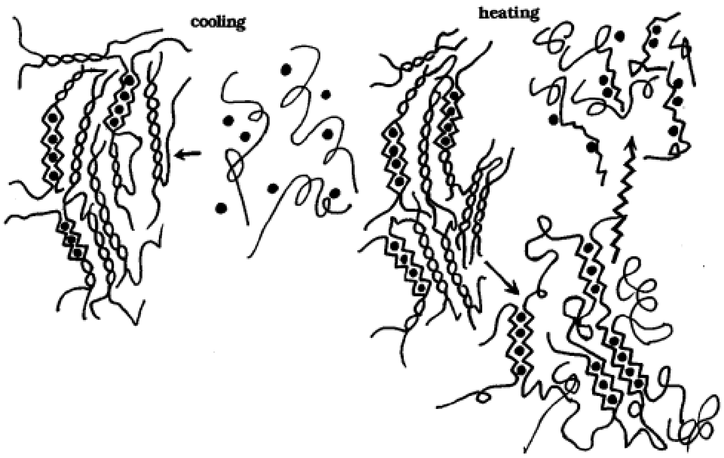


Figure 14 Model that explains the gellan gum gelation mechanism in presence of divalent cations (•). Picture from Miyoshi and Nishinari 1996)

It is important to note that gellan gum needs to be hydratated before coming in interaction with the cations. In milk system calcium is attached with the caseins, during heating there is remanding free calcium but it does not react with gellan gum, owing to gellan gum hydratate in milk above 80°C. Gellan gum can gelate well in yoghurt because it can make cagoules with monovalent ions as the K^+ present naturally in milk. The gellan gum maintains the structure of the yoghurt gel longer even in room temperature (Miyoshi and Nishinari, 1996; Sworn, 2009).

7 Design of the experiment

The yoghurt was produced in the pilot plant at the Department of Food Technology, Engineering and Nutrition, LTH, Lund University. Two standard ambient yogurt formulations with stabilizers provided from CpKelko (Formula 1) and DuPont (Formula 2) were produced, as shown in table 2 and 3.

Table 2 Formula 1 for ambient yoghurt by Cp Kelko

Formula 1 Cp Kelko	
Ingredients	**%**
Whole milk, 3.5% fat	91,82
Sugar	7,00
ThermTex starch	1,00
Kelcogel YSS	0,03
Genu Pectin type LM-106 AS-YA	0,15
Total	100

.

Table 3 Formula 2 for ambient yoghurt by Du Pont

Formula 2 Du Pont	
Ingredients	**%**
Whole milk 3.5% fat	89,2
Skimmed milk powder	2,8
Sugar	6,00
Grinsted® SB 264	2,00
Total	100

Each formula has its own control, without stabilizers. In total, 4 batches were made:

- Control Formula 1,
- Control Formula 2,
- Formula 1 and
- Formula 2.

The experimental design included:

I. Setup of the rig
II. Yoghurt production
III. Applying shear to the yoghurt in the rig
IV. Applying shear to the yoghurt in the rheometer
V. Measuring viscosity and percentage of water retention

7.1 Setup of the rig

In this experiment the point was to develop a rig that simulates a mechanical shear after the fermentation of the ambient yoghurt in line. It was necessary to calculate the shear rate in every step of the process. To do that, the dimension and times of the piping were needed to know from the tank that contains the yoghurt to the product outlet.

The line that was used was a Tetra Pak pasteuriser (Tetra Therm Lacta). To calculate the shear rate, it was necessary to know the dimensions of the pipe and the flow using the tube wall shear rate formula (VDI-Wärmeatlas, 1977):

$$\dot{\gamma} = 2\pi \, \frac{u}{D} \tag{2}$$

Where $\dot{\gamma}$the shear rate (s-1) is u is the mean velocity (m/s) and D the diameter (m) of the pipe.

The shear rates that the yoghurt is exposed to in the line is mainly between 20 to 60 s^{-1} in pipes, this it will be called Low Shear Rates (LSR) and between 70-200 s^{-1}, it will be called Medium Shear Rate (MSR) in the plate heat exchangers (PHE).

It was planned to run approximately 20 litres of yoghurt in the rig, therefore it was need to have a pump for small volumes. A positive pump with a maximum capacity of 500 L/h had the possibility to change the capacity with a frequency changer, to pass the product from 50 to 100 L/h. Due to this facility to change the flow of the pump it was possible to apply the flow rate necessary to simulate the shear rate.

Instead of pipes the yoghurt passed through smooth, flexible hoses with BGA-approved inner rubber layer for food (Bfr food flow CTR 31019 Codan 8021; Codan Rubber Danmark ApS) . The hoses have a standard diameter from the fabric of 16 mm.

Knowing the shear rate, the diameter of the hose and the possibility to change the flow rate of the pump, it was needed to calculate the flow rate that the yoghurt needs to run inside the hoses for a specific time. The time that the yoghurt will suffer the shear rate in the first step of the experiment was decided in 30 seconds. This decision was taken due to the time that the yoghurt spends in every shear is between this time and the length of the hoses need to be adequate to manage them easily.

It was used the volumetric flow rate formula (eq.3), to calculate the length of the hose in order to the product run in the decided time:

$$Q = \mathrm{AV} \tag{3}$$

Where Q flow rate, A is the area of the hose and V is the velocity:

$$Q = A\frac{d}{t} \tag{4}$$

Where d is distance and t time in seconds

Then:

$$d = \frac{Q}{A}\, t \tag{5}$$

Table 4 shows the results of the different lengths of the hoses to simulate the different shear rates in a certain time. The second time that is in the table is 60 seconds. This time was chosen after the results in 30 seconds and only the LSR and MSR were made. It is made another shear rate called Higher Shear Rate (HSR) around 200-400 s^{-1}.

As it is shown in table 4,the length of the hoses are different, to make the hoses larger it was use to put together the hoses used to shear LSR and MSR s^{-1} and have the 21 meters hose, the same procedure when it was needed to run in one minute

Table 4. Hose length for different times and for different shear rates.

shear rate s^{-1}	Pipe diameter mm	Flow rate l/h	hose length (m) for 30 seconds	Hose length (m) for 60 seconds
LSR	16	85	4	7
MSR	16	415	17	34
HSR	16	498	21	----

When everything was calculated the rig was assembled as illustrated in Figure 15.

Figure 15 Rig

7.2 Yoghurt production

As it was mention in the introduction, the milk that it is going to be used in the yoghurt needs a special treatment. The steps to produce yoghurt is illustrated in Figure 16

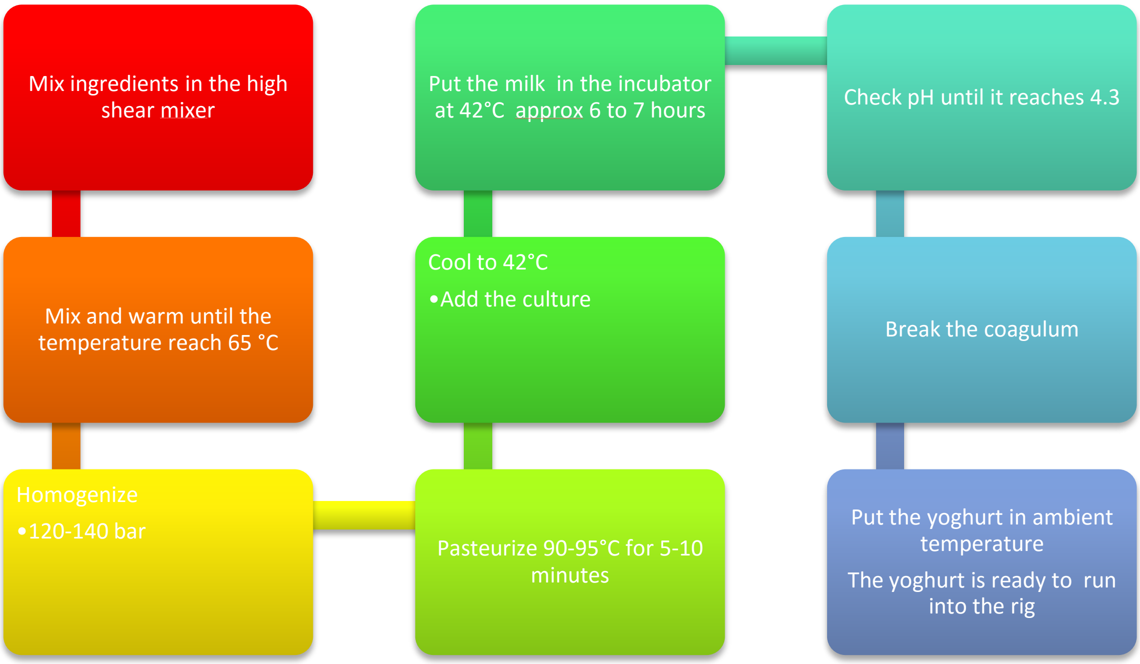


Figure 16Diagram of the production of yoghurt (the method is based on the suppliers recipe

7.2.1 Preparation of the milk

Before inoculation the milk needs to be pre-treated with all the ingredients that are shown in table 5.

Table 5 Ingredients and suppliers used to make the yoghurt

Ingredient	Supplier
Unhomogenized milk (2.9–3.1% fat)	Skånemejerier (Malmö, Sweden)
Sugar	Nordic Sugar (Copenhagen, Danmark)
Skimmed milk powder	Arla Foods (Visby,Sweden)
ThermTex starch (for formula 1)	Ingredion (Hamburg, Germany)
Kelcogel® YSS (for formula 1)	Cp Kelko (Lille Skensved, Denmark)
Genu® Pectin (for formula 1)	Cp Kelko (Lille Skensved, Denmark)
Grindsted® SB 264 (for formula 2)	Du Pont/Danisco (Danmark)

For the treatment of the milk it was used a Tetra Pak Scanima high shear Mixer with capacity of 25 L, fig 17.

Figure 17 Tetra Pak Scanima high shear Mixer

In the mixer, all the ingredients of the respective formulas were added under stirring. The milk was heated to 60-65 °C (recommendation from the suppliers). At this temperature, the milk was homogenized at a pressure of 175-250 bar (internal document by Tetra Pak).

The homogenized milk was then returned to the mixer and pasteurized at 90-95°C for 5-10 minutes, with gentle stirring. After this time, the milk was cooled in the mixer to 42°C in before it was ready be inoculated with the previous activated culture.

7.2.2 Activation of the culture

To activate the culture it was needed previously add it in 500 ml autoclaved milk, as the instructions of the supplier recommend (Sundberg, 2016. Application Scientist - Fermented Milk & Probiotics; Chistian Hansen, Danmark, personal communication 30-mars), in table 6 are shown the material needed for culture activation.

Table 6 Materials and suppliers of the culture and the milk which the culture was activated

Material	Supplier
YoFlex ® Culture YF-L904	Christian Hansen (Hørsholm, Danmark)
500 ml unhomogenized milk (2.9-3.1% fat) previously autoclaved.	Skånemejerier, Sweden

The milk was heated in a water bath until it reached 42°C, the culture was added and stirred for about 30 minutes. The culture was activated and ready to be inoculated in the milk.

7.2.3 Inoculation and fermentation

In this experiment, 20 L of milk at 42°C were inoculated with 10 mL of the previous activated culture mentioned in section 7.2.2. The 20 L of inoculated milk was placed in a incubator at constant temperature (42°C) for 6 hours, or when the pH reaches 4.3.

When the yoghurt was done, it was taken out from the incubator. The coagulum was broken manually with a stirrer fitted with a perforated disk, stirred 25 times in slow helicoidal movements (Afonso et al., 2003, Zhang et al., 2015).

The stirred yoghurt rested all night at ambient temperature before it was run through the rig.

7.3 Run Yoghurt in rig

Before poured all the yoghurt in the rig, two samples were saved in order to make the viscosity and water retention test and other sample was applied shear in the rheometer, figure 18 shows the different steps that the yoghurt had in the shearing application.

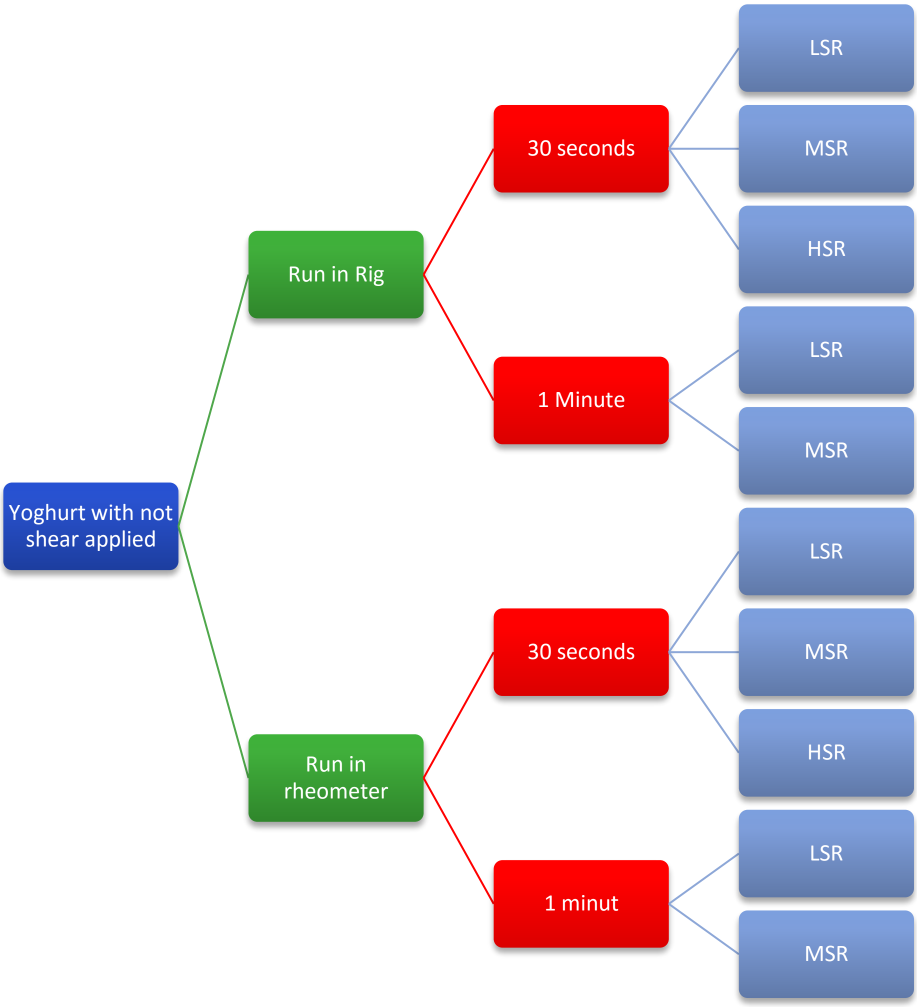


Figure 18 Shear rates and times for one yoghurt formula experiment

7.3.1 Applying the shear rate to the yoghurt in the rheometer

The same shear rate which the yoghurt had in the rig is going to be applied in to a yoghurt sample that has not suffered any mechanical shear. A Kinexus rheometer by Malvern Instruments (Malvern, UK) using a bob geometry (25 mm diameter) and a conical concentric cylinder (27.5 mm diameter). The sample was saved and put in a container to rest all night to measure the viscosity and the percentage of water retention.

7.4 Measure viscosity and percentage of water retention (water holding capacity).

7.4.1 Viscosity

The viscosity was measured using a Kinexus rheometer by Malvern Instruments (Malvern, UK) using a bob geometry (25 mm diameter) and a conical concentric cylinder (27.5 mm diameter). The flow curves of the yoghurt formulations were obtained by varying the shear rate from 40 to 300 s^{-1}.

7.4.2 Water retention (Water holding capacity)

The disruption of a stabile protein system is accelerated when it is exposed to mechanical stress. To measure the stability of the system, the method by Nilsson et al (2006) with modifications by Afoakwa et al (2014) was used.

40 g of the product was weighed into a centrifuge tube and centrifuged at 4000xg for 30 minutes at room temperature. The centrifuge used in the experiment was a Centrifuge Beckman Coulter Allegra X-15R (Beckman Coulter, USA). After centrifugation, the supernatant was decanted and the solid was rested upside down into the tube for approximate 5 minutes. The %Water Retention was calculated according to:

$$\%WR = \frac{s2x100}{s1} \tag{6}$$

Where $s2$ the weight of the sediment and $s1$ is is the weight of the sample.

8 Results and Discussion

The results after the experiments are going to be presented and discussed in this point and they are organized in five sections:

- First section the results of the repeatability of the experiments
- Second section will expose the difference in the viscosity for each formula after different shear rates
- Third section discusses the role that the stabilizers have in the formulas
- Fourth section presents the comparison in viscosity and percentage in water retention in the 2 formulas and their respective controls after shearing 30 seconds and one minute respective.
- Fifth section presents a comparison between the test methods in the rheometer and the rig.

8.1 Repeatability and reproducibility of the experiment.

The repeatability and reproducibility (R&R) in the measures to obtain the results in viscosity and percentage of water retention in yoghurt sample .R&R was done by two operators. To analyse the data the statistical program Minitab 17 was used, quality measurement as R&R gagged nested was chosen, due to the destruction of the sample after making the measure (Gorman and Bower, 2002).

The percentage of study variation (%SV) in the when the percentage of water retention and viscosity in yoghurt samples was measured is 14.03% and 8.48% respectively.

A measurement system is considered acceptable when is less than 10% this means that the measurement of viscosity is acceptable and has robust results in the experiment, to the other hand if the measurement system is between 10 to 30% is considered still acceptable and advice that the measure system needs a minimal improve (Support.minitab.com, 2016).

In other hand the R&R of the making the yoghurt it was not made due to the be short of samples to make statistical results. However the Control formula 2 and in formula 1 was made twice in different weeks and the viscosity results and water retention seems similar between the repetitions, fig 19.

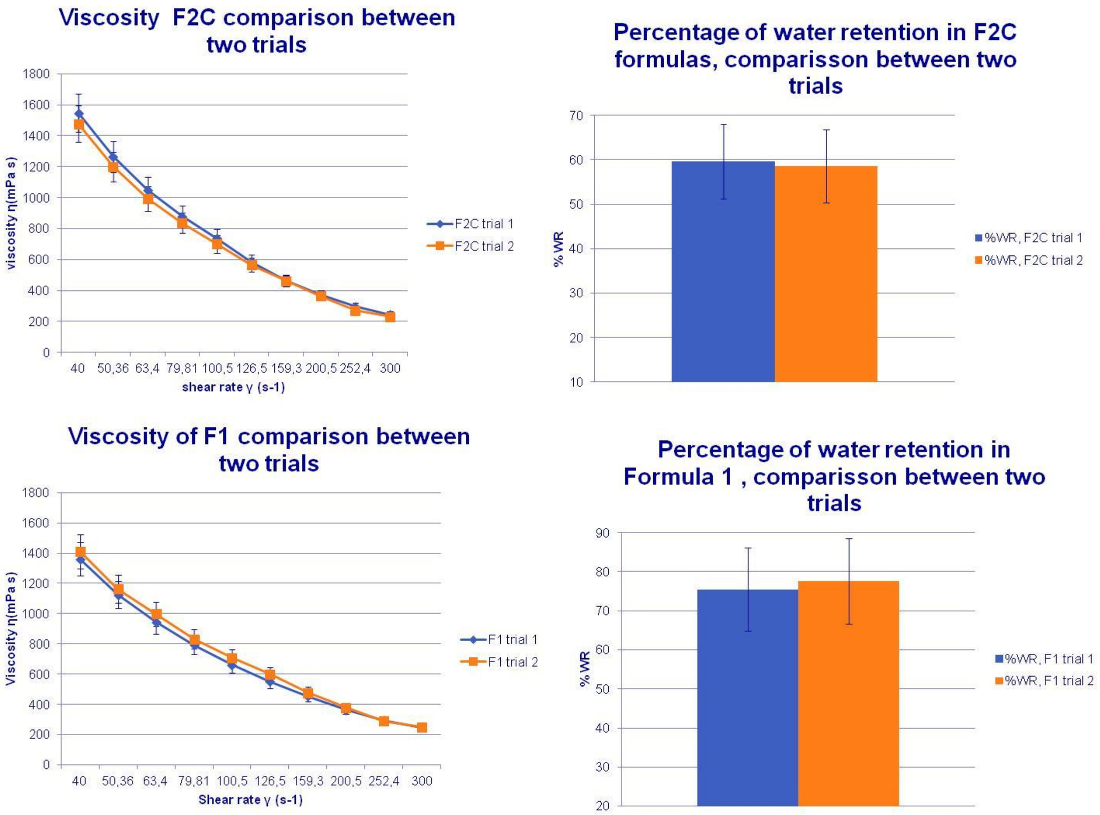


Figure 19 Comparison between viscosity and percentage of water retention between two trials of the formula 1 and the control F2

3.2 Viscosity decrease after running the yoghurt in different shears.

Yoghurt has two dominant structure defects: the variations in viscosity and the syneresis presented after certain time (Keogh and O´Kennedy, 1998). Lucey mention in his work in 2004 that “the excessive shearing in the yoghurt results in considerable loss of structure and may result in a weak body” in Figure 20 shows the decreasing of the viscosity in every formula when different shear rate was applied. In every formula the same behaviour in the loss of the viscosity is followed after the sample run in LSR, MSR and HSR.

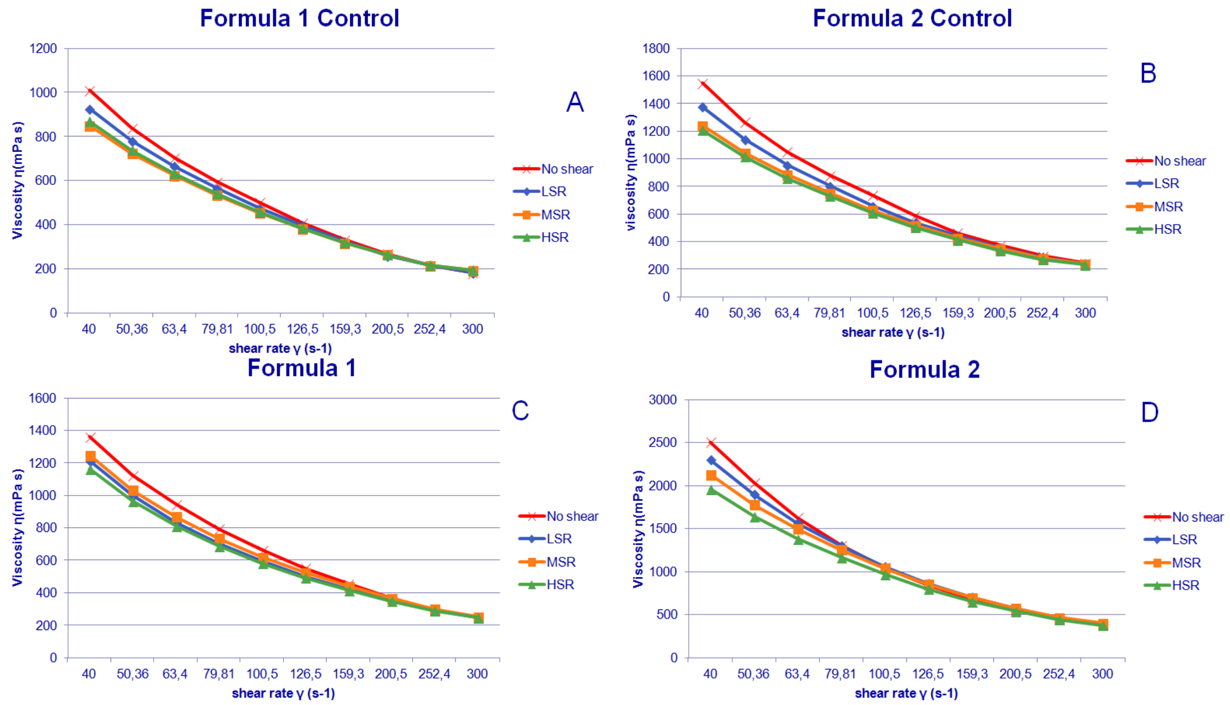


Figure 20 Viscosity in Formula 1 and 2 and their controls after applying different shear rates during 30 seconds in the rheometer.

As it was mentioned in the theory background, stirred yoghurt is a non-Newtonian fluid; it has time-dependent shear behavior and it is shear rate thinning. Due to the stirred yoghurt intricate system the viscosity decreases as a function of shear rate and the time (figure 20) (van Marle et al., 1999). The EPS in the serum phase increase product viscosity and water holding capacity (Lucey 2004). When a high shear is applied the EPS are broken up; large deformations, principally the properties of casein gels were mentioned by Lucey and Singh, in their work in 1997. They mention that only colloidal particles are left in the system and the fluid becomes Newtonian. This explanation is illustrated in figure 21. Nevertheless to transform stirred yoghurt into a Newtonian fluid very high shear rates are needed. In this work it was only measured the viscosity between 30 s^{-1} to 400 s^{-1}, that means that the aggregates does not lose all the structure but the system has more and less stable particles, due to the EPS (van Marle et al., 1999, Weidendorfer et al., 2008).

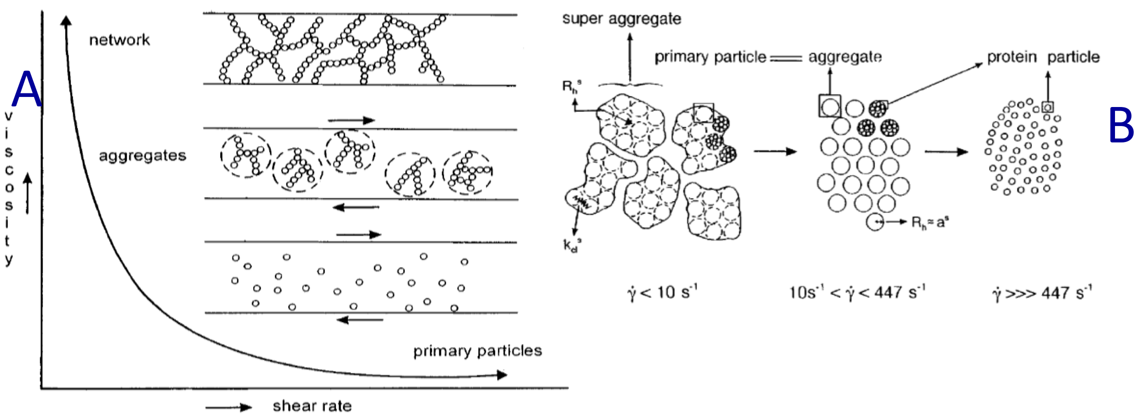


Figure 21 A) Image of the breakup of aggregates in a shear flow. B) Distribution of protein particles in the aggregates of stirred yogurt in three shear rate ranges. Image adapted from van Marle et al. (1999)

In this formulas it was used a texturing starter culture (TSC), a TSC is chosen by it ropiness ability. It increases ropy characteristics for stirred yoghurt 5 times more than the traditional yoghurt starter, increases 2 times the smoothness and helps also 1.5 times the capacity of the percentage of water retention. The bonds between the EPS generated by the TSC have more resistance to shear stress, because they are still incorporated within the casein and continue to maintain some viscosity (depending the shearing) due to the formation of new sticky ends, as the author named it, on the EPS strands, where aggregation happens. It is also important the localization of the EPS inside the system, it depends in the percentage of inoculation of the culture (Sodini et al. 2004). An example of how does the shear affects the EPS are shown in figure 22.

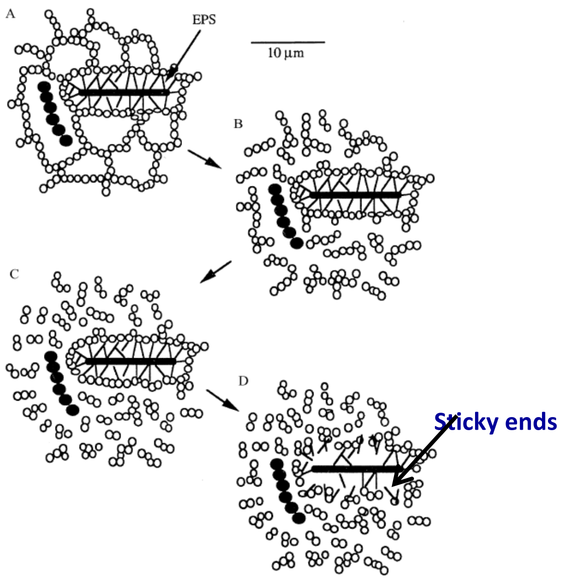


Figure 22 Shear degradation of yogurt structure made with EPS-TSC. EPS is shown as filaments connecting cells of Lactobacillus bulgaricus to the casein micelle network. (A) yogurt without shear , (B, C) represents the progressive destruction of the system (D) after intense shear comes the breaking of the portion of casein network liberating the sticky ends of the EPS. Image adapted from Sodini et al. 2004

The behaviour in all the controls and the formulas with stabilizers has a decrease in viscosity after the application of certain shear rate.

With the difference in the viscosity for each formula after different shear rates it can be said that the gel strength in the yoghurt is affected very much by different shear intensities, which imply a substantial lack of homogeneity in the particles of the broken gel causing high serum concentration and a reduction of water retention, as is shown in table 7. That is why treatment with different stabilizers is made; it has to be assumed that yoghurt is a suspension of weak aggregates (Weidendorfer et al., 2008).

Table 7 Percentage of water retention in the samples at different shear rates

	% water retention			
Shear rate	Control Formula 1	Control Formula 2	Formula 1	Formula 2
0	47,6 ± 3,1	59,6 ± 3,6	75,5 ± 1,6	87,4± 3,2
LSR	50,2 ± 4,5	51,1 ± 1,7	69 ± 1,2	86,2 ± 0,1
MSR	48,4 ± 1,1	50,4 ± 0,1	67,6 ± 1	80,6 ± 0,6
HSR	51 ± 4,6	50,6 ± 0,5	62,7 ± 2	80,2± 1

It is interesting to mark in figure 20 for Formula 1 (F1) and Formula 2 Control (F2C) the samples that run in MSR and HSR (section B and C) are very close in viscosity, there is no significant difference between the two formulas ($p>0.05$). One explanation to this can be the addition of stabilizers that will be discussed in the next point of this section and the other one that between LSR and MSR there is a bigger numerical difference than between MSR and HSR. Through the curves in figure 20 section D look different than section A, B and D, statistical analysis has shown that there is no significant difference ($p>0.05$) between MSR and HSR in Formula 2 (F2).

8.2 Stabilizers roll in the yoghurt formulation

The Formulas used in this study use stabilizers to secure the texture and water holding capacity in the ambient yoghurt when different shear rates applied to it.

After knowing the general viscosity behaviour in the different ambient yoghurt formulas, it is important to visualize and compare the viscosity and the percentage of water retention of the formulas to their respective controls. In figure 23 is illustrated the 3 different shear rates that the yoghurt run in the rheometer and the different viscosities that the formulas present.

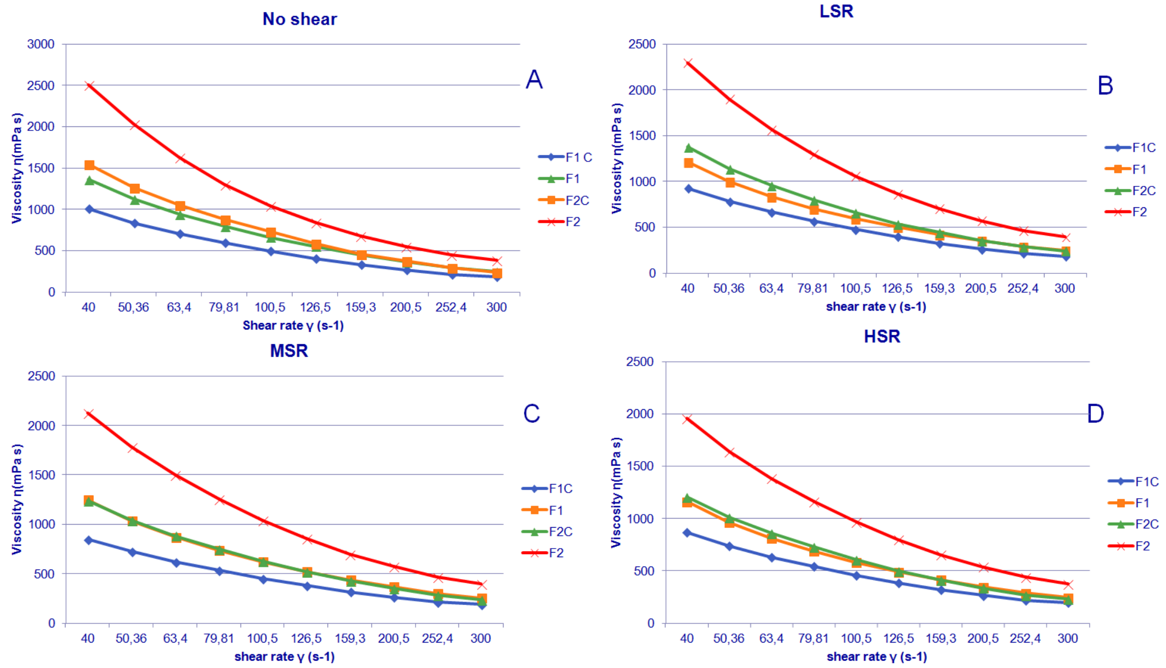


Figure 23 Comparison of the viscosity results between the Formula 1 and 2 with their respective controls in the same shear rate applied 30 seconds in the rheometer.

It is interesting to mark that the F2C and F1, has not significant difference in viscosity ($p>0.05$) after applying shear, even when they run for all the shears tested. F2C has SMP and the F1 not. It could be said that the SMP can give the ambient yoghurt the same viscosity as the stabilizers used in F1. However, in the comparison between the percentages of water retention there is a significant difference between F2C and F1 ($p \le 0.05$), which is illustrated in figure 24.

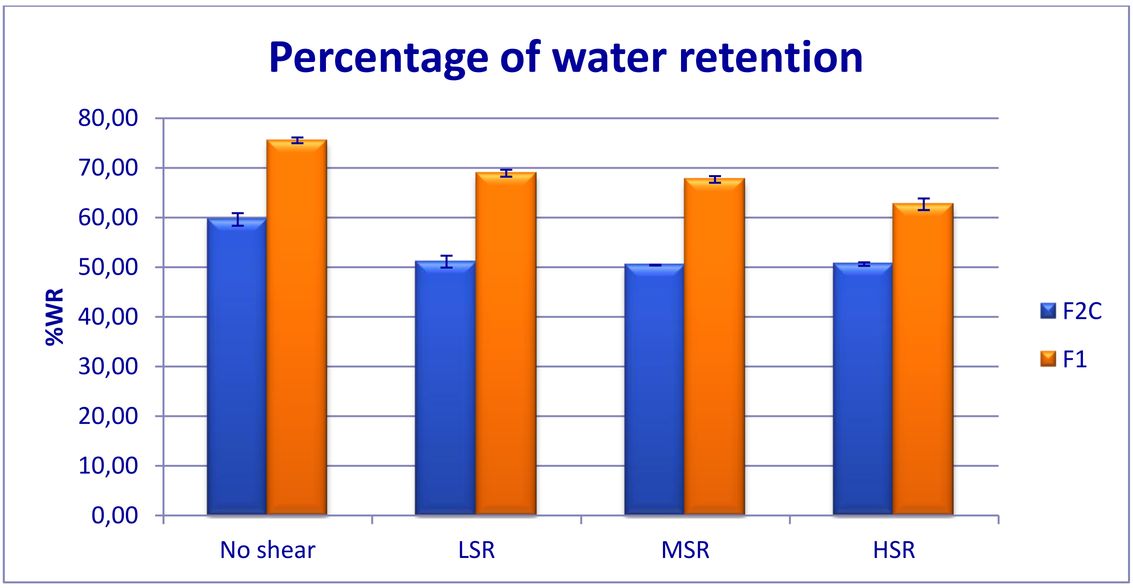


Figure 24 Comparison between F2C and F1 in the percentage of water retention

To add SMP is commonly used in the industry to increase the TS in the milk, this increase gives an improvement of yoghurt texture, as smoothness and viscosity, but not in water retention when high shear is applied (Fiszman and Salvador, 1999; Sodini et al. 2004). The authors doesn´t mention a specific number of high shear rate.

The yoghurt structure is a weak gel; the water in the system can be migrating out the gel during flow. "In this way as the shear rate increases, more water is released from the flocculated network" as Lobato-Calleros et al express in his work in 2014. Having high TS in the yoghurt improve the interactions between the particles in the yoghurt system, due to the increasing concentration of casein. It makes casein chains shorter making consequently the reduction of the pore dimensions and the density of the gel increase. It is important to notice that the lactose concentration could lower the hydration of casein, follow-on smaller casein particles, that can react with the other components in the system (Sodini et al., 2004). This is a reduction in the interisticial space, a space between structures, in the coagulum giving an increase in percentage of water retention (Sodini et al 2004). It can be seen in figure 26 in the control results, but only in the samples without shear induced. It could be said that the F2 network is not strong as F1 when mechanical shear is applied to it.

Sugar is not consider a stabilizer but it has a role in the structure of the yoghurt texture, this it can be explain by their water holding capacity, this capacity increase the viscosity of the serum holding capacity. Sugar also has an influence on the EPS production of the culture that also contributes to the texture (Sodini et al, 2004). F1 has more sugar than F2, 1% more (see section 7, Design of experiments). Watching the figure 25, F1 maintains a decrease in the percentage of water holding capacity than F2 when both formulas run at LSR and MSR. It can be said that this difference in sugar doesn't make a difference in %WR.

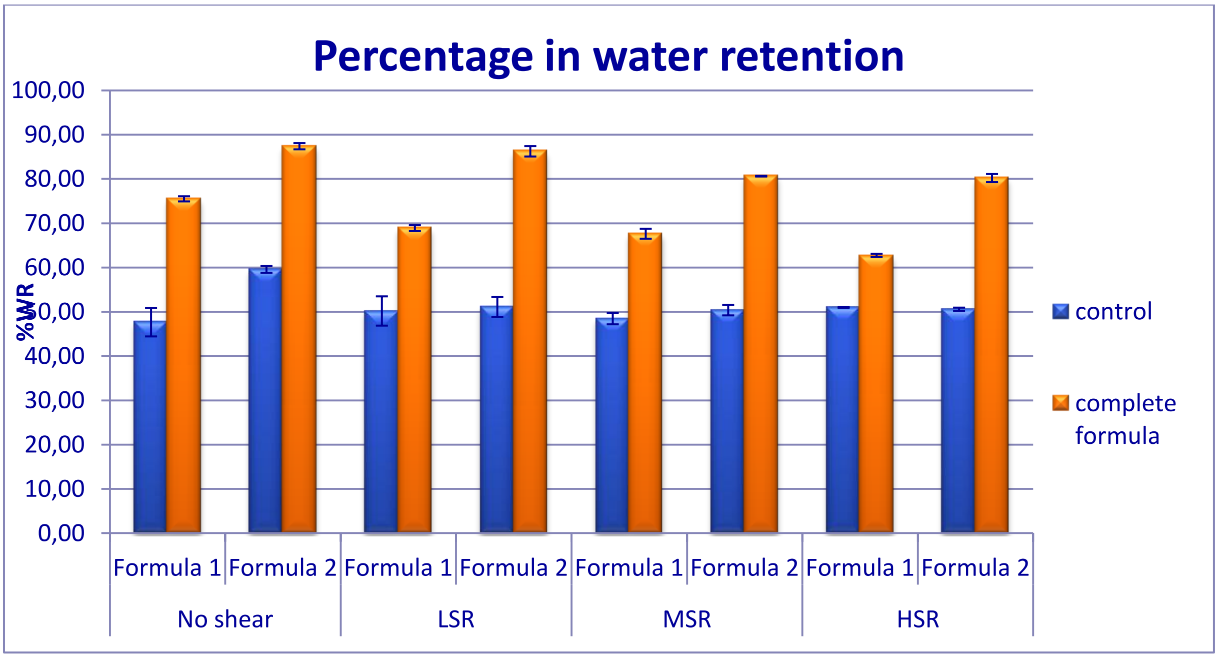


Figure 25 comparison between controls and complete formula in different shear rates

One reason that the F1 has not SMP (see section 7, Design of experiments) is far away for the chemical explanation. In Denmark and Italy is not allowed to fortify the yoghurt with SMP (Tamime and Robinson, 1999), which could be an answer to why SMP is not used in F1.

Apart for the difference of the SMP and Sugar between F1 and F2 has different stabilizers that help to give thickness and reduce the percentage of water retention of the product, see table 8. The percentage of dosage of the stabilizers in F2 is unknown since the supplier gives a prepared mix.

Table 8 Stabilizers used in the ambient yoghurt formulas

Formula 1	**Formula 2**
Modified starch (Hydroxy propyl distarch phosphate)	Modified starch (Hydroxy propyl distarch phosphate)
Pectin	Pectin
Gellan Gum	Gelatin

The addition of stabilizers apart to give stability into the system, is used to minimize the whey separation after the mechanical shearing during the process by increasing the total solids content in the milk, as pectin and gelatine (Lucey and Singh, 1997). Pectin is used in yoghurt for its high calcium reactivity (CpKelko 2011) and the gelatine interactions with the milk that has high solids has better forces to maintain firmer and denser the coagulum (Fiszman and Salvador, 1999), as it can be seen in figure 20 with the difference of viscosities but also in figure 25 that shows the percentage of the water retention in different shear rates. The stability in the yoghurt system and the interactions with the stabilizers, most need the presence of cations like K^+ (potassium) and Ca^{2+} (calcium) that the milk has intrinsically (Pang et al 2015).

As it has seen F1 and F2 have a big difference ($p \leq 0.001$) in %WR as shows figure 25, but in case of viscosity stability see figure 23 the F1 viscosity doesn´t drops to much as F2 when the shears were applied. Both formulations F1 and F2 have HPDSP and pectin but they differ in the third ingredient that is the gelatin in F2 and the gellan gum in F1. It will not be a complete comparison between the two formulations since the F2 has SMP and the F1 don´t, it is not known the quantity of starch, pectin, and gelatine in every mix and F2 is approximately 100% more viscous than F1.

The HPDSP and the milk protein form a continuous network that fortifies the ability of water retention, as it can see in figure 25 in both formulas has a better %WR in comparison with their own control. The better interaction with the acid, heat and shear stability that this starch has is due to the cross-link modification in its structure. These characteristics are very important in the stirred ambient yoghurt (Lobato-Calleros et al, 2014; Pang et al, 2015).

8.2.1 Gelatin

As it was mention in the theoretical background, the addition of gelatin in the yoghurt system can make the product pleasant in the mouth and gives stability to the yoghurt gel and prevents syneresis (Ares et al., 2007).

F2 manufactured with gelatin showed the highest percentage of water retention values. Gonçalvez et al. (2005) mention it their work that syneresis decreased to zero with increasing levels of gelatin around 6000 ppm and also other authors proclaim the absent of syneresis in yoghurt samples with gelatine (Keogh and O'kennedy, 1998; Fiszman and Salvador, 1999). In the mix that it was given by the supplier it is not known the exact quantity of gelatin but it can be assume that is less than 6000 ppm, due the presence approximately 19% loose of water in the system (table 7). It is also important to mention that the centrifugation force was higher than the one used in Gonçalvez et al, (2005) work, since the 4000xg it was proposed for the long life yoghurts and Gonçalvez studies were done on stirred yoghurts.

A coil to helix transition is the mechanism of gelation of the gelatin, the helices formed by heating are similar to the collagen structure, through this transition there is an incorporation of water molecules into the triple helixes, and this water is going to have the name of structural water. This water is going to be trapped in the structure and only very high shears can take it away the system. Nevertheless, the intramolecular cross links doesn´t help to the rigidity of the coagulum, that is why is needed interaction with more stabilizers that have interaction with the milk components. It is important to consider that during the acidification stage gelatin and milk protein doesn´t have strong interactions (Pang et al., 2015).

The effect of the gelatine in yoghurt system is given by the changes in the electrostatic forces; influence the aggregation of casein chains (Fiszman and Salvador, 1999). F2 manufactured with gelatine shows higher viscosity than its control around 153% more; it could be due the gelatin form a stronger three dimensional network with the help of the starch (Ares et al., 2007, Gonçalvez et al., 2005).

Samples made with gelatin or starch as F1 and F2 show a significative higher viscosity ($p<0.05$) than the control sample, it is an indication that the starch or the gelatine contribute to build up the viscosity and helps against the sensitivity that the yoghurt system has to shear and the post heat treatment that the ambient yoghurt has (Imeson, 2010). The addition of thickeners in F2 significantly ($p<0.001$) reduced syneresis compared with the F2C. The pectin that is also in the formulation helps to enhance the viscosity of the product, after a heat treatment, and also to maintain it texture at ambient temperatures (Chandan and Kilara, 2013).

8.2.2 Gellan Gum

Low and high acyl gellan gum can used in stirred ambient yoghurt, due to there is interaction between the two gelation properties that these two gellan gum have (see background information). It could be said that is a gel synergy with other gel, that means that gives a lot of flexibility in the desired texture (Valli and Clark, 2010). The mix of gellan gums add light texture and significantly ($p<0.001$) increase the percentage of water retention (fig 25 formula 1). It could also maintain more stable the viscosity of the system after shear applied (fig 23) in comparison with F2.

The almost stable viscosity in F1 compared with F2 can be explained because gellan gum can maintain the structure against high shear application and heat treatment (step that was not made in this work) (Sworn, 2009).

Sworn (2009) mention that gellan gum with modified starch makes a good synergy in the system structure. Important to notice is that there is not enough studies that talk about the percentage of water retention that this gum and the combination with other stabilizers can help in the stirred ambient yoghurt (Sworn, 2009).

8.3 Comparison between the two formulas with their control in the same shear rate run in 30 sec and one minute

Stirred yoghurt is a viscoelastic fluid; viscoelastic indicates that the product has properties of a solid and some of flow properties of a liquid (Lee and Lucey, 2010). Yoghurt has a time-dependant shear thinning behaviour. Lee and Lucey (2010) mention that yoghurt is not a true thixotropic material, because when the yoghurt structure breaks down after applying certain shearing is not completely reversible once shear stops.

In figure 26 are shown the viscosity of the 2 formulas of ambient yoghurt after applying shear rate in different time, 30 seconds and one minute. In section A and B shows the F1C and F1. It can be observed that there is not a significant difference ($p>0.05$) in the viscosities after 30 minutes and 1 minute shearing. In F2C and F2 in section C and D (fig 26) is interesting to mark that only the samples run at MSR has a significative ($p<0.05$) difference in the viscosity.

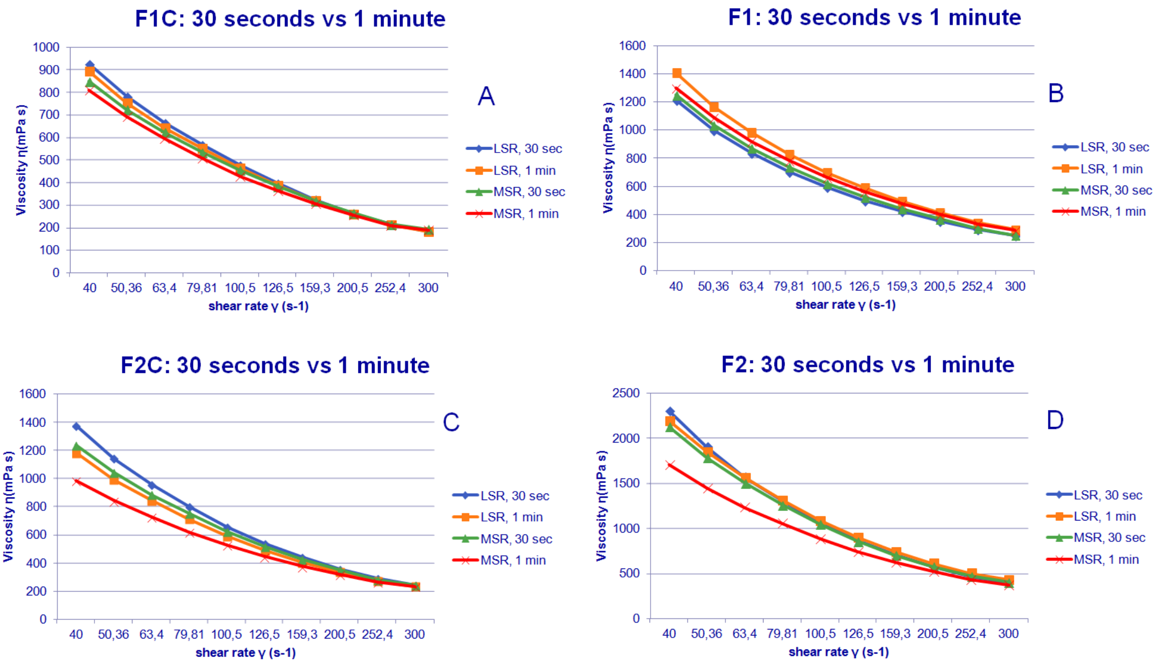


Figure 26 Viscosity of the different formulations of yoghurt that run in a constant shear rate of 46 and 225 s-1 in different times, 30 second and 1 minute in the rheometer.

Viscosity of the yoghurt in F1 figure 26 section B seems more stable that in F2 after 1 minute applying a MSR. The gelatine could be the explanation in this difference of viscosity since the F2 contains gelatine and the sample was at ambient temperature (~20°C). Gelatine is temperature-dependent, the yoghurt gel with gelatin is considered weak in the rise of temperature (Chandan and Kilara, 2013), however the melting point of the gelatine is around 25-40 °C and the samples where at constant temperature of 20°C. It can be assume that the gellan gum, in recipe F1 could be a better answer of these behaviour since the gellan gum, which is in the F1 show more shear-thining behaviour , due the conformational structure (Miyoshi and Nishinari 1999). Also it could be said that the mix of pectins, despite of it is not known the percentage of usage of both pectins, were different in F2 and F1. F1could have more LM pectin than F2 and F2 could have more HM pectin. As it was said in the theory HM pectin make more strength gels but after applying certain shear the gel loose strength. With these it can be assume that the percentage of pectin in the mix in F2 was not enough to maintain the viscosity of the yoghurt after one minute shear.

During the yoghurt process there are many steps that affect the structural changes in yoghurt system, which affect the flow properties. Stirred yoghurt has apparent viscosity and a structure that can recover, called partial thixotropy (Rawson and Marshall, 1997). Applying shear in the yoghurt results in a reduction in viscosity in comparison with the original before the shearing (Lee and Lucey 2010) fig 21. When shear stops and the yoghurt allowed to rest, the viscosity is only partially restored. Recovery of structure is called “rebodying” and is time-dependent phenomenon (Lee and Lucey 2010).

In percentage of water retention, figure 27. There is not significative difference ($p>0.05$) between the 30 seconds and 1 minute. In could mean that the system in the presence of sufficient divalent cations the gellan gum can restructurate the water in the matrix because the abundant OH groups on the gellan gum (Miyoshi and Nishinari, 1999 (Shinyashiki et al., 1999). In F2 the mix with gelatine, pectin and starch has a better %WR

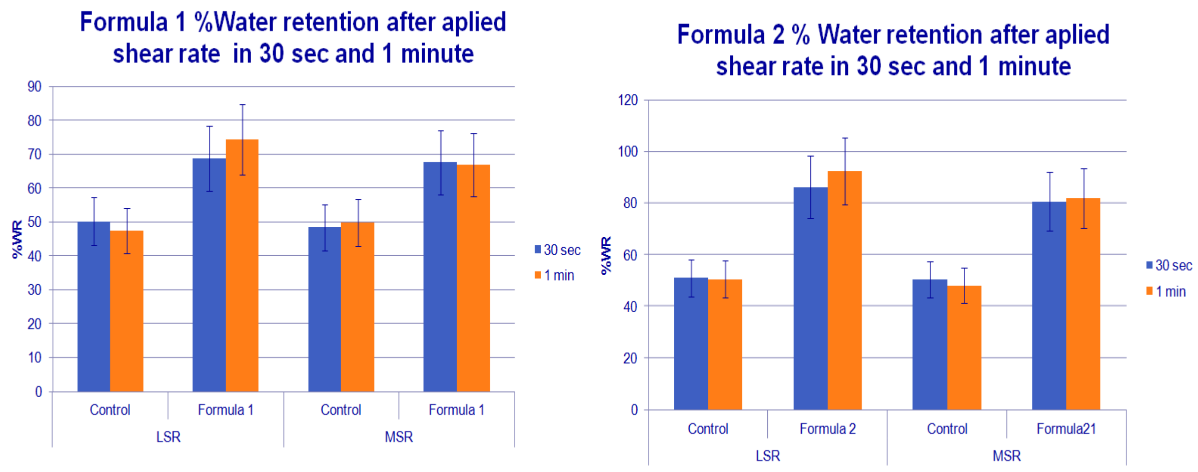


Figure 27 Percentage of water retention in Formula 1 and 2, compared after running during 30 and 1 minute with different shear rates.

8.4 Comparison between the test methods in the rheometer and the rig

Applying specific shear rate to the yoghurt formulas and their controls consisted in two parts. First part was run in the rheometer, which it was used to discuss the results in point 8.1 to 8.3 and the second part in the rig. It was one goal of this work to find out if the results from the rig were comparable to those from the rheometer. The results are illustrated in fig 28.

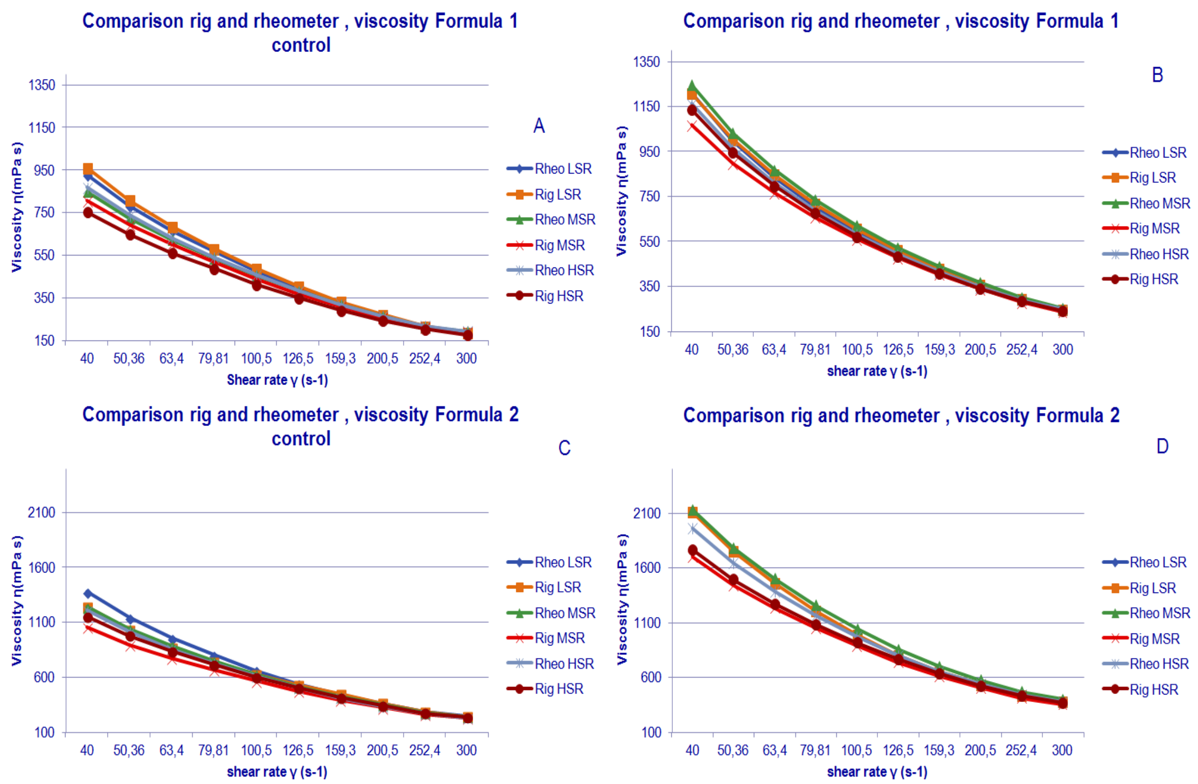


Figure 28 Comparison between viscosity results of the yoghurt formulations and it controls, which run in the rheometer and in the rig.

It is interesting to notice the difference in the results in the rig and the rheometer have significant difference ($p<0.05$) in the viscosity after applying the same shear at the same time. The answer is need more study in the area, it is interesting why the viscosity results after shearing in the rig are not the same in the samples in the rheometer.

To obtain the flow rate that the yoghurt needs to run in order to reach the same shear rate as the rheometer, it was used a Newtonian equation for average shear rate:

$$\dot{\gamma} = \frac{8\,u}{d} \tag{6}$$

As yoghurt is not a Newtonian fluid the further calculations needed to calculate the n' using the power in law (Oswald model) formula:

$$\tau = k\dot{\gamma}^{n} \tag{7}$$

Where τ shear stress, k is the fluid consistency unit, $\dot{\gamma}$ represents the shear rate and n the power law (flow index). The results of n calculated from the viscosity and the shear stress results of the formulas, are shown in table 9.

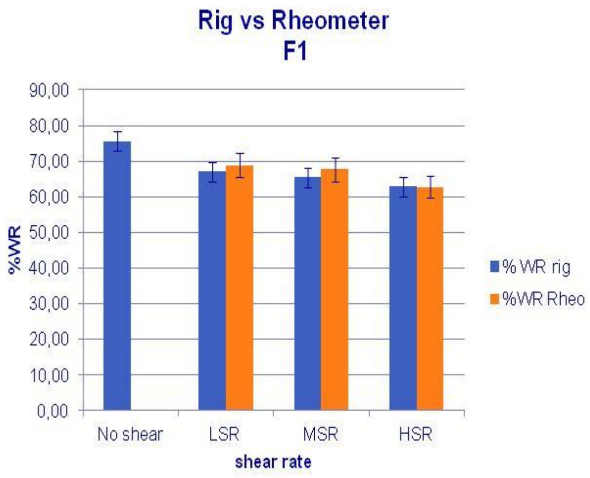
Table 9 Power in law index of the different yoghurt formulas.

Sample	**n Power Law (Flow index)**
Control Formula 1	0.1544
Formula 1	0.1656
Control Formula 2	0.0895
Formula 2	0.064

When $n<1$, the viscosity decrease when shear rate increase, the behaviour is a shear-thinning fluid (Coulson and Richardson 2004).

With these results it could answer why the results in figure 28 in section B, C and D rig LSR and rheometer MSR are almost the same. Even though it was calculated the shear rate in the yoghurt and demonstrate that the yoghurt is a shear thinning fluid, is difficult to obtain the “real-life” shear rate that the yoghurt had in the rig due to the formula used is for straight pipes and the rig has coiled hoses as it is shown in figure 15. One reason that the result where different in the result of the rig and rheometer could be that the degradation of structure in pipe depends in pipe length and diameter. Flow rate and temperature, other factors that influence the final viscosity of the yoghurt are the presence of bends, this varies the velocity and leads higher stresses and lower viscosities (Mokoonlall, Nöbel and Hinrichs, 2016).

It is interesting to see in figure 29 that F2 maintains higher water retention, around 80% ($p>0.05$) after shearing in rig and rheometer. The same in F1 %WR doesn´t drop and almost maintains around 60%.



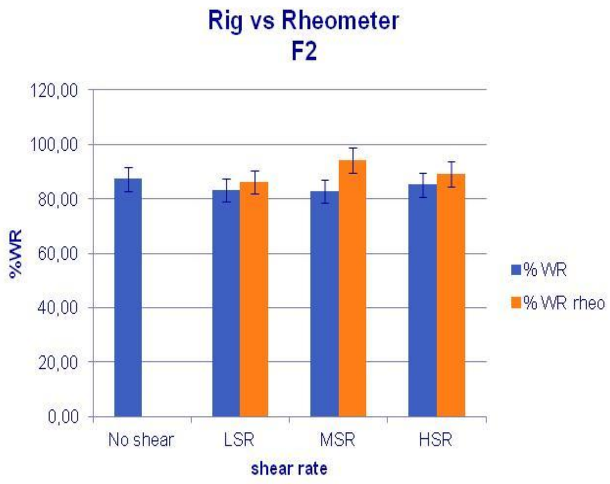


Figure 29 Comparison of the percentage of water retention in the yoghurt run in the rheometer and in the rig.

Previous of the development of the rig it was necessary to consider the flow rate index of the yoghurt to have a better approach of the desired shear rate. However the use of forecast methods to estimate the viscosity of the fluid after certain shear rate, also theoretical approached, in the real life is very important for understanding how the product will perform in the real life.

9 Conclusions

Yoghurt is a very complex system, adding stabilizers enhance the complexity of its behaviour in viscosity and percentage in water retention after certain mechanical shear. It can be concluded:

- Different stabilizers give different behaviour when mechanical treatment is applied in the yoghurt.
- Both formulas used in this work were sensitive to shear but the quality analysis made on the product after shearing show very different results:
  - F1 shows viscosity stability compared with its control when mechanical shear was applied. This could be attributed to the presence of gellan gum in the recipe. However this formula doesn't have high water retention in the product before and after shearing, showing a continuous decrease when shear was applied.
  - F2 had a higher viscosity than its control but, it doesn't show good viscosity stability, this means that viscosity continues dropping when shearing was applied. It could be assumed that the mix of pectins were insufficient. Nevertheless the percentage of water retention was higher and more stable at high mechanical shear; this could be attributing to the presence of gelatin.
- SMP in the F2C recipe shows a similitude with F1 in viscosity of the yoghurt. However not have the same behaviour in the water retention.
- The shear rate applied after 30 second and 1 minute does not make a difference in percentage in water retention in the yoghurt samples. While in viscosity F1 had a better viscosity stability compared with F2.
- A formula with a combination of SMP, gelatine and gellan gum could give stronger ambient yoghurt against mechanical shear as well as a high percentage of water retention.

10 Future Actions

For future studies would be interesting to make a more detailed study about the mix of additives in the yoghurt, visualizing it as a system. Having information about percentage of the stabilizers used in every mix is needed to know in order to explain better the differences in the behaviour of the yoghurt when shear is applied.

The development of a rig that can simulate the real line is essential, next studies can focus in the control of the parameters as coils, diameter and the shear that the pump used gives to the product in order to have accurate and robust results in the sheared yoghurt.

The heat treatment of the yoghurt after fermentation is indispensable to investigate due to the product has this process in line and also to know if the behaviour of the stabilizer mix is significantly affected in the viscosity and water retention compared with the results without heat treatment.

Stirred yoghurt has a different viscosity depending of the market, it is important to consider also the ambient temperatures in different countries. Therefore it is propose in future works continue the study making sensory analysis according tastes and requirements that the customer requires and the shelf life analysis after shearing.

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