Understanding the correlation between molecular structure of chilled dairy creams and the risk of environmental stress cracking in Tetra Pak® carton packages

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MASTER THESIS

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Preface

This thesis is a project in collaboration with the Food Technology and Nutrition Master's program of the LTH faculty of Lund University, and the company Tetra Pak, in Lund, Sweden. The assignment is completed by Alejandra Castaneda, it began in October 2021 and ended in June 2022.

I would like to thank the people who accompanied me on this journey, such as my supervisor Frida Lewerentz, who was always available to answer my questions and help me in all the procedures for the development of the project, in addition to guiding me and offering her valuable knowledge in the dairy area, I also would like to thank my supervisor from Lund University Maria Glantz and my examiner Marie Paulsson for their advice, guidance and patience.

From Tetra Pak to my supervisors Jannika Timander and Helena Wijkander who have been with me closely following the project, offering advice and important feedback that were key to the development of this project. Additionally, thank you to Tetra Pak for the opportunity to collaborate and for the possibility to perform part of the experiments there and using their equipment and laboratories with the help from the kind staff there.

Special thanks to the organization from:

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- Package lab building 316, Lund
- Food packaging safety and interaction lab, Stuttgart

Finally, I would like to express my gratitude for the emotional support that all the people mentioned above gave me and that considered some obstacles that I have gone through in the last few weeks close to the end date of the project. They have been an example to follow academically, professionally, and personally for me.

Abstract

Multilayer food packaging are lined with an inner layer of plastic and has excellent barrier properties. However, plastics lose their mechanical properties after being exposed to certain conditions; the combination of factors generates failures in the material such as the appearance of cracks, this phenomenon is known as environmental stress cracking (ESC). Tetra Pak® has been interested in research on the formation of cracks in the bottom. Previous investigations have been carried out on packaging such as Tetra Top® and products with a high fat content as dairy creams and it was found that in some cases there were “aggressive” agent that accelerate the formation of cracks and increase the risk of ESC. This research focused on the study of chilled dairy creams, investigating the effect of unit operations in the production process (heat treatment: direct or indirect, and homogenization: downstream, upstream, and no apply) that could substantially influence the molecular structure and physicochemical properties of the final dairy cream. Therefore, three different phases were developed in the project; the first was the production of five batches of chilled dairy cream using a lab mini-UHT (with 5 different formulations). In the second, physical and chemical analyses of the dairy cream batches, and in the third phase, the indicative tests of food package interaction as the main objective of investigating the ESC. It is important to note that these tests were carried out on a laboratory scale, the purpose was to simulate the process conditions that occur in the dairy industry, but there are more factors to control, such as the flow speed, the homogeneity of the raw materials, among many other variables that are difficult to control. Nevertheless, the objective of finding significant differences in the final product depending on the applied process was fulfilled

Popular Science Abstract

This is Environmental stress cracking (ESC) is one of the most common problems in polymers, this phenomenon usually occurs regardless of the time of polymer use, therefore, it can occur immediately after starting to use or it can occur in days, months, or years later. It is difficult to establish an analysis methodology that allows early detection of the fractures or cracks formation in the polymer before they are visible to the human eye. This problem has been detected in certain Tetra Top®, Tetra Pak carton packaging, used to package high-fat and refrigerated dairy creams.

Theoretically, it is known that the formation of cracks can begin previously when the plastic material is subjected to stress, or bent, or sealed and increase the risk of cracks formations when it comes in contact with aggressive agents that accelerate the crack formation process. Cracks form and spread resulting in leaks, which is a problem for food producers due to loss of food product and loss of packaging, due to the loss of integrity of the packaging and the product exposed to environmental conditions. The objective of this study was to be able to predict which factors among processing line such as heat treatment and homogenization have a significant influence on the molecular structure of the dairy cream that are packed in the Tetra Top® that can accelerate the formation of cracks and obtain a correlation between the dairy cream product and the ESC in the bottom in the packaging.

In this thesis, it was tried to find relationships between the characteristics of the molecular structure and the responses of the Tetra Top® packaging and its risk factor of ESC. For this reason, the production of chilled dairy cream is proposed using a mini-UHT laboratory scale, controlling variables of traceability of raw materials, the heat treatment and homogenization, as well as cooling phases and storage, among others. As a second phase, it was proposed to carry out an analysis of the dairy cream product obtained previously, such as fat and protein content, as well as analysis of particle size distribution, viscosity, emulsion stability and microscopy with protein staining.

On the other, the heat load of the dairy cream was estimated by measuring the lactulose concentration, also it was carried out viscosity analysis at two temperatures of 6°C and 20°C. Moreover, the stability of the emulsion was analysed in a time of 70 minutes at 23°C using the technology based on the measurement of transmittance as the backscattering of near-infrared light at 880 nm, this study allows knowing the movement of the particles in the dairy creams and creating a prediction model on time of the stability of the emulsion.

As a third phase, analyses were carried out to verify the food-packaging interaction, such as the puncture test using a setup in a force machine, this equipment is used to evaluate the ESC in the inside layer of the package as indicative test. The equipment gives as a result of tip displacement at failure in mm.

The red-ink test was also carried out, which is a dye penetration test widely applied as a leak detection method. The result after to remove the cardboard layers of the material by hand is to visualize if it is the appearance of leak, points, or spots.

Official ESC evaluation tests such as that specified by ASTM D1693, for example the Bell telephone or the Notch Constant Load Test (NCLT), are based on generating biaxial tension and at the same time putting the material in contact with the "aggressive" substance, thus evaluate the crack formation time, managing to quantify the risk of ESC. But these tests are unreliable and slow, they also require the use

of special equipment that quantifies the application of quantifiable forces, in addition to the fact that it is more complex to analyse the data obtained and to be able to generate possible solutions from this.

In conclusion, the risk of ESC depends on many factors, among the main ones are the plastic material, the environmental conditions and the chemical agents that accelerate the formation of cracks. Therefore, in this study, the main focus was to evaluate and summarize all the possible factors associated with the "chemical agent", which in this case is chilled dairy cream, since it is considered a priority to establish correlations between the chemical and molecular structure of the product and the risk of ESC, and thus be able to generate certain recommendations to dairy cream producers that help them reduce the risk of ESC in Tetra Top® packages.

List of Abbreviation

ESC	Environmental Stress Cracking
FPI	Food Package Interaction
ADPH	Adipophilin
ASTM	American Society for Testing and Materials
BS	Backscattering
CC	Concentric Cylinder
CG	Carrageenan
CIP	Cleaning In Place
DDH	Direct, Downstream Homogenization
DNH	Direct, No Homogenization
ESL	Extended Shelf Life
FNCT	Full-Notch Creep Test
FTIR	Fourier Transformed Infrared Technology.
HDPE	High-Density Polyethylene
HMF	5-Hydroxymethyl-2-Fufural
HSPs	Hansen Solubility Parameter
HTST	High-Temperature-Short-Time
IDH	Indirect, Downstream Homogenization
INH	Indirect, No Homogenization
IUP	Indirect, Upstream Homogenization
LDPE	Low-Density Polyethylene
LF	Lactose Free
MDRS	Morphologically Directed Raman Spectroscopy
MFGM	Milk Fat Globule Membrane
NCLT	Notch Constant Load Test
PC	Polycarbonate
PE	Polyethylene
PSD	Particle Size Distribution
TSI	Turbiscan Stability Index Scale.
UHT	Ultra-High Temperature
XDH/XO	Xanthine Dehydrogenase/ Oxidoreductase
β-lg	B-Lactoglobulin
ι-	Iota
κ-	Kappa

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1.Introduction

1.1. Overview

Environmental stress cracking (ESC) is defined by Wright, (1996) as “the premature initiation of cracking and embrittlement of a plastic due to the simultaneous action of stress and strain and contact with specific fluids”. The factors that influence the risk of ESC formation are unavoidable and can be classified into different categories such as the production process, for example, the moulding, the production temperatures of the plastic, the folds and bends that must be made to form the material in a packing. Another category are the fluids that encounter the material that can be accelerators of the phenomenon of crack formation, and end up in leaks, drips, damage to the integrity of the packaging and risk of contamination of the food product, and total loss of the product and the packaging.

Some dairy creams have been highly aggressive agents that perhaps accelerate the risk of ESC. Therefore, the hypothesis could be generated that depending on the production conditions of the dairy cream, the risk of ESC would be increasing. However, it is necessary to highlight that this situation of crack formation in the carton packaging does not occur very frequently and does not occur in all the dairy cream producers that use this packaging material, which makes the situation more complex to analyse.

All the previous research carried out was based on the analysis of samples conclude that the high content of total fat seemed to have a strong negative effect on ESC compared with products with lower fat content. This was found since cream with different percentages of fat content were also evaluated and compared.

The main conclusion is that attention should not only be paid to the packaging material, the filling machine and/or the packaging process. It is also necessary to include the study of the product being packaged, especially the conditions prior to filling, such as the processing line. It is believed that having a wide knowledge of the packaging material, the product and their interaction could prevent, reduce, and mitigate the risk of ESC.

Nowadays, there is a need to understand why different types of creams have different risks for ESC. Some work has been done but understanding this further requires a deeper analysis and more focus in the food matrix. For this reason, it is believed that the molecular structure of the dairy product should be investigated after processing operations, and a pattern of possible causes of ESC hazards should be established when the dairy product comes into contact with the packaging material.

On this occasion, the production of dairy cream samples under specific conditions is planned, for this a mini-UHT by Omve will be used. Five different experiments will be carried out and the main variations will be in the factors of heat treatment and homogenization process. The literature suggests that these two-unit operations (widely used in the dairy industry) have a significant effect on the properties of the cream, differences have been found in the colloidal properties, in the structure and the particle size of the fat globules (Richardson, Booth and Stanley 1993), as well as differences in aggregation phenomena and in the interaction between the protein and the fat globule membrane (Goff, 1997).

In a shelf-life study, the stability of the emulsion in the liquid state also changed depending on the treatment applied (Rodarte et al. 2018), in addition, the crystallization profiles of the fat vary and subsequent aspects such as the whipping time of the cream and foam stability are also affected (Koxholt, Eisenmann and Hinrichs, 2001).

Therefore, one of the hypotheses of the study is that the physicochemical composition of cream can increase the risk of ESC, but the degree of risk varies depending on the unit operations carried out on the process before packaging, such as heat treatment and homogenization parameters.

1.2. Aim

As a general objective, it is desired to establish a molecular and structural analysis methodology of chilled dairy cream products packaged in Tetra Top carton packages, to be able to predict which factors present in the dairy cream product have an impact in the risk of ESC in carton packages.

One of the objectives is to learn the effect of different heat treatment and homogenization in the dairy cream product before being packed, the second objective is derived from the result of the previous objective, it would be to continue with the structural analysis of the samples after the treatments, obtaining a characterization of each; then with statistical analysis to conclude whether there is a significant difference among the samples physical and chemical properties and the risk of ESC.

Thus, it is intended to achieve the general objective of establishing if there is a relationship between the production processes of the dairy cream product and the risk of ESC.

1.2.1. Specific objectives

- Summarize the production conditions as heat treatment process and homogenization condition, that can significantly increase or decrease the risk of chilled dairy creams with highly aggressive characteristics when in contact with the packaging material and produce the ESC,

- To investigate if there is a significant difference among different experiments (chilled dairy cream batches) varying the heat treatment and homogenization, knowing the influence of these on the final product microstructure and the analysis of the food-package interaction,

- Obtain a list of possible improvements that could be applied to the production lines of dairy cream as prevention, mitigation, and reduction measures of the ESC phenomenon in Tetra Top packaging used to pack chilled dairy cream,

- Obtain a possible methodology for studying the food matrix and define the analyses that will allow knowing the level of risk of ESC that another type of product can represent for Tetra Top packaging, extrapolate the methodology to another food matrix different from dairy creams.

1.3. Project Scope

This study will focus on the investigation of some of the physical and chemical characteristics of chilled ESL treated dairy cream with around 36% fat content. Moreover, two analyses will be included that allow learning a little more about the food-packaging interaction, such as the red ink test and the puncture test. In this study, different batches of chilled dairy cream will be produced in a mini-UHT belonging to the Tetra Pak company in Lund. However, it will be limited to chilled ESL-treated dairy cream with fat percentage of around 36% and with the addition of the same concentration of stabilizer (carrageenan) for all the batches. The variables are limited to heat treatment type (direct and indirect) and homogenization (upstream, downstream, no homogenization) the physical and chemical analyses will be carried out in the Tetra Pak laboratory as well as the red-ink test. Emulsion stability analyses will be performed at Kemicentrum, Lund University. The determination of the lactulose concentration will be performed at an external laboratory. The puncture test will be performed at the Tetra Pak laboratory located in Stuttgart, Germany.

The preparation of the dairy cream and the analyses follow a planning designed to minimize errors, clarifying that all the data analysis oversees the principal investigator, Alejandra Castaneda. Moreover, Tetra Top packages that will be used in the red-ink test will be produced at Tetra Pak in Lund, in order to have a "new" package and reduce the risks of error due to aging of the material and trying to reduce unknown external factors.

In this study it is not possible to address some specific tests on the packaging material such as axial or biaxial tension, neither ESC tests for plastics such as ISO 16770: 2019 with Full-notch creep test (FNCT) since it is not within the scope in this phase of the investigation, however, final recommendations will be made regarding how the investigation should continue and be complemented.

1.4. Problem Statements

Previous investigations of the factors that influence the risk of ESC carried out by the Tetra Pak company have concluded that products with a high fat content, such as dairy creams, can be highly aggressive on the packaging material. Interestingly, when samples with the same fat content but from different producer companies (using the same Tetra Top packaging material) were analysed, it was concluded that different dairy creams will be obtained depending on the production process conditions applied by each dairy cream producer. It was found significant differences in the particle size distribution as well microstructure of the fat globules and proteins, and a correlation with the risk of ESC as the formation of cracks and leaks, moreover the results were not sufficient to conclude or make statements.

On the other hand, there were no significant differences in the fatty acid profile MUVA of the samples, nor differences in the content of free fatty acids, then, it is confirmed that the differences are more focused on the physical properties than on the chemical ones of the dairy cream.

On the other hand, the exact reasons why ESC in the bottom form in the polymer and the risk of ESC cannot be attributed to a single factor, as a result a wider view must be taken of each aspect that can influence on ESC. Previous studies do not clearly define the properties of dairy creams dependent on the production process, consequently, the current problem focuses on the differences among the characteristics such as particle size, colloidal dispersion, stability of the emulsion in the liquid state, protein content in the surface and images of the structural arrangement of the fat globule membrane, proteins, and serum phase. All these factors can contribute to the formation of cracks and accelerate the risk of ESC, however so far it is unknown.

2. Theorical Background

2.1. Dairy cream

Dairy cream is defined according to CODEX STAN 288-1976 as a fluid milk product with higher fat content that is obtained by physical separation of milk through processes such as gravitational or centrifugal forces; cream is considered a fat-in-water type emulsion, specifically fat-in-skimmed milk. Creams are generally categorized according to their fat content. However, there is no clarity as to the exact fat content and what denomination it should have. On the other hand, a categorization according to the heat treatment process has begun to be implemented. Currently, dairy products must be pasteurized as a minimum as well the dairy cream, however currently the use of "extended shelf life (ESL)" from Tetra Pak, 2016 "ESL represents the shelf life of a chilled distributed product extended beyond limits of a conventional pasteurized product present in a specific market".

Dairy cream is a very sensitive product that requires a process line with high hygiene standards and proper storage. In addition, the process conditions must be very gentle to avoid the destruction of the milk fat globule membrane (MFGM) and subsequent problems in the product such as the loss of rheological and sensory properties, especially the ability to whipping, desirable firmness, aeration, adequate stability over time, high overrun, among other properties (Wang et al., 2019). The purpose of the heat treatment applied to the dairy cream is the inactivation of the pathogen load, to reduce as much as possible the number of spoilage bacteria, also the inactivation of specific enzymes, some confirmatory tests of the correct heat treatment must be negative phosphatase and peroxidase (Bolling et al., 2005). On the other hand, there is also the inactivation of native milk lipase, which prevents lipolytic fat degradation. Lipoprotein lipase catalyses the hydrolysis of triacylglycerols to free fatty acids. However, the MFGM acts as a protective layer against lipolysis (Calvo et al., 2022).

Consequently, all the industrial production operations of dairy cream must be carried out under controlled conditions, always prioritizing keeping the structure of the globule fat membrane intact. Due to the risk of loss of product quality, it is a product with a high probability of creaming on the surface due to its chemical composition and the difference between the densities of the fat and water phases, in addition to any unit operation such as transport by pipes, the flow rate, the pumps used, the pressures, the agitation, the heating in the heat treatment, homogenization processes, in addition to the aging of the product, among other variables, can affect the membrane of the fat globule and cause serious damage in all product quality, from loss of sensory properties to hydrolytic rancidity and production of free fatty acids (Fox, 2022).

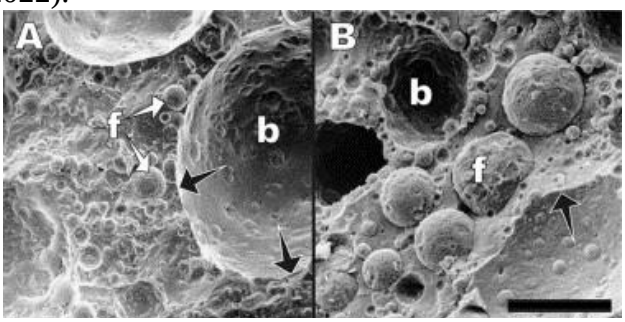


Figure 1 Foam whipped to maximum overrun from A. high temperature short time heat treated B. Ultra-high temperature heat treated, from: Smith et al., 2000

Moreover, it has been studying intensive the texture properties of the dairy cream specially when it comes in the whipped cream because the unit operation will have a big impact in the stabilization of the final product and its desirable texture (Fox, 2022). In Figure 1 it could observe the larger fat globule formed in the cream treated with ultra-high temperature compare with the high temperature short time (Smith et al., 2000).

2.2. Chemistry and structure

Milk fat globules in raw bovine milk are composed of triglyceride cores that are surrounded by the MFGM, which is made up of two layers, a monolayer, and a bilayer. The components in this membrane act as an emulsifier since they contain polar lipids, cholesterol, and proteins. Among the functions of these components are to provide protection to the fat globules and protect them from coalescence. The presence of bioactive components such as xanthine oxidase/dehydrogenase, periodic acid Schiff and other proteins that make up around 1 and 2% of the total protein have been reported. The membrane of the milk fat globules has been studied in depth due to its nutritional and technological properties (Singh, 2006).

Regarding the composition of MFGM, it is mainly glycoprotein and phospholipids and sphingolipids, however the information found in the literature on the physicochemical composition of MFGM is very varied since there are multiple methods of isolation, identification, and purification. The Table 1 shows the estimated average composition given by Walstra et al., (2006).

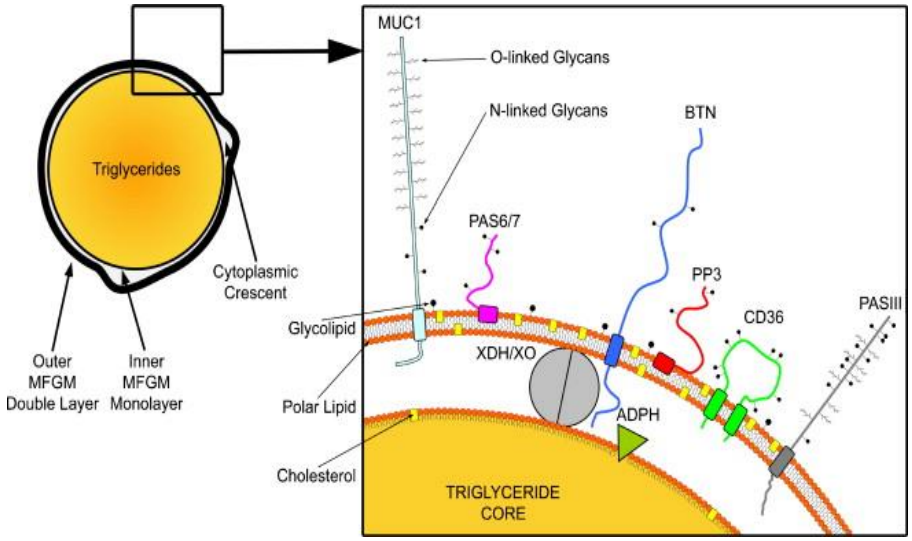


Figure 2 Structure of the milk fat globule membrane, from Dewettinck et al.,2008

In a view of the structure Figure 2, from the nucleus to the outside, the MFGM has an internal monolayer of polar lipids and is surrounded by proteins, on the internal side there is a bilayer composed of proteins and very dense since it has a lot of electron charge. Then there is another bilayer made up of polar lipids and proteins (Dewettinck, et al., 2008).

Figure 2 shows the structure of the fat globule, (the sizes of the components are not to scale), where adipophilin (ADPH) is found in the inner polar lipid layer, and xanthine dehydrogenase/ oxidoreductase (XDH/XO) is positioned between both layers of the polar lipids (Dewettinck, et al., 2008).

In the outer layer is founded mucin 1, mucin 1 is one of the major MFGM proteins, among others. Components such as choline-containing phospholipids, phosphatidylcholine, sphingomyelin, and the glycolipids, cerebrosides, and gangliosides as well are in the outer layer. (Dewettinck, et al., 2008).

Table 1 Estimated average composition of the milk fat globule membrane (Walstra et al., 2006)

Component	mg 100 g^{-1} fat globule
Protein	1800
Phospholipids	650
Cerebrosides	80
Cholesterol	40
Monoglycerides	+[a]
Water	+
Carotenoids+ Vit A	0,04
Fe	0,3
Cu	0,01
Total	>2570

+[a;] present, but quantity unknown

The structural changes in the MFGM and its composition are affected by various factors such as the age of the cow, the microbiological quality of the milk, the lactation phase of the animal, among many other factors. However, the most drastic changes occur after processing of the milk, likewise the cold chain, in this process there is a migration of protein towards the whey phase (Dewettinck, et al., 2008).

On the other hand, mechanical operations can modify and lead to the damage of the MFGM such as the incorporation of air; heat treatment also generates irreversible changes such as protein denaturation. Among the processes that have the greatest positive influence on milk structure and in the MFGM is the homogenization, since there is the formation of a new membrane, composed of more caseins and whey proteins that surround the delicate fat globules, and brings more protection (Dewettinck, et al., 2008).

2.3. Production: Unit operations

Dairy cream is defined as a product where unhomogenized milk fat globules are concentrated by centrifugation processes and subsequently subjected to heat treatment and sometimes homogenized, with or without stabilizer. Cream is defined as an emulsion, then it is kinetically stable over a period, additionally it is thermally unstable; the most common problems in cream are the formation of a dense layer of cream, aggregation, and coalescence (Figure 3), however complete or partial coalescence are phenomena that occur more when the dairy cream becomes whipped cream and there is incorporation of air (Matsumiya, et al., 2017). In this study the focus is the analysis of the cream in a liquid state as an oil/water emulsion.

Regarding the heat treatment and the fat molecules, some authors such as Mulder and Walstra in 1974 affirm that the triacylglycerols in the MFGM nucleus are not affected by high temperature exposure, however, the MFGM outer membrane changes when exposed to heat as increasing the viscosity and part denaturation of some proteins. Usually, the heat treatment is accompanied by homogenization

processes, which can be before the heat treatment known as upstream homogenization or it can be after the heat treatment known as downstream.

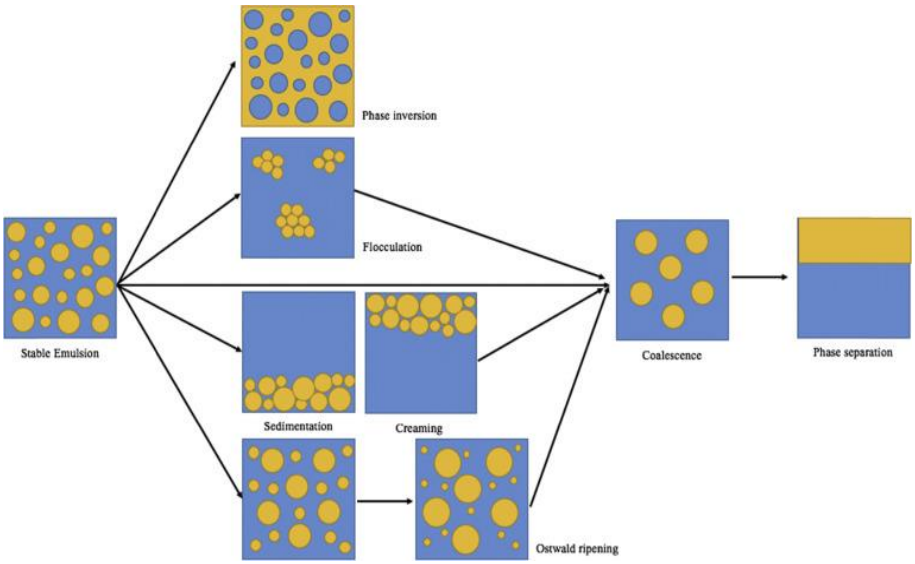


Figure 3 Schematic representation of the emulsion instability mechanisms appearance, from Hu et al., 2017

2.3.1. Process technology

Description for chilled whipping cream 30-35%

A brief description of the manufacturing process for whipping cream could give an overview of the processing line; emphasizing that each country has different high-quality standards, therefore each processing line will have variations. The main raw materials are pasteurized cream 35-40% fat and pasteurized skim milk and stabilizer (Tetra Pak, 2020).

The stabilizer is used to achieve good storage stability of whipping cream. There are several formulations frequently used. Most of them are using just pure κ-carrageenan which is stabilizing the product by building a network but keeping still the product low viscous. Other recipes are keeping the product stable by increasing of viscosity using also other stabilizers like xanthan gum, guar gum or even cellulose. Some mixed stabilizer contains even emulsifiers to speed up the whipping process (Tetra Pak, 2020). Below is a summary of the manufacturing process described by the Dairy centre of expertise from Tetra Pak, updated 2020 (more information Appendix A):

- **Raw material**: pasteurised homogenised skim milk and pasteurised cream. It is of vital importance that the cream prepared for the next heat treatment was handled gently without any excessed shear which will destroy the fat globules and will negatively influence the cream stability. To achieve good storage stability is recommended to use stabilizer as κ-carrageenan which is stabilising the product by building a network but keeping still the product low viscous. Other recipes are keeping the product stable by increasing of viscosity using also other stabilisers like xanthan gum, guar gum or even cellulose.

- **Standardisation and stabilizer addition**: The skim milk is necessary to reduce the fat content to the requested final level, the skim milk is used for dissolving or dispersing of the stabilisers. Depend on the additives the mixing can be done in cold or warm milk or even in water and

always at high shear. The best practise is to make the solution in high shear mixer. After dissolving of the stabilisers, the slurry is cooled down to at least 20 - 25ºC before addition to the cold cream under very gentle agitation.

- **Heat treatment:** Both indirect and direct systems are suitable for production whipping cream.

- **Homogenization:** Homogenization will on one hand minimise the degree of fat separation in the product during storage, but on the other hand it will result in an increased whipping time. Thus, the pressure chosen is always a compromise between the cream stability and whipping properties. Independent of the UHT system used homogenisation of whipping cream is always taking place after the sterilisation in downstream (aseptic) position at temperature 75 - 80 ºC.

- **Refrigeration:** To cool the cream quickly below 10ºC, preferably to 7ºC. Both the whipping properties and the storage stability benefit from rapid cooling, especially due to the crystallization processes of the fat globules in the cream; moreover, this crystallization process will also depend in the addition of stabilizers such as carrageenan, as an even stronger network will form during storage. The final product must always be handled with care, due to the risk of rupture of the fat globules, avoiding shearing and turbulence by using heat exchangers (coolers) and pipes of the correct size. In addition, there is evidence that the homogenized dairy cream seems to be more resistant than the non-homogenized one in terms of the sensitivity of the fat globules to damage (Rønholt et al., 2012). However, all cooling temperatures will depend on the stabilizer used since some stabilizers show highly gelling properties even with cooling between 20 ºC.

2.3.2. Heat treatment

The heat treatment of milk reduces the microbial load, there are many temperature ranges, types of heat transfer and holding times, then, today there are different heat treatment processes. Among the most widely used in the dairy industry is gentle pasteurization high-temperature-short-time method (HTST), other is ultra-high temperature (UHT) with temperatures of $\geq$ 135°C for a few seconds requires chilled distribution (Van den Oever, S. & Mayer, H. 2021).

There is also a process called “extended shelf life (ESL)” that gives a product shelf life of 25 to 27 days for milk, in combination with the use of membrane technologies a shelf life of 30 days can be obtained, and if it is kept refrigerated and chilled distribution for all shelf-life, it can have a shelf life of up to 60 days. ESL technology tries to maintain a balance between spore destruction and preserving milk nutrients as much as possible, avoiding vitamin loss and protein denaturation. These heat treatment processes can be applied directly or indirectly (Van den Oever, S. & Mayer, H. 2021).

On the other hand, the heat treatment will also have an effect on the structure of the chemical components of the cream, even the subsequent properties of the cream can be seriously affected. In creams with >30% of fat are usually used as whipping cream, so the capacity to incorporate air, the overrun, the viscoelastic properties, the loss of the serum phase, among other characteristics, will depend directly on the production process of the cream. It has been reported in the literature that UHT treatment of dairy cream produces creams with lower overrun and also increases whipping time. This is because at temperatures around 80°C and 140°C it causes the denaturation of β-lactoglobulin. This result in the formation of complexes with κ-casein and β-lactoglobulin, and β-lactoglobulin with α-lactalbumin, which increases the viscosity in the serum phase. Changes occurring in the MFGM will have a significant impact on the stabilization of cream and whipped cream (Smith, et al., 2000).

According to Eisner, 2021 in the review of "direct and indirect heating of milk..." it was stated that several authors agree that the concept of heat treatment cannot be strictly limited to time-temperature combinations, since in the line of dairy processes (more commonly on an industrial scale) there are more variables that affect the result. Stating that "not only the holding time, but also the heating and cooling times of the product before and after the holding cell, respectively, will influence the product".

Despite establishing ranges and having high-precision equipment, there is always the risk that these times exceed what was previously established in the heat exchangers. The advantage in terms of process times is to the direct heating of the product by contact with steam and the cooling by sudden evaporation of water (flash cooling), then the time and the heat load of the product can be reduced. This type of process is used on UHT milk in aseptic packaging, it is also used on ESL milk that requires refrigerated storage (Eisner, 2021).

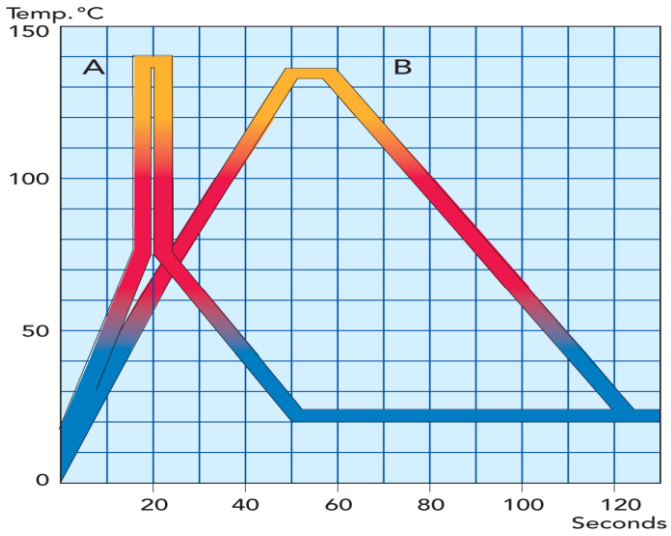


Figure 4 Time-Temperature curve for UHT treatment in direct, A, and indirect, B system, from Tetra Pak Handbook, 2015

In Figure 4 shows representative time-temperature profiles for direct and indirect UHT in milk, of temperature (y-axis) and time (x-axis), a graphical compression of the difference between the processes in terms of time to reach maximum temperature and then followed by cooling time to illustrate the impact of heating/cooling times, the graph is a rough sketch, it also assumes that the homogenization process is downstream (Eisner, 2021).

Direct heat treatment

In the direct heating, steam is briefly injected into the product or by infusion and holding times can be added followed quickly by rapid cooling phases (see Figure 5). The faster the complete process, the higher quality (depending on the customer demands) is maintained in the final product. Since dairy cream is a food rich in macronutrients and micronutrients that can be susceptible to heat and denature, change, or lose its structure. Among the disadvantages of direct heat treatment is the process requires a high energy consumption compared to indirect heat treatment. In this, indirect heat process, the product does not come into direct contact with the heat source, but the product is heated by heat exchangers, for this reason most of the thermal energy can be recovered, and therefore the process it is more profitable than with direct heat (Van den Oever, S. & Mayer, H. 2021).

In direct heating the steam is injected directly into the water and transfers heat to the water, this transfer are by convection and conduction. Then the direct heating medium is mixed with the product, which has a high risk of contamination. On the other hand, indirect heat transfer is the most used method in

the dairy industry, where the heating medium is hot water, in a plate heat exchanger this serves as partition; heat is transferred by convention and conduction. Therefore, it must be remembered that the properties of the product will have an impact on the efficiency of the heat treatment, aspects such as product flow rate, physical properties of the liquids, the programmed temperatures, the design of the exchanger and the addition of homogenization processes will be decisive for the heat treatment process (Tetra Pak, 2015).

For example, in direct UHT systems the whipping cream is preheated with either plate heat exchangers (PHEs) or Tubular heat exchangers (THEs). The final heating takes place with direct contact between the whipping cream and the heating media (steam), and this added steam volume is thereafter flashed off using vacuum cooling. There are two types of direct heating, steam injection and steam infusion (Tetra Pak, 2020).

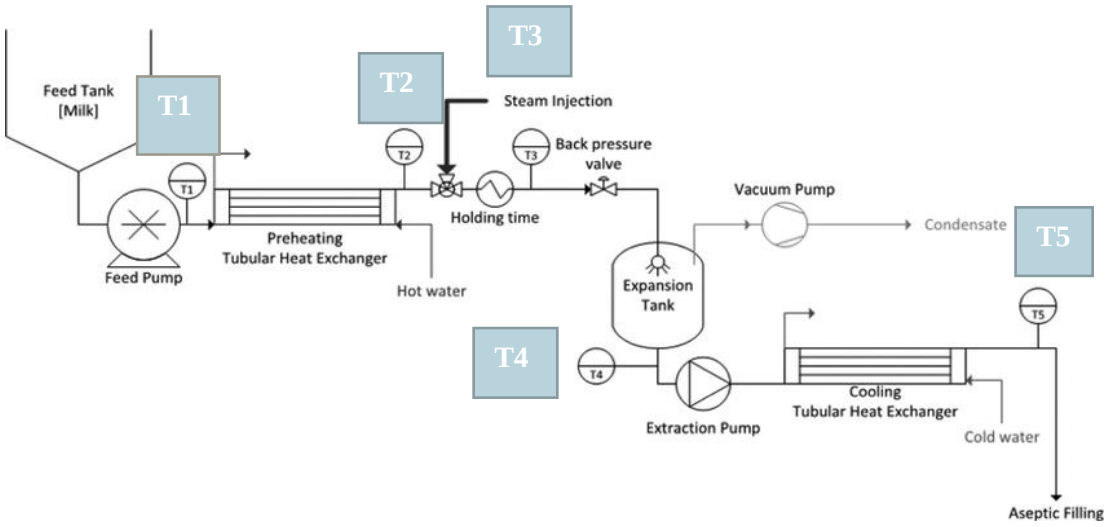


Figure 5 Schematic diagram of Direct Steam Injection process. Example for milk Direct heat treated. T1: milk temperature inlet, T2: milk preheating temperature, T3: DSI process temperature T4: expansion temperature, T5 milk outlet temperature. From: Peterz et al., 2016

Ideally, in steam injection steam is injected to the pre-heated whipping cream around 80 °C to bring the temperature up to minimum 140°C followed by 4 seconds holding cell and then the cream enters a flash vessel. In the flash vessel the amount of steam used for the heating is flashed off and the temperature of the product is by the drop in pressure lowered to the same temperature as the one after indirect preheater - 80°C. The sterilization conditions 140°C/4 s is the minimum, but many customers elevate the temperature 2 - 3°C for safety reasons (see Figure 5). In steam infusion the product is passed through an atmosphere of steam as final heating, otherwise the process is the same as for steam injection. There is an indication that infusion system is slightly gentler to the cream than the steam injection due to a certain shear the cream is exposed to in the injection part (Tetra Pak, 2020).

Indirect heat treatment

Indirect heating usually takes place in a PHEs or THEs heat exchanger that preheats the milk up to 70°C. After homogenization, the fluid is finally heated to 123–125 °C for 2 s. The thermal process by indirect heating is still the most economical technology compared to filtration or bactofugation (Van den Oever, S. & Mayer, H. 2021).

Therefore, the greatest advantage of indirect heat exchangers is the recovery of energy, since the heat of the outgoing hot product is transferred, used to heat the cold product that is entering the system, either directly or through a water circuit. secondary. It has been established that up to 90% of the energy

can be recovered. A combination of indirect heating with electrical heating, sometimes referred to as electric tube heating (ETH), can also be given, offering faster heating rates (Eisner, 2021).

Heat transfer follows the rules of energy balance between two fluids, for example milk and water. The transfer occurs through a wall that divides the two fluids, in dairy it is usually a tube or the plate of a plate heat exchanger (Eisner, 2021).

Several studies have been reported in the literature that compare the effect of direct or indirect heat treatment on milk and dairy products, however, it is difficult to reach definitive conclusions since the methodologies to verify the impacts of these two types of heat treatments they vary a lot. In addition to the fact that the studies are carried out on a pilot scale, so there is a big difference with respect to what it would be like at an industrial level. There are markers to analyse the intensity of heat treatment and thus understand the effect of heat treatment on the product, among the most common tests is lactulose, furosine, 5-hydroxymethyl-2-furfural (HMF), or undenatured whey protein content (Eisner, 2021).

Summary of heat treatment impact on properties

Below is a list of effects on proteins due to heat treatment summarized in the Eisner review, 2021:

- The mechanisms of protein denaturation and aggregation are affected by the combination of heat with high shear forces, specifically in direct heating.
- Heat can induce coagulation of milk due to dissociation of κ-casein, aggregation of κ-casein and whey protein, ultimately loss of micellar integrity.
- The apparent diameters of the casein micelles may be larger due to the association of β-lactoglobulin and κ-casein.
- The association between α-lactalbumin and β-lactoglobulin with casein micelles has been recognized after heat treatment, since the reactions are dependent on kinetics and temperature, therefore variations will be found depending on whether it is direct or indirect heating.

2.3.3. Homogenization

This process is based on the reduction of the particle size going from micrometres to nanometres. The understanding of the final particle size after unitary operations such as homogenization helps to predict future behaviour of high-fat products and the metastability of these emulsions, as well as the viscosity and rheological characteristics in the final food matrix (Masbernat et al., 2022).

The homogenization process aims to avoid the formation of creaming or sedimentation, both phenomena occur due to the gravitational effect. When the appearance of one of these phenomena becomes visible in an emulsion, it is because a total destabilization of the emulsion has already occurred. As reported in the literature, "if the density of the dispersed phase is less than that of the continuous phase, the drops move upwards" and a separation of the continuous phase occurs, giving way to the formation of a layer on top of the continuous phase. Continuous phase, also called creaming.

On the contrary, sedimentation “occurs when the density of the dispersed phase is greater than that of the continuous phase and the droplets move from top to bottom and form a layer at the bottom of the continuous phase” (Fox et al., 2015). However, when these phenomena occur and are visible to the human eye, it is because the kinetic process and the instability in the emulsion began long before.

Objectives of homogenization in dairy products

The following lists some of the main advantages of carrying out the homogenization process in dairy products or in raw materials for the production of dairy products, by Huppertz (2022):

- Avoid the formation of cream: the formation of cream in the upper layer of dairy products is considered an undesirable aspect by the consumer, and homogenization helps reduce the size of the fat globule and slows down the speed of formation of cream. cream.

- Stability: Stability towards coalescence can be improved because there is a risk that densely packed fat globules will clump together in a partial coalescence manner.

- Rheological properties: The viscosity can "improve" when homogenization processes are applied, since homogenizing groups are created and the fat globules may have greater interaction with the proteins in the medium, so the viscosity can increase and be desirable in the products. endings.

- Emulsification: sometimes additives such as stabilizers are added, the homogenization process will help a better dispersion of the components and produce a more stable emulsion.

Factors influencing the homogenization process

In the homogenization process there is a reduction in the particle size and an increase in the surface area and number of droplets. The factors that would determine fat globule size in homogenized dairy products are briefly defined below: mainly the homogenization pressure depend in the fat content of the product. The type of homogenizer especially the valve design as this will affect the passage time, the flow conditions in the valve also drastically affect the final product (Huppertz, 2022).

The number of homogenization stages, because some clusters can form in the first stage of homogenization, thus the second stage helps to minimize these clusters, usually the second stage has pressures of 10% or 20% of the first stage. Another factor is the temperature of the process since the efficiency will be determined by the temperature if the temperature is exceeded there will also be consequences in the efficiency of the homogenization (Huppertz, 2022).

In the Figure 6 it can be seen the physical change that the fat globules undergo after being forced to pass through a very narrow channel at high pressure.

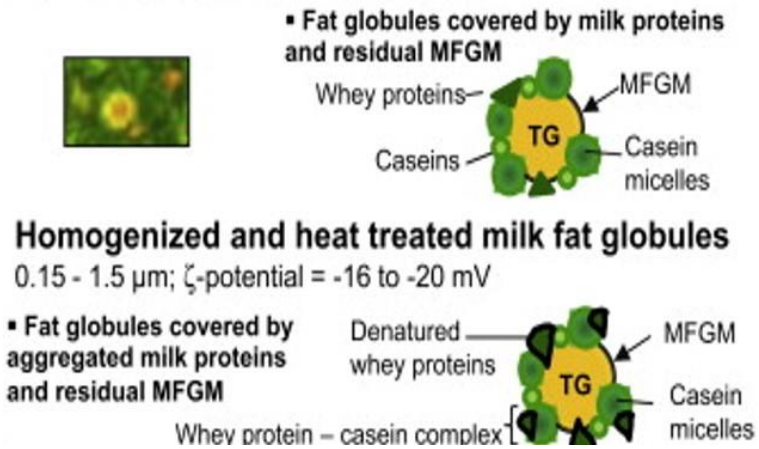


Figure 6 Microstructure homogenized milk fat globules with confocal laser scanning micrographs, showing the triglycerides located in the core of the fat globule. By Garcia et al., 2014

Fat globule disintegration is achieved by high shearing stress, turbulence, and cavitation, as well as a four to six-fold increase in the fat/plasma interfacial surface area. The new fat globule is now covered by a mixture of proteins absorbed from the plasma phase (Garcia et al., 2014).

As previously mentioned, homogenization is used to reduce the size of fat globules and thus improve physicochemical aspects of dairy cream (Ransmark et al., 2019), however the initial quality of the raw materials used for the preparation of dairy cream will have an impact on the final product, as well as unit operation (Paajanen et al., 2005).

Principle of the homogenization

In a homogenizer, dairy cream is forced under high pressure through a narrow gap Figure 7 (Tetra Pak, 2003). However, the homogenization process of each dairy product, depending on the total fat content, will have different types of homogenization, for example dairy cream with >30% fat content it is recommended to use very low pressures since high pressures could have a negative effect on other characteristics of the product such as the foam formation (Ransmark et al., 2019).

There are many reason that process must be very controlled, and it is also recommended to divide it into two stages. Therefore, in a homogenizer, the geometry of the space and the applied pressure are the main factors that will influence the size of the droplets (Ransmark et al., 2019). Other factors to consider is the homogenization temperature, and the position of homogenization device (downstream or upstream).

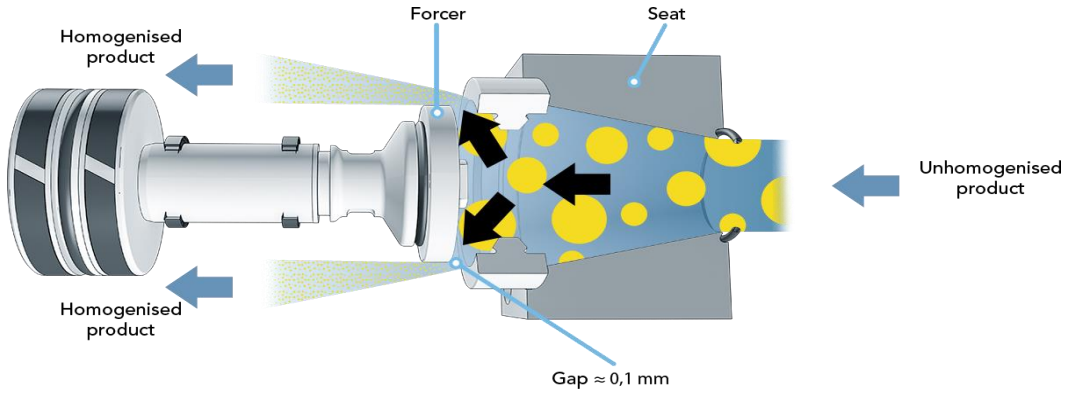


Figure 7 In a homogenizer dairy cream is forced under high pressure through a narrow gap where the fat globules are divided , from Tetra Pak 2003, handbook

The position of the homogenizer (upstream or downstream of the final heat treatment) is one factor that will produce products with significant differences. Whenever the transfer is by direct heat, the homogenizer must be downstream, for example in aseptic filling, just after the high temperature pasteurization process. On the other hand, there is the upstream homogenization where the homogenizer is placed before the final heating section in the heat exchanger (Ransmark et al., 2019). In conclusion all those variables in the processing line will have an impact in the product.

For this reason, today there are analyses such as the study of the stability of emulsions in real time and under storage conditions, as adjustable temperature ranges of storage conditions. These studies can be carried out using equipment such as TurbiScan, whose principle is based on multiple light scattering, with an 880nm NIR light source, sending photons into the sample. This equipment can detect the evolution of stability at an early stage, up to 200 times faster compared to visual tests, it also quantifies the global alteration of the emulsion with the TurbiScan stability index (TSI) scale.

2.3.4. Additives

Due to the unstable nature of dairy emulsions, nowadays the use of stabilizers or emulsifiers of dairy (casein and whey proteins) or non-dairy (carrageenan, guar gum, etc) origin has been extended.

The addition of these additives in the cream helps to improve the rheological characteristics of whipped creams. From non-dairy origin, these molecules are usually large molecule polysaccharides extracted from red seaweeds of the class Rhodophyceae and three different types: kappa (κ-), iota (ι-) and lambda (λ-) carrageenan (CG) contain one, two and three sulphate groups per disaccharide repeat unit, respectively. κ-carrageenan has the main function of increasing the viscosity of the aqueous phase (Lal, et al., 2006), κ-CG and ι- CG are typically used in gelling applications, whereas λ-CG is used in thickening applications, usually apply in dairy product to give a full body with no gummy and desirable creamy texture (Stone and Nickerson, 2012).

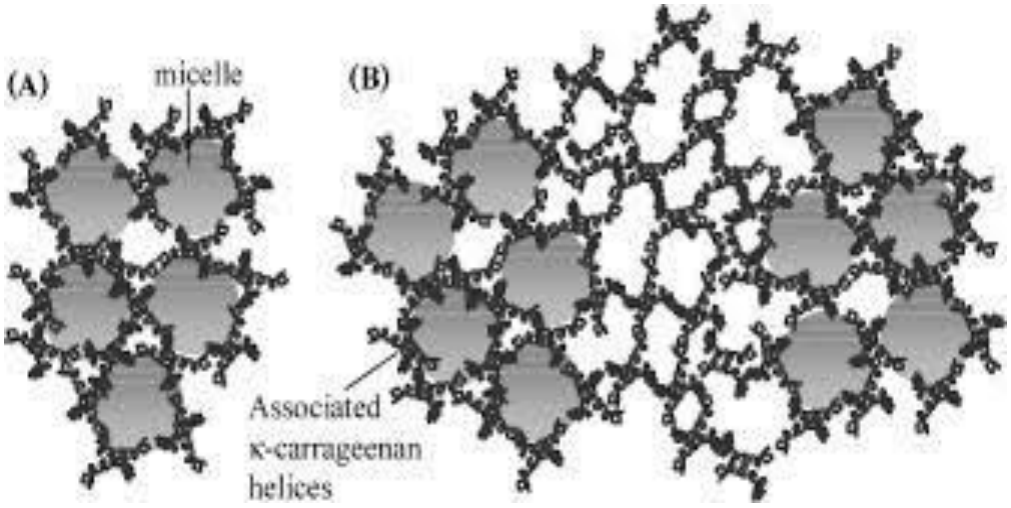


Figure 8 (A) Schematic diagram between κ-carrageenan (0.2%) and skim milk gel microstructures (B) κ-carrageenan (0.7%) Flocculated micelles held together, the gel is dominated by k-carrageenan interactions, from Arltoft, et al., 2007

κ-CG has a direct interaction with the κ-casein-glycomacropeptide complex (Figure 8), it can form aggregates with milk proteins with positively charged segments from the milk proteins and negatively charged from carrageenan (Kováčová et al., 2010). However, previously it was reported the interaction might occur between carrageenan and β-casein and αs-caseins, it is required the presence of the calcium ions and phosphorus ester-bonded of the casein fractions. Moreover, the interaction will depend on the temperature of the system, concentration applied in the formulation and environment conditions (number of ions and ionic strength) (Míšková et al., 2021).

Just as the heat treatment and homogenization have a direct impact on the rheological properties of the dairy cream, the added amount of stabilizer will also have an influence on the whipping time and can reduce any overrum. All these factors in combination can result in a considerable improvement in the properties of whipping cream as confirmed in the study of the influence of processing and κ-carrageenan on whipping cream by Kováčová, Štětina, & Čurda, (2010).

Carrageenan is used in milk-based products due to its properties to be a stabilizer, forming a stable emulsion and well-formed gels. The interaction of whey proteins with carrageenan has been reported. Likewise, carrageenan contributes to the increase in viscosity, helps reduce the mobility of molecules, increases the resistance of the interfacial layer, improves the resistance of potential coalescence; its

functionality depends on the concentration and after the processing line in the production area (Seta et al., 2013 and Arltoft et al., 2007).

However, the schematic diagram above (Figure 8) is an example of the action of the additive carrageenan in a dairy product (skim cream), but in this study a different microstructural behaviour could possibly be observed because the concentration of carrageenan in dairy cream is much lower, around 0.015%, and in the figure is much larger the concentration used.

2.4. Environmental Stress Cracking (ESC)

Polymeric materials have been very useful for the development of society and innovation, but they are materials that are susceptible to changes and loss of mechanical properties, and this increases when the materials are subjected to adverse environments. In some cases, it has been recorded how some organic liquids can cause cracks that increase when an external stress is applied, this phenomenon has been recorded in vitreous polymers such as bisphenol A polycarbonate (PC) (Borisova et al., 2003).

It has also been recorded in crystalline polymers such as polyethylene (PE), according to the studies carried out, it is stated that crystalline polymers are more resistant to these ESC failures. In the examples of these failures, it was recorded in the low-density polyethylene (LDPE) used in wire and cable coatings (used to replace the use of elastomers), that on certain occasions soap was applied to be able to pull the wires and thus reduce the friction of the elastomer, achieving an LDPE with a softer and smoother surface, but over time and with the constant use of soap it was found that cracks formed, which later generated stress-induced failure, which was later called ESC by external agent (Borisova et al., 2003 and De Paola et al., 2021).

Currently, there is an ASTM standard test: D1693: "Standard Test Method for Environmental Stress-Cracking of Ethylene Plastics" use for the determination of the susceptibility of ethylene plastics to ESC when subjected to environmental stress-cracking (Dilara and Briassoulis, 1998).

2.5. Factor that increase the risk of ESC

The factors that intervene in the risk of ESC are extensive and varied, these include the formation of the polymeric material, the manufacturing conditions of the plastic, the melting temperature, the crystallinity of the polymer, the molecular weight, and the slight changes it undergoes (Dooher et al., 2022). Plastics suffer when they are subjected to external forces such as bending, creasing, heat-sealing, among other processes that are common in the manufacture of multilayer packages with polymers such as Tetra Pak carton packages. According to the literature, ESC is more associated with amorphous polymers, however, this problem has also been reported in semi-crystalline polymers such as high-density polyethylene (HDPE) (Dooher et al., 2022).

Several studies have confirmed the importance of understanding the relationship between the polymer and the fluid in contact, but it is known that there are infinite options of fluids that can come into contact with polymeric materials. While some tests, such as solubility parameter and the Hansen solubility parameter (HSPs), are used to predict which liquids pose an ESC risk. HSPs are useful for preparing emulsions and to estimate their miscibility, as a way of predicting if one material will dissolve in another and after developing a new solution.

It has also been established a correlation of ESC severity as a function of fluid hydrogen bonding and polar parameters, with the most severe effects observed in fluids with hydrogen bonding in range 4-9 $MPa^{0.5}$ (Wright, 1996). On the other hand, there are atypical data in this Hansen solubility measurement,

and it is difficult to measure the total components present in these fluids, so it is not a very convenient method (Robeson, 2013).

It has been reported several times in the scientific community that soap and solvents are ESC agents. In addition, an increased risk of ESC has been reported after gas exposure, such as PVC pipe exposed to gas containing benzene (Dooher et al., 2022).

Although there are standardized tests for the evaluation of ESC, the truth is that their application is very limited and restrictive. Therefore, at times, many polymeric material producers can rely on historical ESC failure records and follow history until possible causes can be found and risk factors minimized. Taking into account that the ESC can be mild or extreme, in the mild case the failures can occur days, months and even years later, while in the extreme case the failures occur almost immediately, then it is easy and fast to detect and even prevention, since it is possible to correct or stop the distribution of the batch that presents the extreme ESC, while the mild one is difficult to predict since it can occur a long time later (Dooher et al., 2022).

2.6. Mechanism of environmental stress failure: cracks

The exposure of plastic materials to organic liquids produces a reduction in surface energy, as a consequence there is less stress for the formation of cracks compared to benign or neutral environments such as air or vacuum. In conclusion "the stress required for crack formation is reduced by environmental exposure since the solid/liquid interfacial tension is lower than that expected for the solid/air interface" (Robeson, 2013).

Two main aspects of ESC phenomenon are the critical product and the inherent stress. Some foods that are considered critical for ESC are dairy cream with high fat content and the addition of stabilizers; ESC is mainly initiated by a loss of the inherent mechanical properties of the plastic due to the impact of a liquid on the intermolecular polymer bonds (cohesive failure). In the case of the Tetra Top carton package, an ESC occurs in the filling of the critical product (dairy cream with stabilizers added) while the package is being formed in the filling machine.

In the filling machine for Tetra Top, the material is formed in the forming machine and then filled with the product and can result in failures and crack formation; clarifying, that in case of Tetra Top filled upside down and the bottom is folded after filling. When the package is going out of the machine the package is turned in the standing position. This is when the product comes in contact with the bottom and stressed area.

ESC commonly occurs in the packing area with the highest stress such as folds and corners which resulted in cracks in the polymer, cracks in the corner and fold area of the cardboard package. When the package is formed in the filling machine the tension is high in these areas; therefore, there is a risk of rupture.

2.7. Chilled Dairy cream production

2.7.1. Mini UHT equipment

All the information mentioned in this section comes from the HT220-DSI from OMVE manual. It is used the HT220DSI HTST/UHT (Figure 9 and Figure 10), in this equipment it is possible to produce

different kind of food products with different temperatures to simulate an industrial process but in small scale. It allows to produce batches with even less than 2 litres. The equipment has a touch screen where it can be modifying the parameters as temperature, pressure, and flow rate, the total formulation. It can be distinguished three processes in the operation system: sterilisation, production, and cleaning in place (CIP) (More information see Appendix).

Processing

Below is a brief description of the procedure in Heat treatment system HTST/UHT HT220-. The complete system has been designed to work with product flow of 20 L/h, it uses a tubular heat exchanger for processing.

1. Product can be added to the feed hopper (1 in Figure 9) if all water for sterilization is drained out

2. Product is pumped from the feed hopper to the heat exchanger

3. The product is preheated (2 in the Figure 9 in two tubes, and is finally brought to main temperature in the heat exchanger with two tubes (3 in the Figure 9), in all the tubes the product flows through inner tube with diameter of 8mm

4. Holding time can be from 2 to 15 seconds (4 in the Figure 9)

5. The product is cooled down after heat treatment (5 in the Figure 9) in 2 cooling sections, the first has 2 cooling tubes and the second stage has 4 tubes, this is achieved with normal tap water, or also chilled ice water or glycol, but normally tap water is sufficient

6. At the outlet, a back-pressure-valve is installed to prevent the product from boiling at temperatures higher than 100 °C. Homogenization process in not showed in Figure 9.

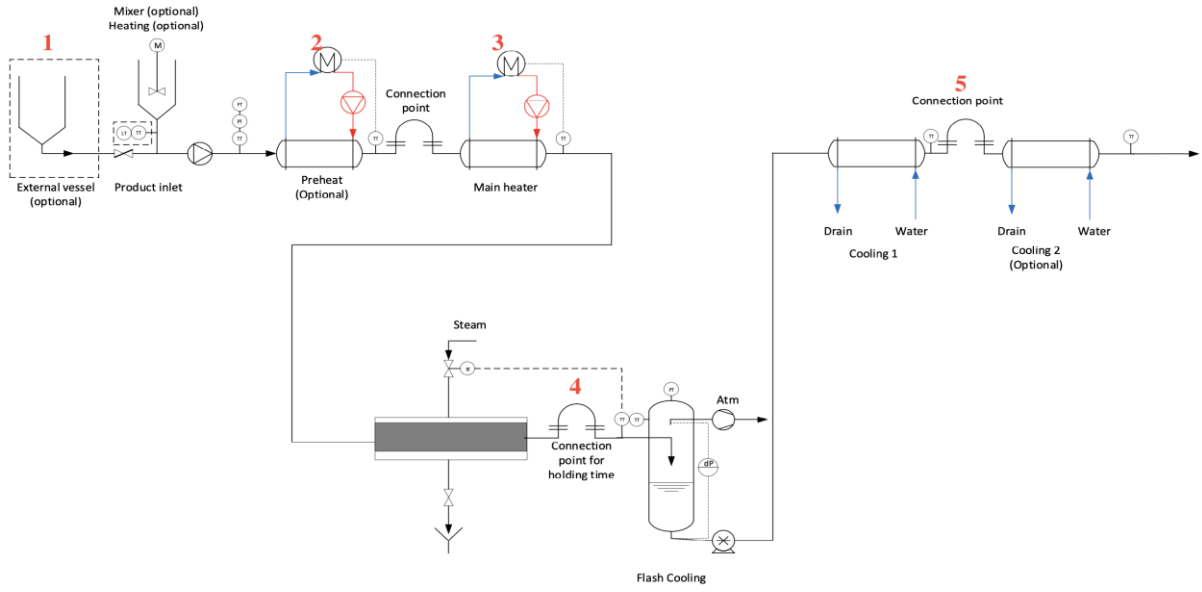


Figure 9 Process flow HT220-DSI, with tubular heat exchanger

2.7.2. Heat treatment and homogenization

Direct steam injection

The product is heated with steam, this results in short heating up times. The steam injector increases the product temperature up to 80°C. After holding the product will be cooled down in the flash cooler. The amount of cooling is depending on the vacuum pressure in the cooler, it can be set among -0.2 and 0.7 bar because the temperature decreases due to a pressure decrease called the flash effect. Consequently, the direct steam injection and flash cooler are connected at the holding section connection of the HT220. The homogenization can be only downstream from manual of HT220-DSI.

Indirect heat with hot water boiling system

The product is indirectly heated with water, which is heated by electrical heaters (tubular). The system heats up softened water with a gear pump through the heat exchanger in opposite direction to the product flow. A homogenizer can be placed upstream between the preheat and the main heater, and downstream between the first cooler and the second cooler.

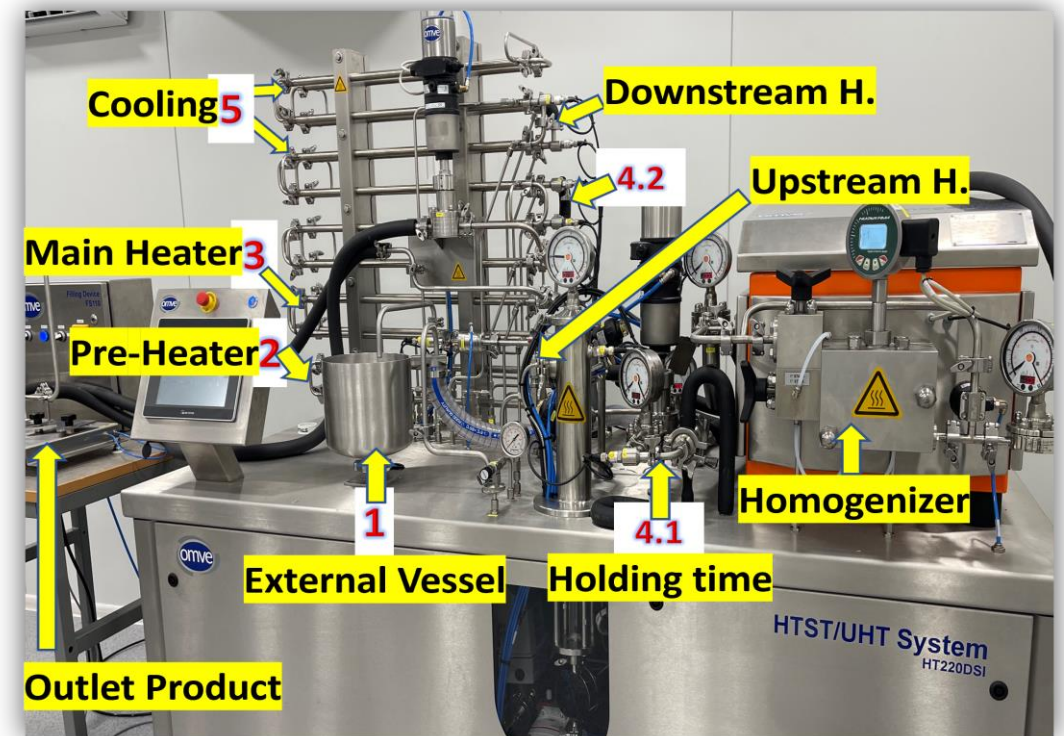


Figure 10 Heat treatment system HTST/UHT HT220-DSI in Acc-Lab Tetra Pak, 1. External vessel product inlet 2. Pre-heater 3. Main Heater 4.1. Holding time in direct heat 4.2. holding time indirect heat 5. Cooling system, Photo from Alejandra Fernandez

Homogenizer

This part is specially developed for laboratories of the food industry, the system has two pistons and is able to do a two staged homogenization with a maximum pressure of 600 bar. This is regulated by a handwheel which rotated clockwise with the pressure increase (Figure 11).

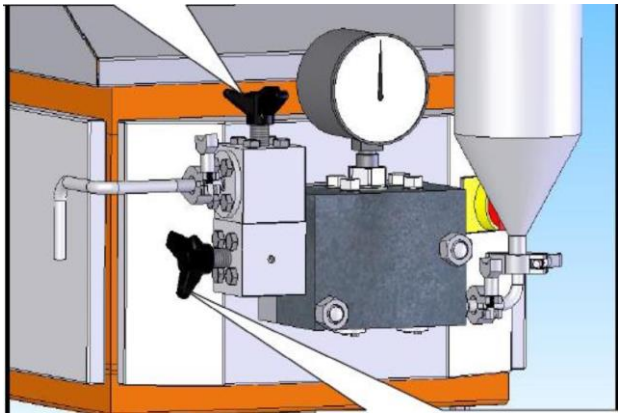


Figure 11 Setting homogenization pressure from Omve

Operating mode has 6 different programs as

1. Indirect heating
2. Indirect heating + homogenizer upstream
3. Indirect heating + homogenizer downstream
4. Direct heating + heat exchanger
5. Direct heating + homogenizer downstream
6. Homogenizer stand-alone

2.7.3. Filling point

It is automatic filling point designed to allow small quantities of food to be filled and sealed conveniently, it can be placed in different environments protected and unprotected (Figure 12). With the foot pedal two hygienic valves are switched. The CIP of the tubing and valves is possible by placing a circulation tube between the filling point and the return connection on the back of the machine.

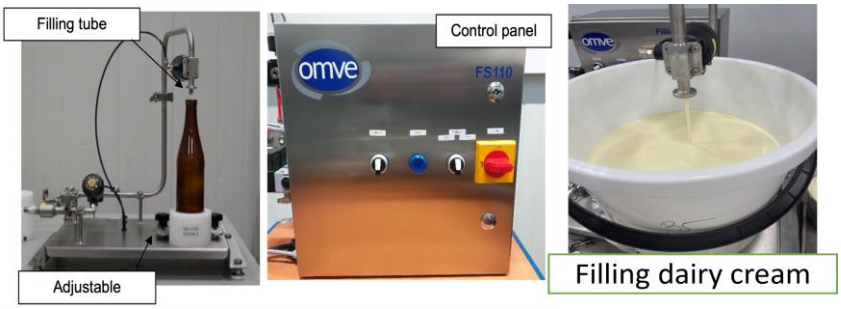


Figure 12 Clean fill system photo from: Omve

2.8. Food package interaction (FPI)

The factors that influence the risk of ESC are multiplex. The investigation checklist should cover the complete description of the packaged product, specifying the processing variables in the production line, as well as the conditions of the packaging and the packaging material, as well as the combination of food- interaction packaging, specifically for the packaging machine and material. As reported by Tetra Pak, some cases of ESC have occurred just after the product touches the surface of the package or directly after filling, which is why it is considered as extreme (failure in the package, as a leakage) and unpredictable ESC, since not all the time it has been reported.

Therefore, it is proposed to investigate the hypothesis that the microstructure of the cream could be correlated to the risk of ESC, moreover the processing conditions previously applied to the dairy cream product could be an indirect factor that would generate a highly aggressive product for the packaging material. It is theorized that there is a correlation between the particle size of the fat globules, the disposition of the components in the serum phase.

Likewise, consider the study of the interfacial interaction between the packages and the product. Previous work by the Tetra Pak team concluded that a list of high-level factors that are likely to facilitate penetration of the PE layer in TT

- Particle size distribution of fat globules, moreover the composition of fat does not have a big impact on how critical a dairy cream is.
- Microstructure properties as formation of casein aggregates and fat globules interaction,
- State of protein denaturation due to heat treatment,
- Since the ESC risk has been detected instantly after packaging the emulsion stability over time is not crucial; however, emulsion stability could be an indicator of the microstructure of the dairy cream and complement to the studies of particle size distribution, microscopy, and viscosity.
- Type of homogenization process and all the variables associated with this process (downstream, upstream, 1 or 2 stages, pressures, temperature of process, flow rate, so on),
- Even if the fat is considering an important factor the total fat content and the lipid profile of the dairy cream does not seem to be a significant factor, however the physical arrangement of the molecules, the particle-particle interactions that occur between all the components present in the dairy cream seem to have an influence in the risk of ESC.

In addition, it was clear indication (still not verified) through the investigation that two products with the same fat content but produced under different conditions, one with homogenization and one without homogenization, presented different risks for ESC. The non-homogenized dairy cream product presented a higher risk of ESC compared to the homogenized product.

3. Materials and Methodology

Next, the methodology, principles, and detailed steps that were part of the general methodology of the project will be explained in detail. As previously mentioned, the project could be divided into three phases.

I. Preparation of the dairy cream, processes from obtaining raw materials, standardization of fat content, sampling of the dairy cream mix before processing, until processing line such as heat treatment and homogenization process in the mini-UHT equipment, among other steps.

II. Laboratory analysis that characterizes each batch of dairy cream produced such as microscopy studies, particle size distribution, emulsion stability, determination of lactulose concentration, analysis of the proximate composition of the product such as fat content, protein, total solids, etc.

III. Interaction between packaging and dairy cream product, performing red-ink tests and puncture tests to analyse how critical the dairy product is on the risk of ESC.

3.1. Sample preparation mini-UHT

Five different batches of dairy cream were manufactured from pasteurized cream 40% fat content and skimmed milk 0,1-0,5% fat content short shelf life (low pasteurization). With the addition of 0,015% carrageenan LP-60 (mostly is kappa-carrageenan).

The final product was formulated to have a final fat content of 36% fat content. An orthogonal statistical design was following with two factors: Homogenization and Heat treatment, and factor level: upstream, downstream, no homogenization, and direct and indirect heat treatment respectively (Table 2). As the process of homogenization upstream with direct heat treatment is not a relevant option because customers do not run it that way.

Table 2 Experimental design for dairy cream production in small scale using Mini UHT with 20 L / h flow

# Experiments	Heat treatment	Homogenization
B1 DNH	Direct	No apply
B2 DDH	Direct	Downstream
B3 IDH	Indirect	Downstream
B4 IUH	Indirect	Upstream
B5 INH	Indirect	No apply

For the manufacture of the cream samples, a mini–UHT HT220DSI HTST/UHT equipment designed by the OMVE company was used (Figure 10). This innovative equipment allows the elaboration of small quantities of product, even less than 2 Liters. Reducing process times, product quantities, start-up time and facilitating the development of experiments and laboratory studies (see flow chart Figure 13).

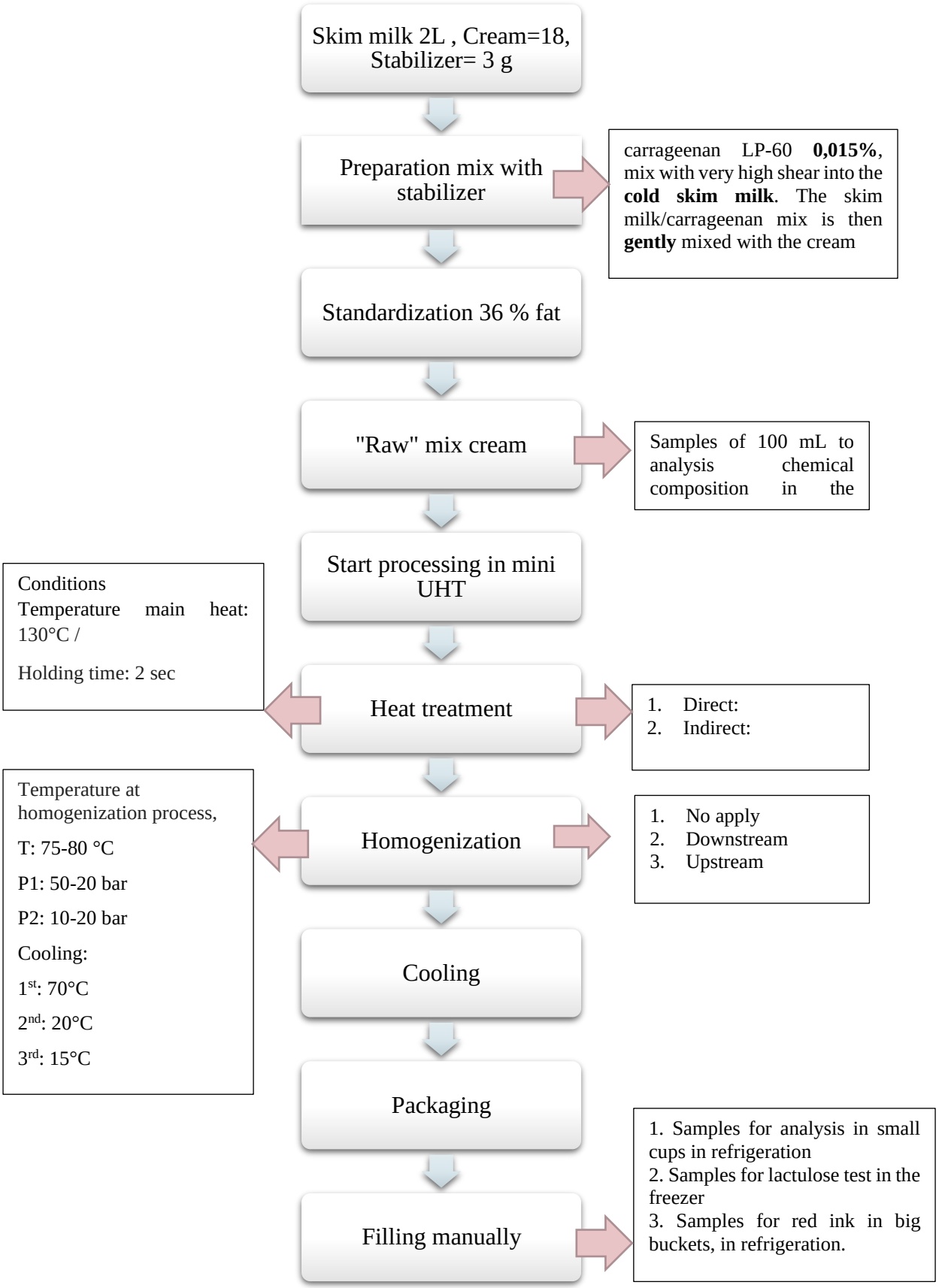


Figure 13 Example of schematic representation of the flow chart production of dairy cream downstream homogenization example, in case of to be upstream homogenization the flow chart will change the position between heat treatment and homogenization

3.2. Test methods

Table 3 Brief summary of the performance of the analysis and main general purpose to which it points.

Table 3 Test methods to study the relation between molecular structure of the dairy cream produced under specific condition and the risk of ESC

Test	Methodology	Purpose
Defect variable test		
Microscopy	A Malvern 4-ID morphology kit was used for this study, a kit that aids rapid, automated, and specific particle characterization in a single, detailed component-specific morphological description of particle mixtures through Morphologically Directed Raman Spectroscopy (MDRS).	Morphology of macro and microstructure
Particle size distribution	Particle size distribution was determined using a Mastersizer 3000 Malvern Instruments. It uses the technique of laser diffraction to measure the particle size and particle size distribution of materials. The analysis were performance without Sol A and with Sol A. The data results analysis are Dx (90) and Mode value.	Fat globule size after processing line
Rheology: viscosity	The viscosity of the dairy cream samples was determined using an Anton Paar Rheolab QC rheometer, with accessory temperature device C-PTD 180/AIR/QC The temperature was programmed at 6 °C and 20 °C for all the analysis.	Viscosity of final product correlated with load heat and homogenization
Emulsion stability with TurbiScan	Dairy creams were analysed as soon as possible after their day of manufacture, glass tubes were filled and analysed with a TurbiScan LAB in backscattering mode of a pulsed near-infrared light source around 850-880 nm as a function of its height. The analysis was carried out for 75 minutes with a period of 25 seconds between each reading.	Macrostructure study of the emulsion (stability on the time)
Lactulose Concentration	Samples dairy cream produced in the laboratory were sent to an external laboratory Muva Kempten GmbH, in Germany. The samples were collected directly from the filling point in the mini-UHT equipment and packed in 100 mL plastic containers and immediately frozen. The analysis was performed by enzymatic route according to 64 LFGB L01.00-31:1988-12 (a), and Muva Kempten GmbH is accredited for this method.	Heat load and changes in the samples
Characterisation of dairy cream composition: MilkoScanTM	With the MilkoScanTM FOSS, it is allowing to keep track on some parameter of the liquid dairy products, the parameters such as fat and protein which are important to characterize the raw materials and final product obtained. The equipment has Fourier Transformed Infrared (FTIR) technology.	Fat and protein content of raw mix and processed dairy cream
Food package interaction (FPI) Defect indicative test		
Puncture Test	Grip-Engineering (Thürmler GmbH), in a force machine. The ESC resistance is evaluated by applying the liquid to the inside layer after fixing it over the hole before the measurement.	The ESC resistance is evaluated by applying the liquid on the inside layer after.
Leakage test: red ink	Red ink test to check package tightness. The ink penetrates holes in the package. The principal is that the paper board absorbs the ink if the inside layers of the packaging material are damaged.	Dairy cream could increase the appears of holes in the packages

3.2.1. Microscopy

The physical, rheological, and textural properties of dairy products are greatly influenced by the three-dimensional arrangement of the different chemical components and their interactions. To understand the disposition of the particles in the emulsion, it is necessary to complement the information from the physicochemical analyses such as total fat content, particle size, protein content, among others, with imaging techniques to visualize the interaction between the components and affect the spatial arrangement and the organization of the components present in the dairy emulsion at the nano and micro scale. Subsequently, it would be possible to make correlations between viscosity data, microscopy, and particle size distribution, as well as the stability of the emulsion and the risk of suffering separation or defects such as creaming, sedimentation, flocculation, and coalescence (El-Bakry et al., 2018).

A Malvern 4 ID morphology (Figure 14) kit was used for this study, a kit that aids rapid, automated, and specific particle characterization in a single, detailed component-specific morphological description of particle mixtures through Morphologically Directed Raman Spectroscopy (MDRS) (Malvern Panalytical, 2017).

The preparation of the samples was carried out the sample: light green. Pasteur-pipette was used, mixed one drop of sample, one drop of light green SF yellowish Certistain®. The analyses were carried out in duplicate. Samples were at room temperature. Morphologi ID software was used with 10X, 20X and 50X magnification, light and colour mode were modified.

Figure 14 Morphologi 4-ID from: Malvern Panalytical

3.2.2. Particle size distribution

Particle size distribution was determined using a Mastersizer 3000 Figure 15 (Malvern Instruments). It uses the technique of laser diffraction to measure the particle size and particle size distribution of materials. It does this by measuring the intensity of light scattered as a laser beam passes through a dispersed particulate sample. This data is then analysed to calculate the size of the particles that created the scattering pattern (Malvern Panalytical, 2021).

Figure 15 Mastersizer 3000 particle size analyser, from: Malvern Panalytical, 2021

For this analysis it is important to carry out the measurements with the addition of Sol A since Sol A helps to break down the aggregates of proteins, fats and complexes and the simple fat globule can be visualized by itself, and without Sol A it can be observed the presence of the micelle caseins, the different aggregates, and the "natural" state of the dairy cream. The sol A solution contains Duran, deionized water, tween 20, EDTA/NaOH, the objective of the addition of Sol A is to be able to dissociate the casein micelles, thus reducing the influence of the casein micelles, since these are found in the same size range as homogenized fat droplets (Malvern Panalytical, 2021).

For these experiments, the result is presented as the average of six replicate measurements. Without Sol A 1 µl sample of cream is directly dissolved in the measurement cell until the obscuration rate between 2.4-3.5%, all the sample were measurement around the same temperature 20°C. It would be recommended to dilute cream sample in deionised water before measuring, to fit the obscuration easier, (more information see section Future work).

With sol A, the cream samples were mixed with sol A and deionized water in a 1:1:1 ratio. After mixing, the cream sample with Sol A was added dropwise to the water in the measurement cell until the browning rate reached 2.4-3.5%.

In this analysis the aim is to visualise if there are fat flocculates or not, focus on the fat globules. When it is added the Sol A the casein micelles would be disappear, this displace of protein on the surface of the MFGM; in this case if it is analyse the D (4,3) value (volume weighted mean) it will be an average of all the particles present, then it should be consider which of the data could be more representative for the aim of the study, perhaps it could be recommended to study the data of Mode and Dx(90).

Dx (90) value describes the diameter where ninety percent of the distribution has a smaller particle size and then ten percent has a larger particle size and the Mode value it is where the top of the curve is, the peak of the frequency distribution. Moreover, the parameters are statistically analysed to determine if there is a significant difference between the means of the results of the different batches of dairy cream.

3.2.3. Rheology: viscosity

The rheological properties of dairy products are one of the most important characteristics. Since viscosity data can become decisive for the processing of dairy products such as transport through pipelines, processing conditions, it even gives a description of the final product such as textural profile and consistency. These properties depend directly on temperature. Kristensen et al., 1997 stated that milk and cream generally have a Newtonian behaviour, under conditions such as be a fresh product, at temperatures above 40°C, and with a fat content below 40%.

Figure 16 Rotational Rheometer Rheolab, with accessory temperature device, from: Anton Paar, 2022

The viscosity of the dairy cream samples was determined using an Anton Paar Rheolab QC rheometer (Figure 16) with accessory temperature device C-PTD 180/AIR/QC.

The temperature was programmed at 6 °C and 20 °C for all the analysis, because around 6°C is the temperature of cooling approximately and 20 °C could be consider room temperature. The rheological measurement data was analysed using RheoCompass® software (Anton Paar, 2022).

Output from software analysis is shear stress and absolute viscosity, respectively, as function of shear rate. The viscosity in (mPa·s) was calculated using the best-fit-power-law-equation, then with the software RheoCompass®. The dependence of apparent viscosity on shear rate was $100s^{-1}$, this is the pre-established measurement condition by Tetra Pak.

3.2.4. Emulsion stability with TurbiScan

The cream is an emulsion, and the proteins help to give the cream stability, moreover the addition of the stabilizer carrageenan as well contribute with this stability, however the stability in dairy products is affected by several factors such as pH, ionic strength, the heat treatment applied to the raw materials and the product before being packed, the homogenization and the applied pressures, among others (Guggisberg et al., 2012).

However, the heat treatment has a very crucial role in the stability of products such as dairy cream, as well as the homogenization process. Regarding heat treatment, this is due to the denaturation suffered by whey proteins and the fat globule membrane. Mostly because an increase in the associations between β-lactoglobulin and the fat globule membrane, especially at temperatures around 65°C, when it is started to denature but very slowly, due to the low temperature (Guggisberg et al., 2012).

In this study, the stability of the dairy cream was studied and whether the type of heat treatment, such as direct or indirect heat, and the type of homogenization (upstream, downstream, do not apply) have a significant effect on the stability of the cream. Dairy creams were analysed as soon as possible after

their day of manufacture, flat wave glass tubes were filled and analysed with a TurbiScan LAB (Figure 17) in backscattering (BS) mode of a pulsed near-infrared light source around 850-880 nm as a function of its sample height. The analysis was carried out for 75 minutes with a period of 25 seconds between each reading.

Figure 17 TurbiScan LAB enables fast and sensitive identification of destabilization mechanisms, from Formulaction smart scientific analysis

TurbiScan Lab measurement principle is based on the dispersion of the particles which is analysed in a cylindrical glass cell. The TurbiScan works in scanning mode, the light source is a near-infrared light emitting diode (Sifontes et al., 2015). Two synchronous optical sensors receive light transmitted through the sample at 180° from the transmission sensor and light backscattered through the sample at 45° from the backscatter detector. The optical reading starts scanning from the head the length of the sample up to around 55mm. The curves give the flux of transmitted and backscattered light in % relative to the standard suspension of monodisperse spheres and silicone oil as a function of sample height in mm. It could consider the information as the macroscopic footprint of the sample at the given time (Sifontes et al., 2015).

Delta Backscattering

In the TurbiScan it can be define three different phenomena (Table 4), one of this is the cremation by particle migration, average value at the top and bottom of the sample for a quick approximation of the phase separation behaviour.

Case 1 Sedimentation

The backscattering increases at the bottom of the sample due to an increase of the concentration in dispersed phase (sediment) and decreases at the top of the sample due to a decrease of the concentration (clarification).

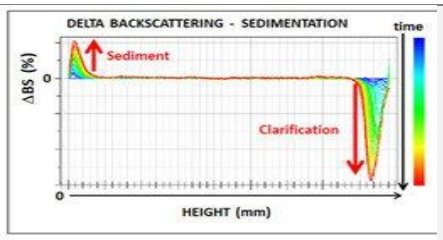
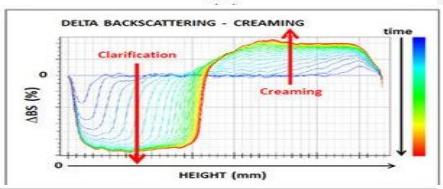
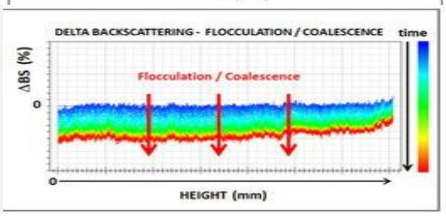
Case 2 Creaming

Very easy that the backscattering flux decreases at the bottom of the sample due to a decrease of the concentration on the particles in this part as clarification and it increases at the top of the sample due to an increase of the concentration of the dispersed phase (creaming).

Case 3 Flocculation or coalescence

It can observe that the backscattering flux decreases at the bottom, middle and upper part of the sample due to a decrease of the concentration of the particles in these part.

Table 4 Delta backscattering, possible variation, and data interpretation

Delta BS	Graph BS Vs Height	Bottom	Middle	Top	Instability phenomena
Case 1		Up	-	Down	Sedimentation
Case 2		Down	-	Up	Creaming
Case 3		-	Down or up	-	Flocculation or coalescence

TSI TurbiScan index

It is a fast, robust, and quantifiable measurement of the stability of the sample in a certain time, the value of TSI can be correlated with a vision observation and to be able to make predictions of the behaviour of the emulsion in time (TurbiScan®, Formulaction, 2022). In simple terms the TSI is a dimensionless number that is the result of summing all occurring destabilization processes in the sample that can be noticed by backscattering or transmission signal intensity along the sample height.

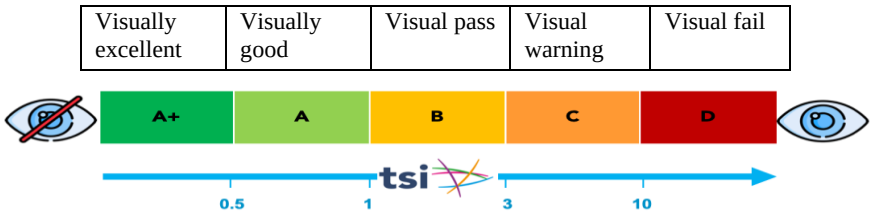


Figure 18 Scale of TSI

The TSI is a time-dependent function, it is important when several samples are analysed and they want to be compared with each other, the analysis must be carried out in the same time interval. Each TSI value corresponds to a state (Figure 18). Therefore, the higher the TSI value, the more unstable the sample will be, that is, the greater risk of destabilization in a given time.

3.2.5. Lactulose Concentration

Lactulose (4-O- β-D-galactopyranosyl-D-fructofuranose) is a disaccharide, which is formed by isomerization product of lactose, in basic media and is also produced during heat treatment of dairy product (Fox & McSweeney, 1998). Nowadays, it is used as an indicator of the severity of the heat treatment, the greater the amount of lactulose present in the analysed samples, the greater the severity of the heat treatment, with the analysis of lactulose it is possible to differentiate between a sample that has been treated UHT at temperatures 135–150 °C for 2–10 s or sterilised milks 110–140 °C for 20–30 min, however by having a sensitive analytical method for its detection, lactulose could be used to differentiate between UHT milks and pasteurized (pasteurization is usually done at 71–74 °C for 15–20 s). The main methods used for the determination are gas and liquid chromatography but also by enzymatic methods (Montilla et al., 2005).

Regarding the values of lactulose in dairy cream, a study by Boitz et al., 2015 evaluated the content of lactulose and other components, as indicators of heat load in samples of whipping cream at the retail in Austria and found that lactulose concentrations were 29 ± 10 mg/L in pasteurized samples, 56 ± 41 mg/L in ESL samples, and 201 ± 24 mg/L in UHT samples (Boitz et al., 2015).

In other investigation of the whipping cream (30-38% fat content) the value were 29 ± 41 mg/L in pasteurized samples, and 57 ± 28 mg/L in heat treated samples, and 56 ± 41 mg/L in ESL samples and the highest value in UHT 195 ± 39 mg/L (Boitz et al., 2016). In addition, it is mentioned the lactulose and β-lactoglobulin concentrations are use as indicator of differences between UHT and sterilised milk, in the European Union (EU) it is maximum 600 mg/L lactulose as an upper limit with a minimum of β-lactoglobulin of 50 mg/L for UHT milk (Boitz et al., 2015. Corzo et al., 1996).

Samples of the 5 different batches of dairy cream produced in the laboratory were sent to an external laboratory Muva Kempten GmbH, in Germany. The samples were collected directly from the filling point in the mini-UHT equipment and packed in 100 mL plastic containers and immediately frozen, to avoid aging of the sample and stop the enzymatic reactions in the dairy cream, also due to density difference, the freezing process facilitates the removal of the solid part of the sample corresponding to the fat globules and thus study the aqueous phase.

Two samples per batch were sent to the laboratory. the analysis was performed by enzymatic route according to 64 LFGB L01.00-31:1988-12 (a), and Muva Kempten GmbH is accredited for this method.

3.2.6. Dairy cream composition, MilkoScan™

With the MilkoScan™ FOSS (Figure 19) analytical solutions for food quality improvement and control analyser, it is allowing to keep track on some parameter of the liquid dairy products, the parameters such as fat, protein, lactose, total solids, solids no fat content, which are important to characterize the raw materials and final product obtained. The equipment has Fourier Transformed Infrared (FTIR) technology, so it is not necessary to handle chemical components or sample handling. High repeatability and precision but a very short time compared to chemical methods (IndiFoss, 2022).

For this study, a MilkoScan^{TM} FOSS belonging to the Rheolab, PDC department, of the Tetra Pak company was used.

Figure 19 MilkoScanTM FOSS, milk and dairy liquid solutions analyser from IndiFoss, 2022

3.3. Food package interaction (FPI)

3.3.1. Puncture Test

The Grip-Engineering machine (Thürmler GmbH) was used belonging to the Surface and material characterization of the Tetra Pak company in Germany.

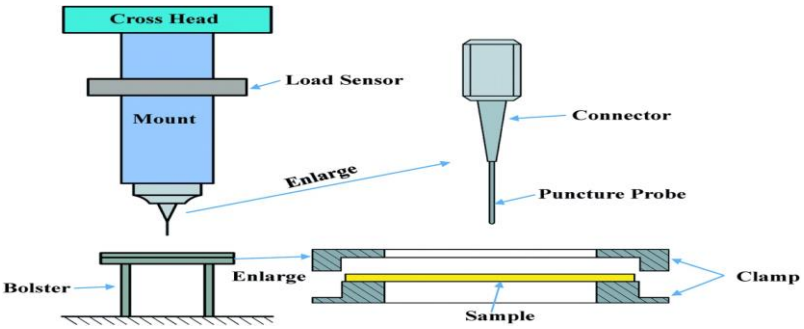


Figure 20 The puncture resistance test device exemplification, author Jinan Horizon Tester

The ESC resistance is evaluated by applying the liquid to the inside layer after fixing it over the hole before measurement. Internal reference tests were used with water and an oil emulsion.

The purpose of this analysis is to evaluate the strength of the material and whether any critical fluid accelerates the distance of maximum elongation of the material (see representation of the method Figure 20). Therefore, the 5 samples of the dairy cream batches were evaluated in this test and compared with neutral agents such as water or aggressive agents such as oil. Results are sampled as tip displacement at failure the mean and standard deviation.

The study of ESC is an important indicator of the performance of plastic materials such as low density polyethylene, unfortunately there are still no methodologies or equipment that allow the determination of ESC in materials in a fast, simple, low cost and reliable way. There are official tests such as ISO 220088-3:2008 and ASTM D543 “Standard practice for evaluating the resistance of plastics to chemical reagents”, tests that are based on the measurement of cracking strength progress, the principle is based on applying a known stress to the polymer under conditions test and expose it to external

environments such as aggressive liquids (detergents, lubricants, vegetable oils, etc); the presence of the ESC agent accelerates the effect of stress on the plastic material until the failure (Cheng et al., 2008).

Therefore, there are other alternative methodologies that are indicative tests of the risk of ESC, as Puncture tests, with a practical approach that correlates the risk of ESC with the parameter of interest is the “elongation at failure”, the distance until reaching material failure and complete deformation. The longest the distance, the greater the resistance of the material and there is less risk of ESC. This test allows the evaluation of polymeric material in contact with food samples and uses water as the reference of a neutral agent and an oil emulsion as a critical agent.

3.3.2. Leakage test: red ink

Dye penetration is a testing method, it is evaluated if there are leaks or possible formation of leaks in the packaging materials in the packages, the principal is that the paper board (outside layer) absorbs the ink if the inside layers (polymer) of the packaging material are damaged. These are put in contact with a red substance and then it is removed and allowed to dry, to then remove the layers of paper board from the multilayer material and locate spots or leak points either in the corners or in critical areas.

In this study, it focused only on the appearance of spots or leak points only in the bottom area. An average of 100 units of Tetra Top® packages that were produced to be used in this study and that were filled with water were analysed, in addition, the test was also carried out for packages with only water, as a result in total there were 6 experiments, 5 with batches of dairy cream and 1 with only water.

The objective of this test is to evaluate the different 5 batches of dairy cream produced in the laboratory, by contacting the Tetra Top® packaging, (simulating machine filling conditions) after a contact time (same time and temperature in all the experiments around 10°C) the dairy cream is removed and then the red-ink test is continued and it is verified if there is the possibility that any batch of dairy cream increases the risk of leaks in the packaging previously producing deliberately “bad” production settings.

Perhaps, some dairy cream batch is highly aggressive with the material and therefore presents a higher percentage of leaks and that this correlates directly and positively with the increase in ESC. Likewise, a "baseline" should be evaluated, in this case it was water, which is not usually considered an aggressive agent and will serve as a base reference.

When the production of packaging was conducted, red ink tests were implemented on the newly produced packaging and it was verified that if there was any presence of spots, even if it was only filled with water; the results were around 8% (1/13 total packages) had spots, a relatively low value consider the machine setting was modified to cause more stress in the package bottles; therefore it was decided to conduct the red ink test instead the blue ink with the dairy cream experiment.

The red ink test is more "aggressive" test than blue ink test. Both blue ink and red ink tests are used to detect holes, cracks in the bottom among others defect in the packages, however, blue ink is used in packages that are chilled packages with shorter shelf life without aluminium foil, and red ink is for the other packages.

3.4. Statistical analysis

Results are presented as the mean with standard deviation. The number of replicates for each experiment was variable, so each analysis specifies how many replicates were performed. For the analysis of particle size distribution and an analysis of variance (ANOVA) was performed and the Tukey test was applied to compare the means with a significance level of $\alpha=0.05$ using the Minitab 19 Statistical Software.

4. Results and Discussion

4.1. Dairy cream

Raw materials:

- Cream as fresh as possible with 40% Fat content
- Skim milk 0,1% from 0,1% Fat content low pasteurized short-life shelf
- Genulacta ® carrageenan type LP-60 0,015%

((see Figure 21)For more information on the formulation, production dates of the batches, and the analysis dates; carrageenan specification see Appendix). After package, the product, and samples they were refrigerated in 4 °C as soon as possible.

4.1.1. Dairy cream preparation

Figure 21 Result of the production tests of the dairy cream product in the accelerated Tetra Pak laboratory. Photos from Alejandra Fernandez Castaneda

Table 5 Setting input in the mini- UHT in the LAB in Tetra Pak

Product inlet	**Settings**
Speed	15%
Flow	20 l/h
Water inlet time production	15 sec
System pressure	4 bar
Heat treatment	
Indirect main Heat	Temperature 130 °C
DSI Direct main Heat	Temperature 130 °C
Pre Heat	50 °C

Cooling	
1st cooling	70°C
2nd cooling	20 °C
3rd cooling	15 °C
Holding time	2 sec
Homogenization	2 sec
Pressure 1st stage	50-20 bar
Pressure 2nd stage	10-20 bar

In a description of the visual appearance of the dairy creams obtained, it can be seen in the Figure 22 of the final product that cream B1 and B5 had bubbles, and circular dots of possible fat globules on the surface, it was also more yellowish, and texture was noticeable.

For samples B3 and B4 in the Figure 22, they were very similar, white, and with a smooth surface, without the appearance of fat globules, however when they were poured, sample B4 was denser, these two batches have in common indirect heat and homogenized but different types of homogenizations. For sample B2, it was slightly more yellow but equally smooth and without fat globules in sight or the presence of air bubbles on the surface, it looked quite smooth and flowed more similar to batches B3 and B4, however B2 was treated by direct heat and also homogenized.

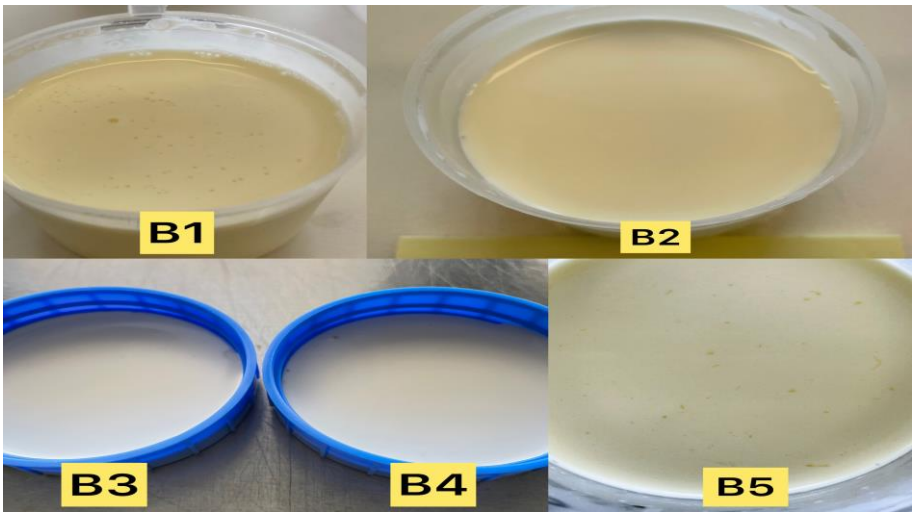


Figure 22 Final product: visual appearance (photos by the author)

From the description of the visual appearance of the samples obtained, it is possible to verify the hypothesis that the cream after being homogenized is whiter, and this is due to the finer dispersion of the fat globules and "thus greater light scattering" (Fox, 2022).

4.2. Analysis results and discussion

The microscopy, particle size distribution, emulsion stability, chemical composition, and viscosity analyses was carried out in two different days, in the case of batch B1, B2, and B5, they were performed the day after the production of the batches. However, the analyses of B3 and B4 were carried out on the third day after production, due to logistics inconvenience. The samples for all the analyses was refrigerated at 4 °C, except for the samples for the lactulose concentration study, which were

immediately frozen after the product was produced in -18. (For more detail in the schedule of the analysis dates see Appendix)

The red ink analysis for batch B1 and B2 were performed on the 5th day after of production of the dairy cream; for batches B3 and B4 they were analysed on the 4th day after the production of the cream. For batch B5, the analysis was performed on the 3rd day after of production. An attempt was made to follow a similar agenda from the day of raw material collection, the day of production of the dairy cream to the days of laboratory analysis, however it was not possible to accurately carry out the analyses on the initial dates proposed, since it is required of the coordination and availability of the laboratories. For future work, it is recommended to take into account the day since the product is produced, because the "aging" of the product could be an external factor that alters or leads to erroneous results (more information section 8)

4.2.1. Microscopy

The results of the microscopy study can be seen in Figure 23 and Figure 24, dye was used for proteins such as light green SF yellowish Certistain®, for this reason the coloration that is observed is due to the presence of proteins. The results generally give a description of the molecular structure and the spatial arrangement of the components in the dairy cream, it can be inferred that the samples B1 and B5 without homogenization look very similar.

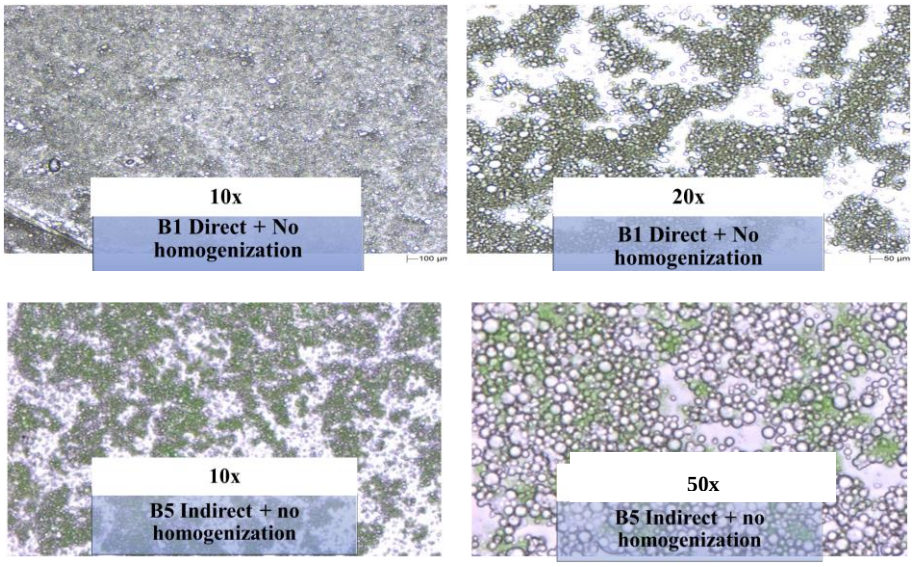


Figure 23 Digital photographs from microscopy, stained for proteins. Samples dairy cream B1 Direct + No Homogenization. B5 Indirect + No Homogenization.

In a close-up with 50X objective in Figure 25, the roundness and spherical shape of the fat globules is visualized, it is not aggregate or partially coalesce to each other in both samples B5 raw mix and B5 after are observed in greater detail.

The spherical shape of the fat globules is a typical property of milk fat due to the insolubility of oil and water, an interfacial tension is produced in the two phases that results in a spherical form, however, the presence of carrageenan must be highlighted in this study, since it will reduce the interfacial tension, then the globules will coalesce or unit more slowly (Fox, 2022).

The principal difference between B5 raw mix and B5 after, is the after processing with heat treatment some portion of the proteins suffered denaturation, likewise, parts of aggregated proteins corresponding to the bluish colour are observed, this could be correlate with some of the microstructural observations and analysis by Matsumiya et al., 2017.

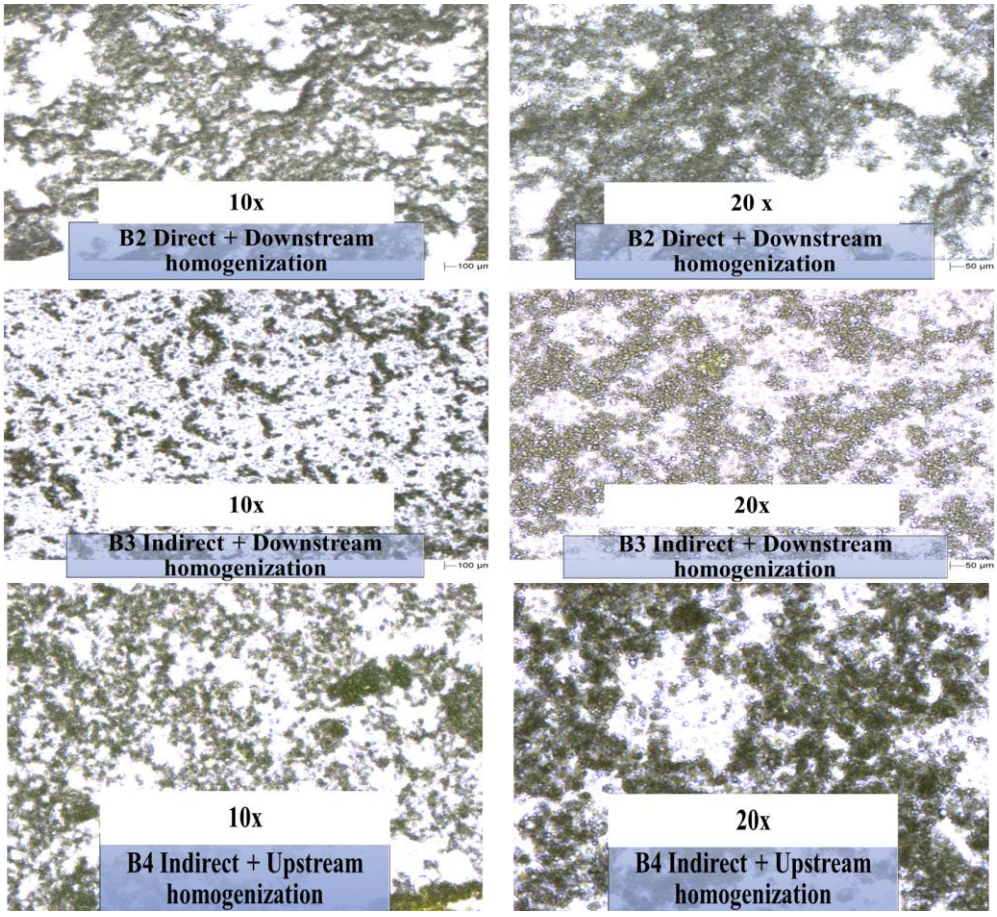


Figure 24 Digital photographs from microscopy, stained for proteins. Batches of dairy B2, B3 and B4 cream with heat treatment and homogenization process in different settings

On the other hand in comparison between B5 and B3 both after processing observing in the Figure 23 and Figure 24, the structure of the molecular components of sample B3 with indirect heat treatment and with downstream homogenization, the fat globules roundness and spherical shape are not clearly visualized due to the homogenization process and on the contrary it is observed in B3 how the aggregates between proteins, and proteins- MFGM "attach or interact", moreover it is observed in the image possibly the coating of the new membrane of the fat globule contains more proteins and this is observable, likewise greater dispersion and homogeneity is observed in the sample, while B5 after the process specific parts are observed agglomerations of green-bluish colour, and around are those round fat globules.

The results from B2, B3 and B4 (Figure 24) could be confirmed with the results found by Cano-Ruiz and Richter, 1997 in their publication "Effect of homogenization pressure on the MFGM proteins" where it was shown that after the milk homogenization process, the new MFGM contained more protein, in fact there was a process of "adsorption of plasma proteins at the fat globule surface", and this directly depends on the heat treatment and homogenization previously carried out, accordingly this description could be visually verified in the images found in this study for the dairy cream, presence of proteins that "hoards or covers" and are more present in the new MFGM.

In general terms, the expected results were found in terms of the morphology of the fat globules, the proteins were visualized with the help of dyes. The dimensions of the particle sizes of the fat globules are confirmed to be bigger in the samples without homogenization than the samples homogenized; moreover, the significant impact of both the heat treatment and homogenization is verified.

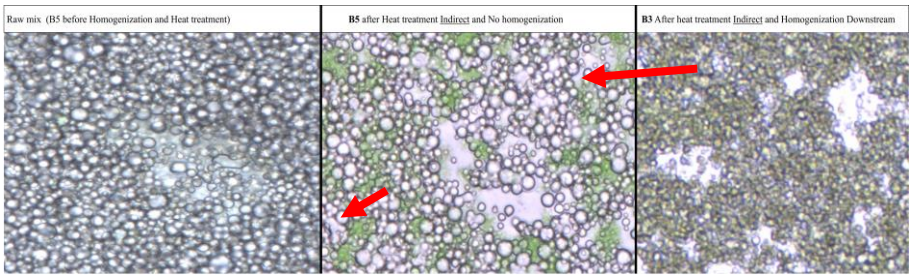


Figure 25 From left to right →, Raw cream mix B5. B5 after processing. B3 After processing

In Figure 25, Compared with the results found in the sample of raw cream mix B5 and B5 after, it is notified the importance of the physical and chemical characterization of the composition of the dairy cream, since apparently in the results found in the MilkoScan composition analyser (results part 4.2.6) the raw cream samples (with the addition of stabilizer) and then the samples after process at the compositional level do not present significant differences, in terms of fat and protein content, however the impacts of the unit operation and the process line that is followed have a significant impact in the molecular structure.

It is confirmed that the samples of dairy cream without homogenization do not have that complete attach with the proteins, therefore it could be stated that in samples without homogenizing the fat globules are more "individual" and perhaps there is a greater risk of ESC when it comes in contact with the PE plastic film; however in the literature it has been confirmed the free fatty acid could have a negative impact when they come into contact with the PE plastic film (Saad et al., 2020) but it has not been mention about the fat globule without homogenization in products with high fat content as the dairy cream.

In conclusion, such a hypothesis could not be affirmed since only the microscopy images are being analysed in this part, and it would be necessary to confirm this with further analysis as diffusion test.

4.2.2. Particle size distribution

Below is show the table 6 with the data results; however, in this study for the statistical analysis we were focus on the results Dx (90) and Mode without Sol A and with Sol A.

Table 6 Results particle size distribution with sol A and without sol A for all the 5 different batches

Sample Name	D [4;3] (µm)	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)	Weighted Residual (%)	Mode (µm)
B1 DNH	2.63	0.03	2.11	6.51	0.54	3.67
B1 DNH SOL A	4.4	1.59	3.77	8.09	0.6	3.8
B2 DDH	2.81	0.03	2.13	6.98	0.5	3.81
B2 DDH SOL A	1.21	0.03	0.92	3.08	0.78	1.96
B3 IDH	1.4	0.02	0.15	4.11	0.76	2.78
B3 IDH SOL A	1.19	0.02	0.94	2.95	0.93	1.86
B4 IUH	3.98	0.03	2.36	11.05	0.54	7.28
B4 IUH SOL A	1.23	0.02	0.61	3.35	0.92	2.14
B5 INH	2.73	0.03	2.57	6.83	0.65	4.01
B5 INH SOL A	4.18	2.05	3.92	6.8	0.7	4.03

The results obtained in the studies of the samples of dairy cream B1 and B5, have similarities in the graphs (Figure 26), especially when sol A is added (blue curves), the first peak on the left side disappears, this did not happen in the analysis of the other batches B2, B3 and B4 (Figure 27).

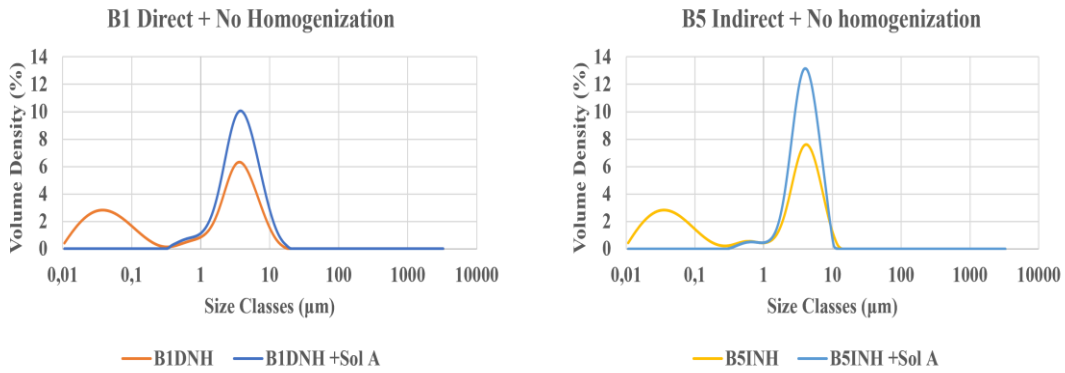


Figure 26 Graphs Volume density % (x axis), Size classes (µm), particle size distribution, B1 and B5with Sol A (blue curves) and without (orange and yellow curve), both batches are heat treated and no homogenized

The results presented in the Figure 26 were to be expected since both batches B1 and B5 are dairy creams without homogenization, fat globules have average particle sizes larger than homogenized creams. In the graphs it is observed how Sol A fulfils the function of dissociating the protein-fat globules aggregates and allows the fat globule to be observed by itself, as well as how Sol A dissociates the casein micelles. It cannot be compared with some data from literature due to mostly the data are reported as a value D [4;3]. Then the comparison it is mostly among the samples prepared in the same equipment, under pre stablish conditions.

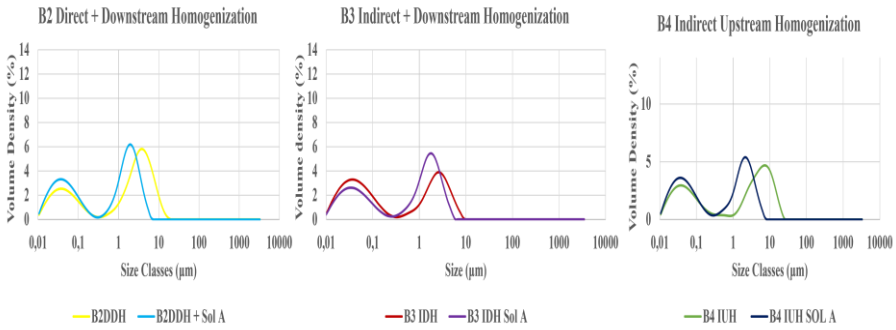


Figure 27 Graph particle size distribution batches B2,B3, and B4 (from left to right) (the blue curves and violet are with Sol A)

The first peak in both graph without Sol A (orange-B1 and golden-B5 curves) on the left side are the casein micelles, then with Sol A these disappears since they are casein micelles due to the action of the EDTA component (present in Sol A) that chelates the ions and forms a complex with the Ca^{2+} ions found in the dairy cream, this produces an imbalance in the solution, therefore the calcium phosphate inside the casein micelles will dissolve, resulting in a dissociation of the casein micelles, however EDTA will not act on the protein aggregates that are linked by the sulphur bridges (Malvern Panalytical, 2021).

Moreover, regarding the size of the casein micelles, the value recorded in the results in the graph corresponds to what is theoretically known, the size is on average between 0.15 µm but its size can range from 0.05 µm to 0.6 µm (Glantz et al., 2010). Then the second peak in B1 and B5 without sol A corresponds to the fat globules with average size between 1 and 10 µm (Walstra et al., 2006), no peaks after these to the right side are observed, therefore there was no presence of air bubbles or interferences.

However, In the PSD analysis in batch B1 when Sol A was added to sample, a small peak was observed that extend up to 100 µm (more detail see Appendix), which was very unusual and difficult to explain the reason for this peak, since the dimensions would not be considered bubbles of air, in this case the peak was like a continuation of what would be the fat globule measurement. The most typical expected would be a behaviour like that observed in sample B5 with Sol A that there are no peaks to the right side after the peak due to fat globules. For this reason, it was decided to modify the results of B1, and a "cut" was made to the graph, eliminating the presence of this peak, which was defined as a contaminating external material.

The Dx (90) and Mode value of the particle size in B1 (Table 6) increased with sol A, possibly because the elimination of the smallest particles such as proteins was achieved, therefore the average value by not taking into account these small particles tend to be larger than the average value.

Regarding the data found in the samples of B2, B3 and B4, the presence of two peaks is observed in the graphs (Figure 27) of No Sol A and with Sol A, it could be affirmed that the homogenization process and in combination the heat treatment produces stronger protein bond and stronger interaction between the casein micelles and the fat globules, as well as the decrease in the particle size of the fat globules would need greater participation by proteins for the reconstruction of the new membrane of the fat globule of dairy cream, for this reason despite Sol A is added, the results still show the first peak corresponding to the casein micelles; this statement could be supported by the results reported by Wang et al., (2019) in a study of the changes in the fat globule membrane protein components due to different homogenization conditions, it was reported that when the milk is homogenized, the milk proteins are more involved in the "reconstruction" of the new fat globule membrane. As well as the protein load in the new fat globule membrane increases considerably after homogenization because the particle size of the fat globule is reduced, the surface area increases, and more proteins are needed for the new formation of the fat globule.

Therefore, if there are more proteins, in combination to the heat treatment would have disulfide bonds, confirming the theory that some proteins interact and aggregate with proteins through disulfide bonds. In some studies, in heat treatment milk, it has been confirmed the aggregation of heat-denaturable whey proteins with κ-casein via disulfide bonds (Lowe et al., 2004).

Perhaps, these disulfide bonds exist in the dairy cream, then Sol A will not be able to break these bonds since it has no influence on the disulfide bonds. In conclusion, possibly the heat treatment and the homogenization process in combination, increased the disulphide bonds and for this reason Sol A could not dissociate the casein micelles and these still appear in the particle size measurements, present as the first peak of the graph (Figure 27)

In addition, there is also the present of the stabilizer carrageenan, which would help with the ionic strength of these bonds ($Ca2^{+}$ and carrageenan). In conclusion, the main difference between B1-B5 compared to B2, B3 and B4 was the homogenization process, which was expected, moreover it is still appear the first peak in the samples with heat treatment and homogenization then both factor have a significant impact in the results of the PSD.

However, when Raw mix cream samples were analysed (before processing the sample) it was also found that the first peak continued to appear even with the addition of Sol A and even if the sample was neither homogenized nor heat treated (Figure 28).

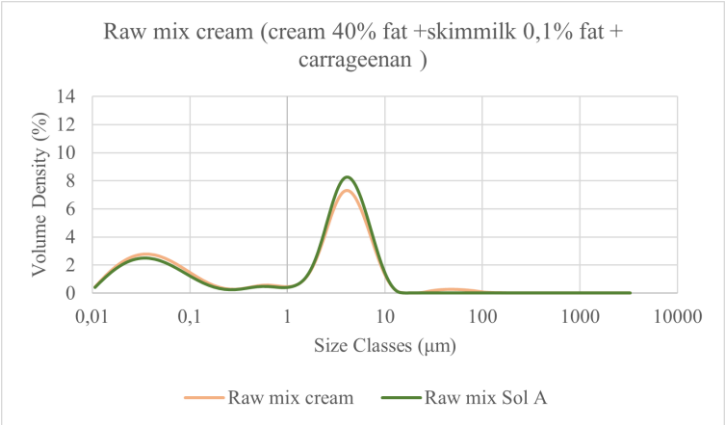


Figure 28 Raw mix cream without heat treatment neither homogenized

However, sol A fulfilled the function of dissociating the fat aggregates and for this reason the measurements in the Dx (90) and Mode value decrease with sol A for B2, B3 and B4 (Table 6). Thus, when the fat droplet size decreases after addition of Sol A this means that there where flocculates in the original cream.

In summary, in interviews with experts in the management of the Mastersizer equipment, from the Rheolab laboratory from Tetra Pak, it was revealed that the same thing happened in samples of raw milk, the graph obtained was similar to the graphs in Figure 27 and Figure 28, since the first peak did not disappear even after applying sol A, and even if the sample has not been heat treated and without stabilizer.

In conclusion, there is not enough evidence to prove that the appearance of two peaks in the samples with Sol A in samples B2, B3 and B4 is due to a consequence of the effect of the homogenization process, since these two peaks were also found in samples of raw mix cream (40% fat cream and 0.1% fat skimmed milk and carrageenan), as well as in other studies carried out in Tetra Pak as in samples with raw milk

Continuing with the analysis of the results, a study of the means of the tests was carried out, each PSD analysis was carried out in six duplicates, the data is shown in Table 7, an ANOVA and Tukey test were carried out. Tukey's HSD is a multiple comparison technique that tests the null hypothesis that two means are equal.

In this case, from the P value, the graphs (Figure 29) and the Table 8 shown by the Minitab 19 software, it was found that only the mean of the samples of B2 and B5 in Dx (90) value without Sol A it is not a statistically significant difference, this it is observed in Table 8 and confirmed in the Tukey. For which all the means of the samples of the other batches with sol A and without sol A for values of Dx (90) and Mode there is a significant statistical difference. The summary of the statistical analysis of PSD can be seen in greater detail in Appendix).

Table 7 Results from Particle Size Analyzer Expert Instrumentation, of the particle size distribution for value of Dx (90) and Mode for all the batches of dairy cream with Sol A and without sol A

Samples	Dx (90) (µm)	StDev	Mode (µm)	StDev
B1 DNH	6.51	0.19	3.67	0.03
B2 DDH	6.98	0.05	3.81	0.01
B3 IDH	4.11	0.02	2.78	0.01
B4 IUH	11.05	0.15	7.28	0.16
B5 INH	6.83	0.33	4.01	0.04
B1 DNH SOL A	8.09	0.00	3.80	0.00
B2 DDH SOL A	3.08	0.02	1.96	0.03
B3 IDH SOL A	2.95	0.03	1.86	0.01
B4 IUH SOL A	3.35	0.01	2.14	0.00
B5 INH SOL A	6.80	0.07	4.03	0.01

Table 8 Tukey test multiple comparison, for value Dx (90) without Sol A

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping			
B4 IUH	6	11.05	A			
B2 DDH	6	6.9817		B		
B5 INH	6	6.832		B		
B1 DNH	6	6.51			C	
B3 IDH	6	4.10667				D

Means that do not share a letter are significantly different.

However, although there is no statistically significant difference between the Dx value (90) between B2 and B5 (Table 8 and Figure 29), it could only be stated that the value found in B5 probably depends only on the intact fat globule since there was no homogenization, on the contrary, the value without Sol A of B2 depends is as a result of the fact the agglomeration between particle-particle since this sample was homogenized in downstream, and also when Sol A is added, the value decreases drastically, thus confirming that the fat globule is much smaller.

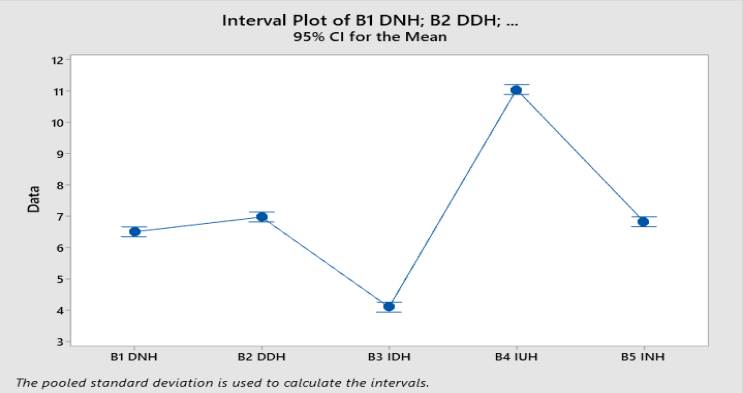


Figure 29 Graph of data for value Dx (90) (y- axis) all the batches of dairy cream without sol A.

In conclusion, although B2 and B5 had similar average particle sizes and no statistical difference, the deeper analysis would show that there is a significant difference between the two batches, since they do not behave exactly the same in all the analyses carried out, especially in microscopy and the graph of the PSD study, where it can see clear differences in the shapes of the fat globule and the participation of protein complexes.

4.2.3. Rheology: viscosity

Below in Table 9, are the results of the viscosity measurements at two temperatures previously established at 6°C and 20°C. The data is presented as the shear rate of 100 reciprocal ($100s^{-1}$) units, and the viscosity in (mPa·s) was calculated using the best-fit-power-law-equation, then with the software RheoCompass® it was applying the "regression" and calculate the viscosity at that shear rate from the n and K values extracted from the shear sweep in the equipment. The complete data table of the viscosity study is found in

Appendix , information on shear rate, shear stress, viscosity, temperature of each measurement point, torque, status, and time can be found for each batch of dairy cream in intervals 3 and 6 corresponding to temperatures of 6 °C and 20 °C, respectively.

Table 9 Viscosity results with rheometer Anton Paar

Sample	Viscosity (mPa·s) 100-s shear rate	
	6.0 °C	20 °C
B1 DNH	25.5	16.4
B2 DDH	59.5	35.2
B3 IDH	92.2	56.2
B4 IUH	241.6	231.1
B5 INH	31.4	19.4
C1 36% fat	35.3	22.5
D1Lactose-free 35% fat	49.4	37.1

The highest viscosity was recorded in the batch sample B4 Indirect heat treatment and Upstream homogenization (results Table 9), in addition to the 5 batches of dairy cream produced, the viscosity of two samples (C1 and D1) purchased at the Lund supermarket was also studied.

The viscosity profile of sample B4 could be explained by making correspondences with other data found in this investigation; in the PSD study, sample B4 showed a particle size Dx (90) without Sol A on average 11.05 μm and with Sol A 3.35 μm (Table 6), when the other samples (with heat treatment and homogenization) B2 and B3 in size Dx (90) without Sol A on average 3.3 μm and with Sol A 1.91 μm, denoting that there were big aggregations of fat globules in B4, and larger particles aggregated together, then Sol A solution was able to disperse them. Consequently, more aggregated in the dairy cream will increase the viscosity.

The value without Sol A could denote that the sample had large agglomerations, consequently this could explain the reason for the high viscosity found in sample B4, as explained by Blankart et al., 2022 in their most recent article on the study of the homogenization process in creams, stating that the agglomeration in creams after being homogenized is the cause of the increase in viscosity, due to the resistance that the previously agglomerated fat globules have when they are subjected to shear forces; in this same study it was also found that the homogenization process decreases the diameter of the fat globules and this produces an increase in the interfacial area and leads to a greater interaction between fat globules and the other particles, which relates with an increase in viscosity due to increased particle-particle interactions.

With regard to the other samples analysed, the samples with the lowest viscosity were the samples without homogenization, which is to be expected since the fat globules are individually and tend to agglomerate less. The heat treatment seems not to be difference between direct and indirect regarding to the viscosity in B1-B5. However, indirect heat tends to produce slightly higher viscosity dairy creams than direct heat comparing B1 with B5 and B2 with B3.

Supermarket samples were analysed to have a benchmark of what is currently on the local Swedish market. However, only one sample was analysed per brand and no affirmations could be made.

It is important to mention that the results of the viscosity measurement for sample B4 are strange and it is difficult to affirm that the measurement is 100% correct, because the viscosity is very high compared to the other samples. However, the initial hypothesis is that the dairy cream with indirect heat treatment in combination with upstream homogenization would have higher viscosity, mainly due to the effect of the homogenization process before the heat treatment. But the results from B4 are outliers and for this range of viscosities it is recommended to use another geometry of the cylinder. Therefore, the result B4 could be considered an error, and it is something to take into account in future investigations.

Correlation Viscosity and Lactulose content

Correlation was found in the value of the content of lactulose found in sample B4 (Table 10), since it was the highest in B4 with 123 mg/kg compared to the other 4 batches studied, this high value of lactulose is an indicator of the heat treatment load, therefore batch B4 suffered more heat load; then it is predictable that sample B4 has higher viscosity due to the effect of heat treatment and protein denaturation, confirming the theory that whey proteins and caseins are modified with high heat loads and these are responsible for the increase in viscosity. Accordingly, the proteins have a significant influence on the physicochemical characteristics of the product, which in turn has an impact on the appearance, colour, mouthfeel, texture, and subsequent rheological properties such as thickening (Morison et al., 2013).

It was confirmed for sample B3 with a viscosity value of 92.2 mPa·s and 56.2 mPa·s at 6.0 °C and 20 °C, respectively, being the second highest in viscosity, likewise the second highest value in lactulose

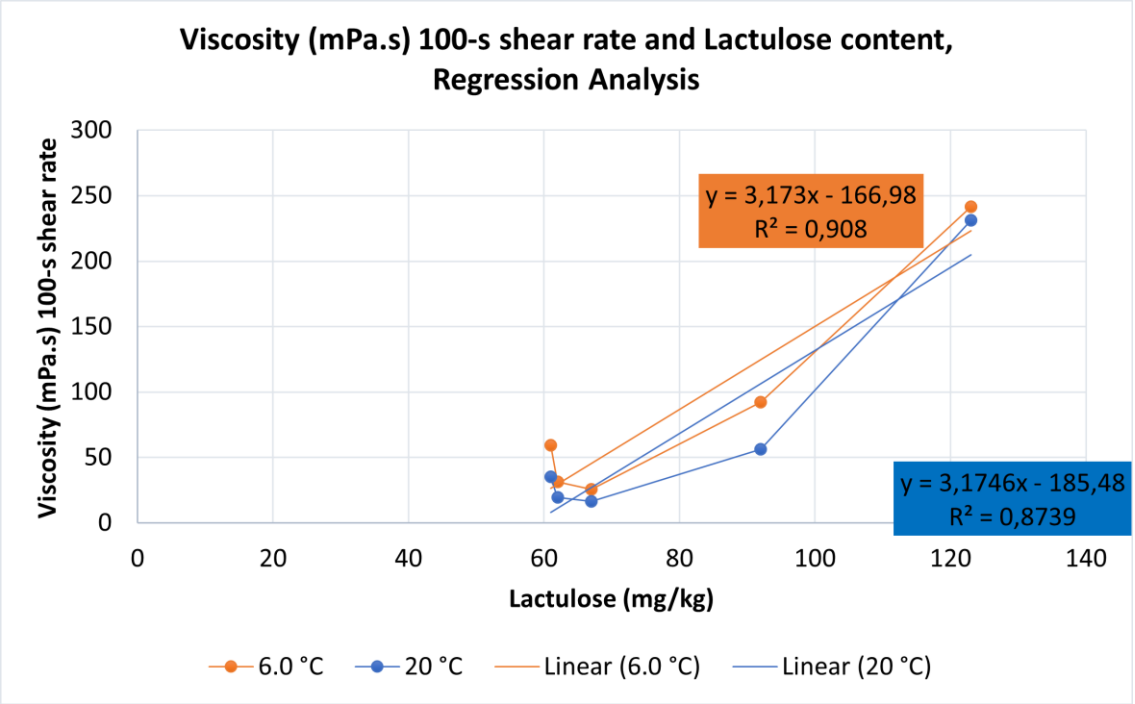


Figure 30 Fitted line plot of Viscosity in two temperatures of 6.0 °C and 20 °C, vs Lactulose. R-sq can be visualized

concentration with a value of 92 mg/kg. Therefore, a regression analysis was applied to verify the R-squared and the correlation between the lactulose concentration of the samples and the viscosity, a high coefficient of determination was found, because the R-squared was greater than 0.8 (see Figure 30).

In this case, the response variable would be viscosity and the predictor variable would be determined by the lactulose concentration of the sample; however, the lactulose concentration is determined by the heat treatment load during processing in the mini-UHT. The heat treatment could not be the predictor variable since the variables “Temperature” and “Holding time” were constant during the production of all batches of dairy cream (high uncertainty in real industrial scale processing line), therefore direct or indirect heat transfer have a different and significant effect on the samples, which will be explained in more detail in the results section of lactulose concentration. (More detail about the Regression results viscosity with Lactulose concentration from Minitab19 is in Appendix).

Viscosity and temperature

Regarding the other viscosity values, it was found that the lowest viscosities were for batches B5 and B1 (figure 31), both batches without homogenization. Viscosity also seemed to have an inversely proportional relationship with temperature, the higher the temperature, the lower the viscosity, which explains probably that at higher temperatures there is greater thermal energy in the particles, and they overcome the forces of attraction that keep them (Matsumiya, et al., 2017).

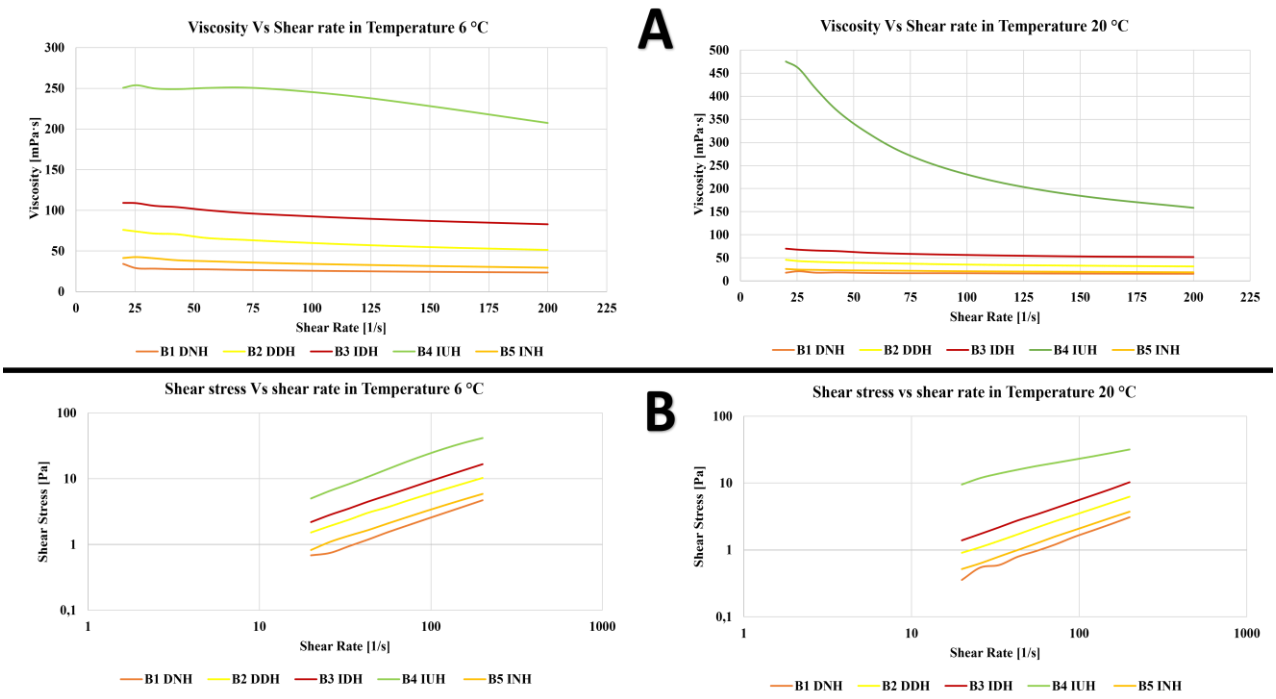


Figure 31 A. Viscosity (mPa.s) profile in temperature 6 °C and 20 °C depend on shear rate (1/s) (x-axis). B Shear stress (Pa) in temperature 6°C and 20°C depend on shear rate (1/s) (x-axis) (same colour for the batch in both graph A and B)

In addition, from the analysis of the graph in figure 31 it can be seen that in the viscosity data (A) and in Shear stress (B) dependent on the Shear rate, there are probably no significant differences in the samples of batches B1 and B5, even B2, which is possibly related to the results of no significant difference in the particle size of the samples between B5 and B2, since the particle size, the spatial arrangement and the molecular structure of the fat globule have a direct impact in the viscosity of the cream.

Despite the fact that the dairy cream elaboration process was carried out on a small scale, with a production volume of 20 Liters, under laboratory conditions, it can be observed that part of the results denote the influence of the heat treatment processes and homogenization in the “response variables” as viscosity, PSD, microscopy and lactulose concentration; this is similar to what was reported from a study published Matsumiya et al., 2017, the study of heat treatment and homogenization on fat globules and proteins in whipping creams, specifically in the viscosity results comparing samples made on a laboratory scale with samples of commercial products produced under industrial scale conditions, as a final result it was suggested that the small-scale samples imitated very well the samples produced on a large scale, regarding viscosity and PSD results, in this study the samples of dairy cream were elaborated in a plate heat exchanger FT74, Armfield Ltd, UK and an APV LAB1000 SMT Co. Japan homogenizer. Taking into the consideration the data from the samples analysed from the local supermarket Table 9.

4.2.4. Emulsion stability

The stability analysis of the five batches and two samples of commercial dairy cream was carried out as a variation of the backscattering profiles (delta BS) depending on the time and measured in the tube the height is visualize in the X-axis. In this case, only the backscatter data corresponding Figure 32 is analysed, where the profile of the 7 samples (5 samples from dairy produced and 2 commercial samples

from the local supermarket) is observed at the same time determined one hour and 11 minutes after starting the analysis.

The time was established by the author of the research, because one hour could be enough time to appreciate the changes in the emulsion at room temperature controlled and stable).

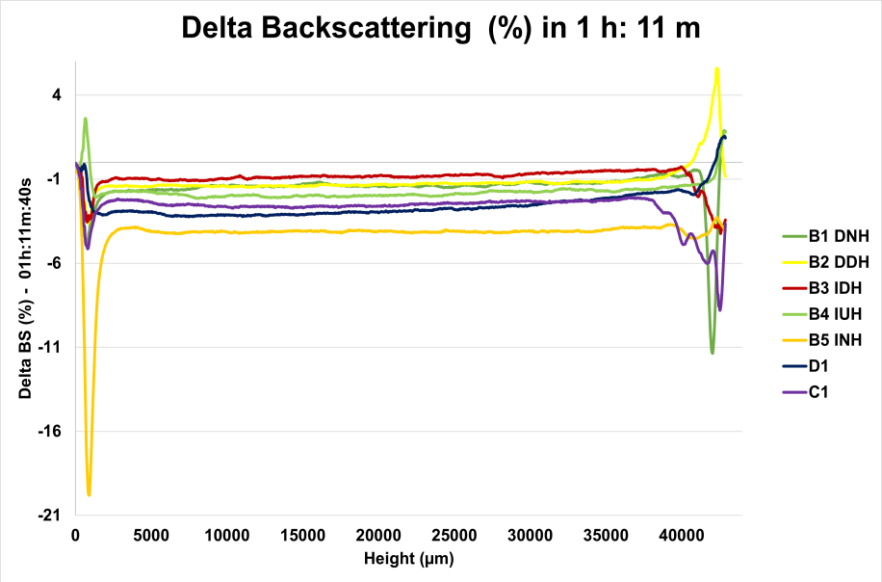


Figure 32 Delta backscattering profiles vs Height, data was reported as a function of time in 01 h:11m:40 s after the first measurement in the TurbiScan

Backscattering measurement occurs as a function of time and particle migration, that is why there may be positive peaks (corresponding to increased backscattering) for example sample B2 in top of the tube (between 35000 and 40000 µm would be the top of the tube).

Negative peaks (corresponding to decreased backscattering), as an example, the profile for batch B5 (indirect heat and without homogenization) of the dairy cream, a progressive decrease in the concentration is observed at the bottom of the sample (x-axis is the height of the tube, between 0 and 5000 µm would be the bottom of the tube), there is a decrease in the backscatter signal, a negative peak. (More information on the graphs of Delta backscattering vs. height as a function of time of the 7 samples analysed can be found in Appendix)

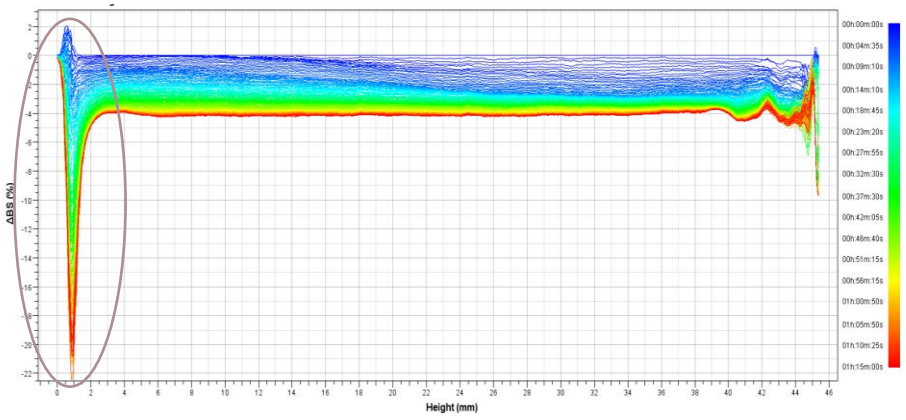


Figure 33 Delta backscattering profile sample batch B5 Indirect + No homogenization, data was reported as a function of time from 0 s until 1 h:15m, in the TurbiScan

The complete graph of delta backscattering vs height (Figure 33) gives information on what is happening at the molecular level in the entire sample, therefore it is easier to analyse a specific part of the tube. The TurbiScan software itself offers the options to analyse to detail the bottom part, the middle part, and the top part. Regarding these parts, some authors such as Celia et al., 2009 affirm that there is no significant variation in the size of the particles when the backscattering profile is within the interval ± 2%. However, when the variations are greater than ±10% (negative or positive peaks), they are an example of an unstable formula.

Celia et al., 2009 also affirm that phenomena such as sedimentation and creaming are reversible but particle size variation such as coalescence is usually irreversible. It must also be considered that an increase in the size of the particles due to cases of coalescence leads to a decrease in the backscattering profile. This could explain what happens in the middle part of the tube in the example of the results in B5 (Figure 34) in the middle part, probably coalescence phenomena are observed.

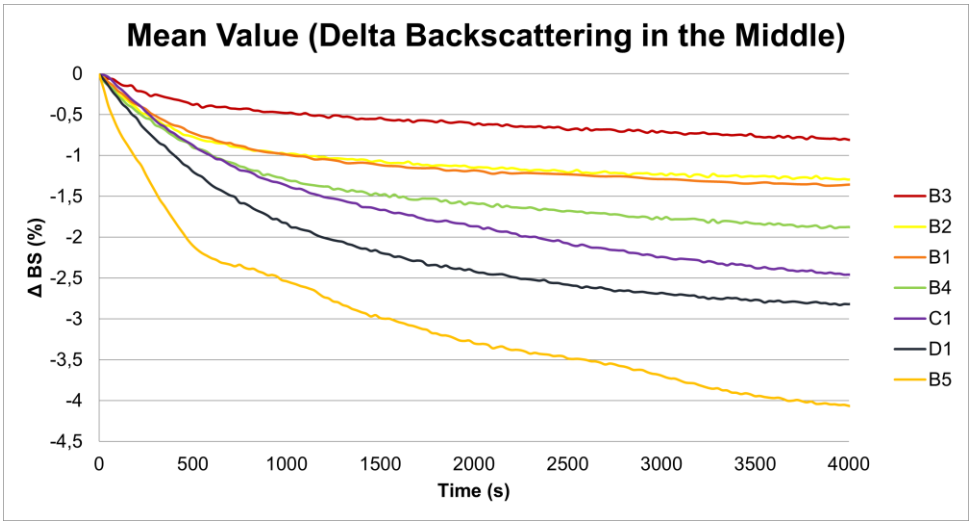


Figure 34 Mean Value in the Middle of the tube using for the Turbiscan analysis, all the samples were analysed included two samples from the local supermarket

Figure 33 and Figure 34 and shows how the greatest destabilization change occurs in sample B5 indirect heat + non-homogenization, it can also be seen how B1 and B2 seem to be quite close throughout the study time, which is against all odds since the hypothesis suggested that the samples without homogenization would be the most unstable samples and B1 was non-homogenization and B2 was direct heat treatment + downstream homogenized, and therefore with high variation in the delta backscattering study.

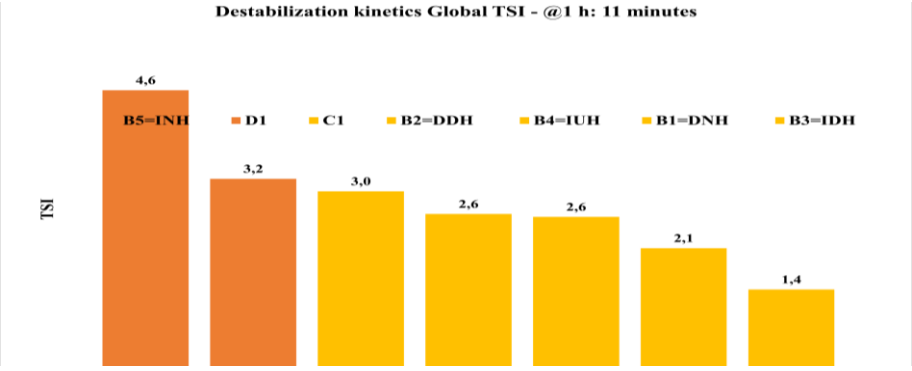


Figure 35 TurbiScan Stability Index (TSI) is a parameter to easily rank the stability of the dairy cream and samples from supermarket, doing by a data table and the TSI values were associated with a colour that allows for direct analysis and sample validation

However, apparently only B5 was the sample with the greatest instability, and on the contrary B3 indirect heat + downstream homogenization it was the most stable sample with quite stable delta backscattering values in the middle part and without many variations over time.

According to the TSI scale (Figure 35), it can be affirmed that there is no significant difference between batches B1, B2, B3 and B4 since they are all less than 3, therefore they are categorized as "visual pass" in the visual scale with the letters “The variations detected are higher than the “early” stage, correspond to the beginning of the destabilization, however, the destabilizations remain non-visual in most cases (>90%)”.

However, samples B5 and D1 and C1 have values ≥ 3, so they are categorized as “visual warning” meaning the destabilization is due to sedimentation or creaming regarding to the variation in the particle sizes and it is starting the phase separation (TurbiScan®, Formulaction, 2022).

4.2.5. Lactulose concentration

All the results can be seen in (Table 10), the highest content of lactulose can be observed in sample B4 with 123 mg/kg corresponding to the cream with indirect heat + Upstream homogenization, the lowest value was for B2 indirect heat + Downstream homogenization.

In general, no major difference is observed between batches B1, B2, B3 and B5, and even the values are in the low range, however when the 5 batches produced are analysed, it is found that B4 has a fairly high value in comparison, almost the double of B1 B2 and B5.

Table 10 Results of lactulose concentration in cream samples

Lactulose in mg/kg	
Batch	**Results**
B1 DNH	67
B2 DDH	61
B3 IDH	92
B4 IUH	123
B5 INH	62

It can be noted that the samples treated with indirect heat and homogenization B3 and B4 were the samples that have the highest value of lactulose concentration, however batch B5 does not have a high value and is not significantly different from B1 and B2, possibly because B5 only has heat treatment by indirect heat and does not have homogenization, since it could be considered that the homogenization process occurs with preheating at 75-80 °C, then this could increase the total heat load, however it is not high enough to start the formation of lactulose.

There is a difference between the heat transfer process between samples B2 and B3, both have downstream homogenization under the similar process conditions, however the main difference is the heat transfer. Which confirms the hypothesis that the heat transfer through the indirect process, it exposes the product to a greater heat load, since it takes more time to reach the heating point compared to direct heat, as an example the Figure 4, of the behaviour in milk, considering that for cream it has difference due to the physical chemical properties of each product, however it is a good illustration to try to understand the mechanism of the heat transfer.

Regarding the homogenization process, sample B4 was first homogenized and then continued to the process of indirect heat treatment; performing homogenization first (upstream) this reduced the size of

the fat globules, increase the surface area of the globules, increase the amount of small new fat globules, there is greater absorption of casein on the surface of the new MFGM, and consequently all this increases the viscosity of the cream, This would be confirmed by the hypothesis of the consequences of upstream homogenization and heat treatment processes (Calvo et al., 2022).

The processing conditions in the heat transfer will also vary in a direct proportion with the cream viscosity after being homogenized, it has therefore an increase in the time of the complete heat treatment cycle, where the sample would suffer more heat load which have been seen in the lactulose concentration in B4. Which agrees with the literature that viscosity affects the heat transfer coefficient, a liquid with higher viscosity will increase the thermal resistance in the heat transfer process, resulting in lower heat transfer coefficients (Gonçalves et al., 2017).

In the heat treatment, chemical changes happen and changes in the macrostructure also follow, corresponding with the data from the literature where have been reported in milk, at temperatures around 80 °C increases the incorporation of whey proteins, especially β-lactoglobulin (β-Lg) in MFMF, which increases the content. total membrane proteins. In addition, heating also has an impact on the free sulfhydryl groups in the MFGM and an increase in disulphide bonds, possibly in the bonds between β-Lg with membrane protein (Lee and Sherbon, 2002). Finally, the heat treatment process in combination with homogenization has significant consequences on the final dairy cream product.

4.2.6. Dairy cream composition, MilkoScanTM

Five batches of dairy cream were analysed before and after processing. The results are presented as the average value of two measurements that the equipment performs and the standard deviation in table 11.

The raw samples correspond to the mixture of pasteurized 40% fat cream, 0.1% fat skimmed milk and 0.015% carrageenan well mixed, and then after processing all the samples with heat treatment and in combination with homogenization processes for samples B2, B3 and B4. It should be noted that the MilkoScan instrument was not calibrated for cream samples in total solids (%), non-fat solids (%), and lactulose (%). Neither is the equipment calibrated for samples of cream with stabilizer. For this reason, the samples were studied before and after processing. Factors to consider as possible sources of error.

Table 11 MilkoScan result composition

Sample	Fat (%)	Protein (%)
Raw mix B1	33.34 ± 0.12	2.22 ± 0.01
B1 DNH	33.32 ± 0.16	2.19 ± 0.01
Raw mix B2	33.38± 0.16	2.22 ± 0.00
B2 DDH	32.97 ± 0.00	2.16 ± 0.01
Raw mix B3	33.34 ± 0.12	2.22 ± 0.00
B3 IDH	33.74 ± 0.03	2.16 ± 0.00
Raw mix B4	35.51 ± 0.04	2.26 ± 0.00
B4 IUH	35.19 ± 0.00	2.20 ± 0.00
Raw mix B5	35.09 ± 0.11	2.21 ± 0.00
B5 INH	35.26 ± 0.01	2.21 ± 0.00

It can also be observed that there are no great differences in terms of the composition of the raw cream before being processed and after being processed, since the values of fat content, for example, increased slightly (B3 and B5) but decreased a little in other samples (B1, B2 and B4), the complete analysis is in the Appendix however in general terms these variations can be predictable due to the high degree of confidence with which the samples are analysed, however they were only in duplicate per sample . In conclusion, it is considered that there were no modifications or significant changes between the samples before being processed and after being processed.

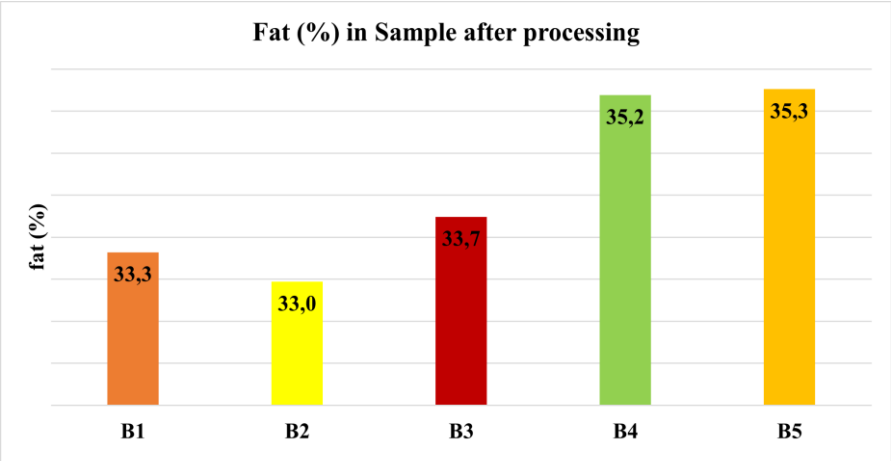


Figure 36 fat (%) in the samples of the dairy cream after processing in mini-UHT. B4 and B5 have no statistically significant difference, however B1, B2 and B3 have statistically significant difference

The data was analysed in the statistical software using one-way ANOVA, in addition to the Tukey test, differences in the means of the samples after being processed in mini-UHT, significant differences were found in the content of fat (%), B1, B2 and B3 are statistically different from samples B4 and B5, therefore B4 and B5 have no statistically significant difference (Figure 36) for more information in the ANOVA and Tukey test in the fat (%) see Appendix. Regarding the protein content, batch B2 presented the lowest value of 2,16% protein and was significantly different from the other batches.

In general, it could be affirmed from the analysis of the raw samples and after processing that the mini-UHT equipment produces products maintaining the integrity of the product, since there were no dilutions or concentration of the product.

4.3. ESC Indicative test of food package interaction

4.3.1. Puncture test

Bellow Table 12 shows the values of tip displacement (mm) in the package mat made in the Stuttgart laboratory, Tetra Pak.

Table 12 Data results puncture test for the 5 different dairy cream batches and the Oil (aggressive) and Water (neutral)

Tip displacement at failure (mm)		
Batch	**Average**	**STD**
B1 DNH	10	0.6
B2 DDH	9.7	1.4
B3 IDH	10.4	0.7
B4 IUH	10.1	0.6
B5 INH	10.1	0.5
Oil	12	1.4
Water	17.6	1.2

From previous studies carried out in Stuttgart with different dairy cream products with variable fat content, the puncture test was studied on the same package material specification, and the sensitivity of the different products was compared as a function of fat content. The main conclusion was that the increase in fat content produced a tip displacement response with low values, that is, a higher risk of breakage, so there was not enough elongation. Same supplier more fat more risk.

The in resume it was found in the previous investigation that dairy product from different supplier and same fat content present different risk of ESC. However, significant differences were found when two samples from same supplier, but different production batches present two different results in the puncture test, then two different risk of ESC were studied.

Figure 37 shows the samples of the 5 batches of cream and other additional samples from previous studies for comparison. From left to right, the value of the tip displacement at failure is observed in mm, water has the highest value of tip displacement, which is interpreted as a high elongation capacity of the material before breaking, then oil emulsion with a value of 12 mm, after the dairy creams are observed, the values are between 10.4 and 9.7 mm, no significant differences were found between the samples of the batches prepared for this investigation.

Then the results found in previous studies were added for comparison, Sample A (S-A) samples from left to right lactose-free (LF) with 36% fat content is S-A1, with 40% fat content is S-A2 and with 36% fat content is S-S3 light orange colours. Next a Sample S (S-S1) 35% fat content (dark blue), followed by Sample C (S-C) 36% fat content and finally another Sample S (S-S2) LF 35% fat content, but from another production batch (Then sample S (S-S) are come from the same producer but in two different batches, and samples S-A are come from the same producer but with different fat content or lactose free).

From the batches of dairy cream produced in this experiment B2 (direct + downstream) has the lowest value with 9.7 mm and B3 (indirect + downstream) 10.4 mm the highest, however there is no significant difference in the impact between the five cream samples.

It is concluded that the dairy cream samples have lower values compared to the oil emulsion. The oil is used as an "aggressive" agent that increases the risk of ESC. This result suggests that also these cream samples (B1, B2, B3, B4 and B5) are critical with respect to ESC in the bottom formation.

However, with the results obtained in this test, there is not enough information on the reasons to understand why the dairy cream, independent of the unit operations applied in the process line, shows to be an aggressive agent, because the data found were very similar among the batches of dairy cream. Nevertheless, it is observed that the 5 samples of creams prepared with different configurations on a laboratory scale using the mini-UHT seem to be less critical than the samples of S-S1, S-S2 and S-C. The higher the number of tip displacement at failure, the lower the risk of ESC.

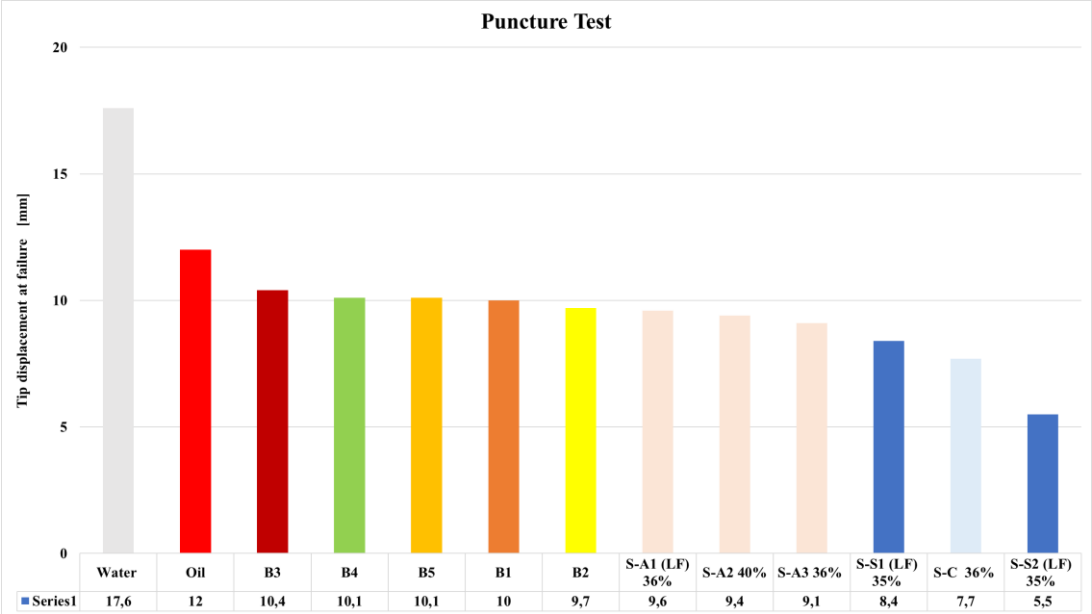


Figure 37 The tip displacement at failure in (mm) obtained for the inner layer plastic, together with the results from the previous research, as well as water, and the oil emulsion.

In conclusion, this test is only an indicator, which must be correlated with other tests in order to reach more robust conclusions and define which factors in the food samples are the ones that increase the risk of ESC, it is relevant test, but it needs to be complemented with more indicative test to have a complete overview of the critical product.

It should be noted that this test, similar to the official tests mentioned above, are not reliable enough for this study, since the problem in this case is when there are leaks in the packages that have previously been subjected to bending, folds, cuts, and modifications and after coming into contact with the food product and leaks are visualized.

All these stress conditions in dubbing and other factors are not part of the official ESC tests, since in these tests only a very small part of the material has not been subjected to all these prior stresses. In addition, only the layer of the polymer material is evaluated, and in the multilayer Tetra Top packaging there are other materials that will also have a significant influence on this type of problem.

4.3.2. Leakage test: red ink

To understand these results, it should be mentioned that the packages used in this study were manufactured with the purpose of being used for this evaluation with the different 5 batches of dairy cream produced in the laboratory.

These packages, as mentioned in the methodology, were made by making modifications to the packaging machine (bad settings), with modifications that would increase the physical stress on the package in order to later obtain scalable results.

Table 13 Leakage test results of TT packages and description

Sample	Specification	Crack in the bottom	Corner cracks	Leakages	Total packages	% Cracks in bottom	Description
B1 DNH	Direct+ no homogenization	14	5	0	117	12.0	Large dot that can be seen from the outside of the package
B2 DDH	Direct+ downstream homogenization	4	3	0	103	3.9	Very small and fuzzy spots and barely visible after removing the outer cardboard layer of the package
B3 IDH	Indirect+ downstream homogenization	12	1	0	113	10.6	Very small dots, difficult to visualize so it was necessary to remove a lot of cardboard material
B4 IUH	Indirect+ upstream homogenization	6	1	4	113	5.3	Very small dots, difficult to visualize, it was necessary to remove a lot of cardboard material
B5 INH	Indirect + no homogenization	8	0	4	102	7.8	Large dot that can be seen from the outside of the package

In Table 13 shows the results of the tests performed on the packages, each test about 100 packages were analysed.

In summary, batch B1 presented the highest number of red-ink with 12%, the description of the spots and cracks is specified in the Table 13, since there were visual differences in these cracks, B1 presented large spots and identified from the outside of the complete package without the need to remove the layers of paper, while B3 showed very small spots, and hardly visible, despite B3 presenting the second highest value of cracks.

The results confirm the theory that dairy cream without homogenization could present higher cracks in the red ink test, however, B5 without homogenization and with direct heat showed a value of more than 7.8% cracks.

Despite the fact that the cracks in the corners are not part of the ESC risk study since they are not in the ESC area in the bottom (area of greatest tension), the presence of 5 double cracks (in both sides the packaging) is reported batch B1, and 3 from batch B2. The Tetra Top package has 4 corners, then 1 crack corner is considering a single crack-corner, and two crack in two different corners is double crack-corner, and successively.

The Table 13 also gives a description of the spots observed, since the spots found could be considered different, some were much larger and with greater absorption of the penetrating liquid that is even observed in the outer layer of the packaging and other spots were very small and even difficult to observe and with less ink absorption.

5. Summary of results

It was verified how the fat globule was disrupted by the homogenization process, this process reduces the size of the fat globules and produces an increase in the surface area of the new fat globule; as a result of this reduction, the particle-particle interaction increases, the proteins have a greater participation in the microstructure of the new membrane of the MFGM, this new layer contains a greater load of caseins and whey proteins. The schematic representation of the different process line regarding with the homogenization process is in Figure 38. (For more details see Appendix)

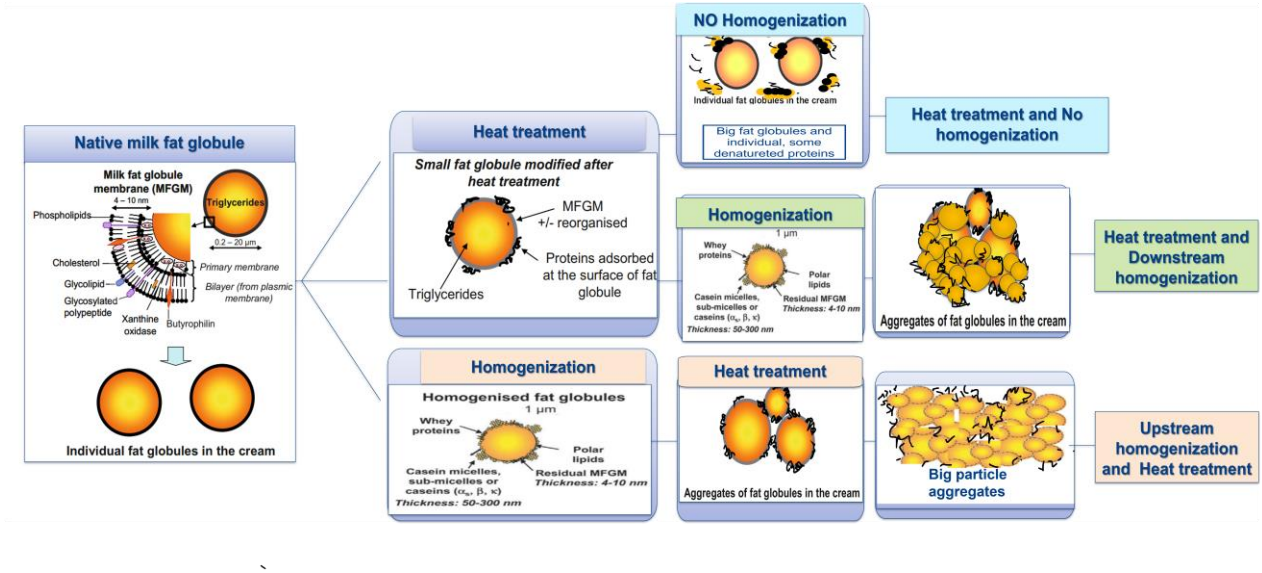


Figure 38 Schematic representation of the micro molecular re organization of the dairy cream and changes after processing line with heat treatment and no homogenization, downstream homogenization, or upstream homogenization, NOT TO SCALE, source Gassi et al., 2008 and Lopez and Briard-Bion, 2007 with some editions by Alejandra Fernandez

The heat treatment also had an influence on the physicochemical characteristics of the samples, although it is mainly used to ensure that harmful microbial components are eliminated, and the shelf life of the product is prolonged, significant changes also occur in the physicochemical composition of dairy cream.

Changes as the increase in size of the particles distributed in the emulsion, possibly due to the swelling of the proteins after being denatured, in addition to greater interaction between particles that promotes aggregations between the fat globules. Heat treatment also influences the bonds formed between the proteins and the fat globule membrane.

The analysis of the graphs and the data of the particle size distribution study without Sol A (natural state of the samples) and with Sol A demonstrated the impact of the homogenization process and the heat treatment. In addition, the theories described in the literature that the homogenization process generally increases the viscosity of the final product due to the higher number of fat globules, and the higher adsorption of casein on the surface of the product, were confirmed. Moreover, the viscosity is also seriously affected by the heat treatment and homogenization process, which was proved that the higher the thermal load, the higher the viscosity, consider the impact as well to homogenization first

and after heat treatment, as in the case of B4 (indirect + upstream), since the lactulose content was high and therefore was the viscosity.

The increase in viscosity can be attributed to molecular entanglement of denatured proteins, there is also a swelling of the proteins, which contributes to the increase in viscosity. Then, higher heat load will contribute with the formation of more aggregations, and it will increase the viscosity.

The data found in the PSD can be confirmed with the analysis of the microscopy with the protein staining it is proved there is a new formation of the MFGM with greater participation of the proteins and greater interaction protein-fat globules.

Regarding the PSD and the stability analysis of the samples, the theory that the non-homogenized samples would probably be the most unstable with the highest TSI value was confirmed for batch B5 (indirect + no homogenization), being the most unstable sample, however, sample B1 (direct + no homogenization) presented a lower TSI value and is considered more stable (All the data see table 14).

About the composition of the samples, the dairy cream had fat content between 33 % and 35%, two of the batches did not have significant differences in terms of fat content B4 (indirect + upstream) and B5 (indirect + no homogenization), however B2, B3 and B4 were significantly different in the fat content compare among all five batches.

Despite B4 processed with indirect and upstream homogenization, with high viscosity and with PSD values for Dx (90) and Mode the highest (due to aggregations); therefore the aggregation give a stability profile to the sample B4, because it was a sample with stable TSI values, and with low value red ink, which is interpreted that despite the size of the particles and aggregates due to the upstream process and the heat load, it is still more stable than the non-homogenized sample B5.

Table 14 Results of the analysis of the dairy cream batches, it is showing the most relevant data

Phase I **Cream**	**Phase II** **Analysis dairy cream samples**										**Phase III** **Analysis (FPI)**	
Analysis	Dx (90) (µm)		Mode (µm)		Viscosity [mPa·s][1]		Lactulose	Emulsion Stability	MilkoScan		Red ink[2]	Puncture test[3]
Sample Code	No Sol A	Sol A	No Sol A	Sol A	6.0 °C	20 °C	mg/kg	TSI	Fat %	Protein %	%	(mm)[4]
B1 DNH	6.51	8.09	3.67	3.8	25.5	16.4	67	2.1	33.3	2.19	12	10
B2 DDH	6.98	3.07	3.81	1.96	59.5	35.2	61	2.6	33.0	2.16	4	9.7
B3 IDH	4.11	2.95	2.78	1.86	92.2	56.2	92	1.4	33.7	2.16	11	10.4
B4 IUH	11	3.35	7.27	2.14	241.6	231.1	123	2.6	35.2	2.20	5	10.1
B5 INH	6.83	6.8	4.01	4.03	31.4	19.4	62	4.6	35.3	2.21	8	10.1

[1.] Shear rate 100 s-1
[2.] Red ink leakage frequency crack in the bottom
[3.] Inside polymeric layer
[4.] Tip displacement at failure

Tetra top packages suffer stress in the forming process in the packaging machines, in addition to mechanical stress there is the possibility that the packaged food product is an "aggressive agent" that increases the risk of ESC considering the food as an aggressive environment for the inner layer of the packaging that is composed of a polymeric layer. If the risk of ESC increases, there is a risk of crack formation that leads to embrittlement and premature failure of the packing.

In this research, a methodology was followed that included the characterisation of the critical fluid (dairy cream) and indicative food-packaging interaction tests with the aim of obtaining more information on the possible factors that influence the production of highly "aggressive" cream due to that previous investigations found creams with exactly 36% fat content but with totally different responses in the interaction with the packaging.

In general, the main hypothesis was that some of the batches B1 and B5 without homogenization would be the samples that would present all the highest values of PSD, as well B4 due to the upstream homogenization process and the heat treatment after.

Consequently, greater instability in the TSI analysis in TurbiScan, greater percentage of cracks in the red ink and with a more critical value for the puncture test study, however only sample B1 seems significantly more aggressive and may increase the risk of ESC; however, there is not enough information and data to confirm the hypothesis. This analysis is performed only in comparison among the batches of cream produced.

On the other hand, it would be statistically difficult to compare these results with the studies carried out from previous investigations analysing supermarket samples since much information would be unknown in the process line, production dates, origin of the raw materials, formulation, process conditions (temperature, pressures, flows of flow, pumps, transport among others), interaction with other ingredients, among many other noise factors.

Despite having information from indicative studies such as leakage test with red ink and blue ink, the characteristics of the packaging and the conditions in which it was produced were unknown.

In this study, it can be concluded that the dairy cream produced in the five different experiments have medium/low risk to be aggressive product and increases the risk of ESC according to the indicative study of the puncture test. However, the red-ink test does not seem to be significant considering it was done with red-ink and deliberately badly produced packages in this study, which should yield magnified results because it is a "more aggressive" test than blue- ink.

Then, focusing more on the results of puncture, the differences in the particle size distribution, microscopy picture and emulsion stability, it could be concluded that the cream produced in this investigation under known conditions have a medium/low risk to be aggressive agent for the Tetra Top of packaging, however, compared to previously studied samples, it can be said that it is less aggressive, afterward possibly other factors could be influencing the risk of ESC, including the use of food additives, stabilizers, emulsifiers and vegetable oil.

Moreover, it is necessary clarify that the leakage: red ink test, was done on specification, however the puncture test was done with another specification (the main objective of the puncture test was only to compare the products with a certain material specification and not with the actual specification material is used in the packages for chilled dairy cream).

5.1. Overall discussions

With the preparation of dairy cream on a laboratory scale in the mini-UHT, it was tried to control the variables raw materials, heat treatment and homogenization. An attempt to reduce the "noise" factors that could increase the error in the analyses subsequently carried out. After achieving the preparation of the dairy cream under the desired conditions, the physicochemical analyses were carried out (PSD, microscope, compositional analysis, lactulose concentration) that would give a description of the behaviour at the molecular level of the cream, being able to correlate the impact at the microstructural level of the cream with other analyses (viscosity and emulsion stability) and thus obtain a more complete description of the changes in the dairy cream due to these unitary operations. This dairy cream preparation was used in analyses indicative of Tetra Top cream-packaging interaction in order to obtain factors that correlate with the impact on the risk of ESC.

With the main objective accomplished, it was possible to understand a little more in detail the molecular behaviour of dairy cream and the influence of the unitary operations of heat treatment and homogenization have depending on the process conditions such as the type of heat transfer as direct or indirect heat, whether or not to perform homogenization, including the position in which the homogenizer is located with an upstream or downstream process.

Unfortunately, it was not possible to confirm which factors due to the heat treatment and the homogenization process are exactly the ones that could have an impact on the increased risk of ESC. However, it was confirmed that dairy cream is an agent with a tendency to be medium/low risk to be "aggressive" for the packaging material, depending on the product's processing line as heat treatment and homogenization process. Confirming that the product chilled dairy cream, due to its high fat content, tends to be aggressive and increase the risk of ESC in the integrity of the inner layer of the packaging.

Significant difference was found between heat treatment and homogenization in the dairy cream in the PSD analyses. Significant differences between the samples due to the homogenization process, especially in comparison between homogenization and non-homogenization in the descriptive analysis of the microscopy images. Significant differences were found in the viscosities depending on the treatments applied, giving as a general result that the creams heat treated without homogenization tend to have lower viscosities than the homogenised and heat-treated samples; and the impact of heat treatment was also confirmed on the increase in viscosity by correlating the lactulose concentration with the viscosity of the dairy cream samples produced.

An understanding at the micro-structural level of the behaviour over time of the dairy cream was possible by monitoring the movement of the particles in the emulsion stability study with the Turbiscan analysis equipment. It was found that the B5 samples without homogenization and with indirect heat presented high instability (high value of TSI) in comparison with the dairy cream samples and also with two supermarket samples analysed in this study.

Therefore, the emulsion stability study could be part of the methodology to complement the characterization of the dairy cream, then it could be a parameter that could be correlate in the study of the risk of ESC because, even if it is a time-dependent study, still give an information that could be correlate with the PSD, Microscopy, and viscosity data results.

6. Conclusions

Summary list of the objectives achieved and main conclusions:

- The use of the mini-UHT and the homogenizer unit of the Acc-Lab produced dairy cream samples with expected successful results on a laboratory scale, regarding the decrease in particle sizes due to the homogenization process and the heat load was analysed.

- The downstream homogenization process in combination with direct or indirect heat contributes to obtaining a stable dairy cream product that is visually white and homogeneous, moreover it was not found increase the risk of ESC after the predictive analysis as leakage test: red ink and puncture test.

- The non-homogenized and heat-treated samples have large fat globules, which confer properties of yellowish colour, presence of bubbles in the cream layer, low viscosities compared to the homogenised ones and more unstable emulsions, especially sample B5 with indirect heat without homogenization, as well it was found the sample B1 and B5 have slightly medium/low risk of ESC.

- Confirmed with the samples produced on a small scale that upstream homogenization process in combination with indirect heat treatment is not recommended on an industrial scale by Tetra Pak, due to the increase in the viscosity of the product prior to being heat treated, which hinders the heat transfer process, and therefore can generate negative consequences such as excess increase in viscosity, Maillard reaction and too much total heat load, among other consequences derived from this.

- The newly formed interface layer of fat globules from homogenized cream increases the surface area available for lipase (an increase in free fatty acids may occur in theory), therefor the homogenization process must be controlled due to the big impact in the full quality could have

- The average particle size of batch B4 without sol A was the largest because it suffered a greater impact from the heat treatment, possibly it was exposed for a longer time (holding time) or perhaps there were variations in temperature, however when sol A was applied the average particle size decreased.

- The production of packages in "deliberately bad" conditions is difficult to achieve if only the configurations of the forming machines are modified; then bad enough packages are not obtained to be able to study the risk of ESC. Then to use packaging material in “bad conditions” could be consider as dry material that could increase the risk of the formation cracks and leakage.

7. Sources of error

Small-scale cream production has as a source of error aspects such as low flow, different internal processing conditions due to the behaviour of the fluid when it is being processed, then trying to simulate industrial-level conditions turns out to be a bit difficult. However, with the study of PSD, Microscopy, Viscosity and Lactulose concentration, it was confirmed that the small-scale process in a mini-UHT can become significant and comparative with the industrial scale. But more studies should be carried out as duplicates under the same conditions to confirm the obtaining of homogeneous data.

In the filling section in the mini-UHT equipment for the B2 direct + downstream homogenization product, the product came out splashing and with a non-constant flow, therefore the product could be seriously modified due to this sudden exit that hit the walls of the used packaging, taking into account that the fat globules are very sensitive, and any extra damage could cause irreversible damage to the product and accelerate other processes such as enzymatic degradation, release of free fatty acids, among other harmful processes.

Although raw materials from the same supplier were used, the studies were carried out on 3 different dates, so 3 different batches of raw materials were used, which increased the risk of obtaining different final dairy cream products. In fact, the initially desired fat content of 36% could not be obtained, since there was no study of the composition of the raw mix before it was processed. The actual fat content of the raw materials was not verified either, so there could be variations.

The production dates of the raw materials, the production dates of the dairy cream, and the dates of the analyses should have been done at the same time initially indicated, but for external reasons the initially marked scheme could not be followed. Therefore, the aging of the samples can be a determining factor in the results found.

The puncture test only evaluates the polymeric material (inside layer), so it is not a complete and reliable study that can characterize what can really happen between the cream-packaging when they come into contact.

Making comparisons of the five batches of cream produced in this study with the commercial creams studied in previous investigations turns out to be unreliable, since the experiments were carried out under different conditions, so it would be wrong to compare, for example, data from the blue ink test carried out at 300 packages when in this study a red ink test was carried out with approximately 100 packages (number of package analysed B1:117, B2:103, B3:113, B4 113, B5: 102, water: 100).

8. Future work

8.1. Dairy cream production samples

The experiments carried out here should be carried out at least twice more, specifically the preparation of the cream, after continuing with phase II and phase III of the FPI. Obtain small-scale dairy cream samples in triplicate and analyse the data together, looking for significant differences. After this, it would be possible to reach more precise conclusions and continue with other areas of the project, which will be mentioned in these recommendations.

Therefore, the use of the mini-UHT would be recommended again to be able to minimize the error of the results using the same equipment, with three replications it could be statistically concluded, in addition the mini-UHT equipment demonstrated to produce dairy cream with quite similar results to what expected depending on the heat treatment and homogenization processes applied.

Trying to produce creams under "deliberately bad" conditions, such as higher process temperatures, or using raw materials with a longer production time (old creams or skim milk), among other factors, to try to produce "more aggressive creams" possibly. Then you would have the data of creams that are produced under non-recommended conditions.

However, in further investigations, it could be considered the use of an industrial scale equipment and produce the batches of dairy creams, producing on a bigger scale, this will allow to have samples more similar to what is produced in the dairy industry and have a higher chance of approaching a commercial installation; in PDC they have Ultra pasteurizer equipment that could be used in this investigation.

8.2. Food package interaction: indicative test

In the FPI, the leakage test: red-ink or blue-ink could be considered to study the effect of the oil emulsion and analyse the percentage of crack found in the packaging, since the oil emulsion is analysed in the puncture test and is considered an aggressive agent, likewise, water is considered neutral. Therefore, it is necessary to carry out the cracks test with the two substances to establish parameters and have wider comparison among the test as well the measurement of the cracks.

In relation to the FPI indicative test, it should be noted that the production of the packaging must be "deliberately bad”, then the indicative test could be evaluated on packages produced with packaging materials in different conditions or different specification, for example using "older" and dry packaging material, since the humidity of the material has an impact on the entire package it could be important to verify which of those condition increase the risk of ESC. Different specification of packaging material could be an alternative study, as well to be focus in the two/three batches of dairy cream, like B1 (shows the highest cracks in red ink test) and B2 (the lowest cracks in red ink test). Regarding the pack mats could be study in different specifications.

It is recommended to carry out the leakage test: red ink with 300 packages and include the cracks measured in 30 packages and quantify the number of cracks found in each individual package.

8.3. Analysis performance

Regarding the analysis of dairy cream samples, due to the high density and thickening of the sample, it is recommended to make dilutions with deionized water to analyse in PSD and microscopy studies; this would facilitate the measurement and more homogeneous results could be found.

In this study, the use of a single stabilizer, used at the concentration specified by the supplier, was investigated. According to previous research data, some creams that were "aggressive agents" for packaging contained more ingredients than dairy cream, so a list of the ingredients used should be made and an attempt should be made to continue the study focused on the effect of stabilizers, thickeners, and ingredients

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10. Appendix

Appendix A

- **Process technology description**

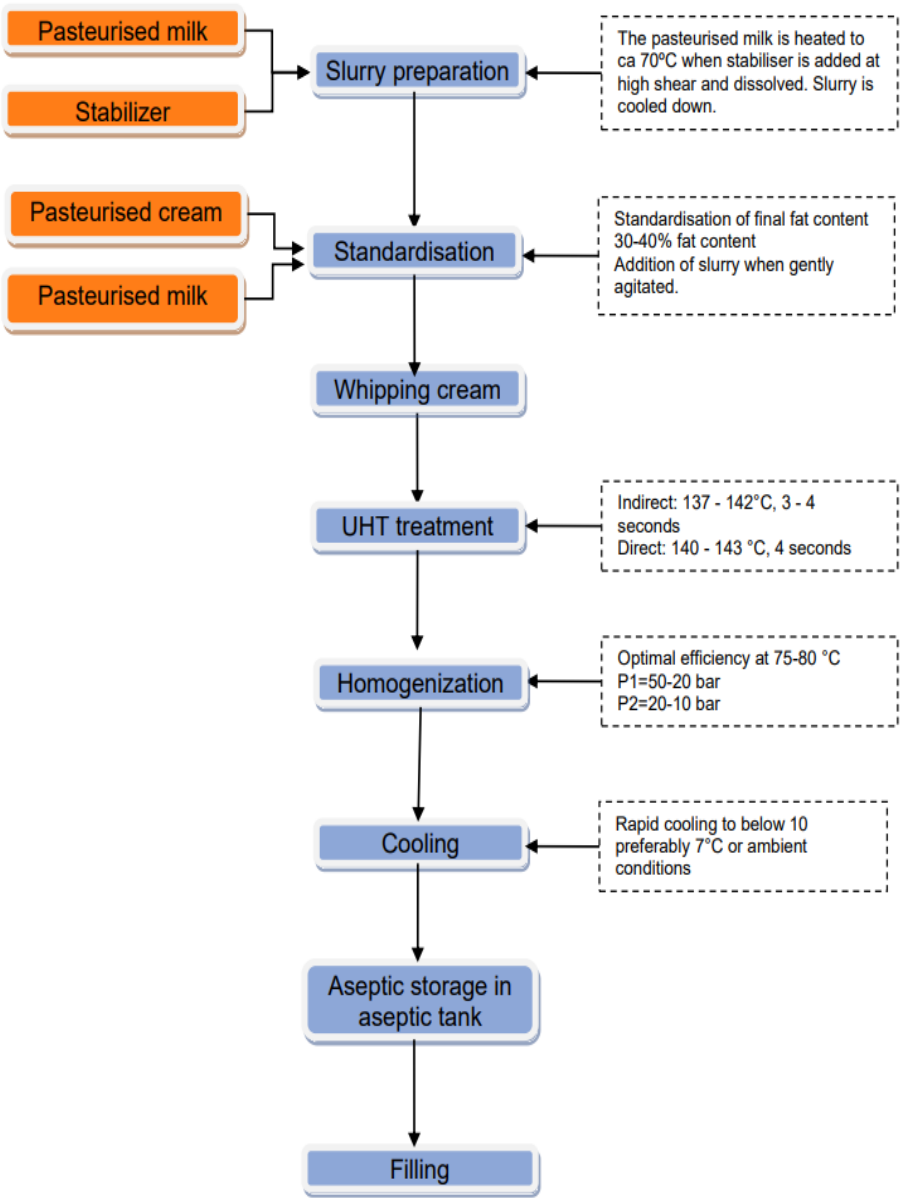


Figure 39 Technology diagram, Process technology description, recommendation from: Tetra Pak

Appendix B

- Specifications from Lab & Pilot Equipment OMVE

Main system specifications	
Mechanical	
Type	OMVE HT220 DSI
Construction material dimensions l x w x h	Stainless steel AISI 304 and 316 11850x880x1750 mm
Weight	Ca. 680 kg
Volume feed hopper	5 l.
Electrical system	
Voltage	230 V
Frequency	60Hz
Nominal	31A
Maximum current	32A
Phase connection	Three phases
Control	PLC with touch screen control
Feed pump	
Type	Non-progressive cavity pump
Flow	10 – 150 l/hr
Max. Temperature max.	70
Max lift pressure	12 bar
Max. Viscosity	2000 cp
Stator material	Acrylonitrile butadiene rubber
Indirect heating Pre-heat	
Type	Tube in tube heat exchanger counter flow
Number of tubes	4
Internal diameter tube	8 mm
Length of the tube	740 mm
Temperature range	0-120
Heating medium	Water
Indirect heating Main-heat	
Type	Tube in tube heat exchanger counter flow
Number of tubes	4
Internal diameter tube	8 mm
Length of the tube	740 mm
Temperature range	0-150
Heating medium	Water
CIP Setting, cleaning	
Flush water 1	Sets the time of rinsing phase 1
Cleaning CIP 1	Sets the time of the first CIP cycle
Flush water 2	Sets the time of rinsing phase 2
Cleaning CIP 2	Sets the time of the second CIP cycle
Flush water 3	Sets the time of last rinsing phase

Appendix C

- Formulation for prepare 20 kg around of dairy cream

Ingredients	Cream product 36% fat content 20 Liters	Final product
Fresh Cream 40% fat Pasteurized	90%	18 kilograms
Skim Milk 0,1% fat Pasteurized	10%	2 kilograms
Carrageenan type LP-60	0,015 %	3 grams

Appendix D

- Information batches production and date of analysis

Procedure	Date					Where
	B1	B2	B3	B4	B5	
Cream 40% Date of reception (in PDC)	2022-03-10	2022-03-10	2022-03-24	2022-03-24	2022-03-30	Refrigeration in PDC, Lund, Sweden, Tetra Pak
Cream 40% Date of expired	2022-03-16	2022-03-16	2022-04-01	2022-04-01	2022-04-06	Move to Container outside 316 4 C Lund, Sweden, Tetra Pak
Skim milk 0,1% Date of production	2022-03-07	2022-03-07	2022-03-22	2022-03-22	2022-03-27	Container outside 316 4 C, Lund, Sweden, Tetra Pak
Skim milk 0,1% Date of expired	2022-03-16	2022-03-16	2022-03-31	2022-03-31	2022-04-05	Container outside 316 4 C, Lund, Sweden, Tetra Pak
Production of cream 36%	2022-03-10 / 10:00:00	2022-03-10 / 15:14:00	2022-03-25 / 10:00:00	2022-03-25 / 13:00:00	2022-03-30 / 10:00:00	Acc-lab, Lund, Sweden, Tetra Pak
Date of the analysis and more details						
Microscopy	2022-03-14 / 08:00:00	2022-03-14 / 09:00:00	2022-03-28 / 08:00:00	2022-03-28 / 09:00:00	2022-04-01 / 8:00:00	PDC, Rheolab, Lund, Sweden, Tetra Pak
Particle size distribution	2022-03-14 / 09:30:00	2022-03-14 / 09:50:00	2022-03-28 / 09:30:00	2022-03-28 / 10:30:00	2022-04-01 / 9:00:00	PDC, Rheolab, Lund, Sweden, Tetra Pak
MilkoScan	2022-03-14 / 11:00:00	2022-03-14 / 11:30:00	2022-03-28 / 11:35:00	2022-03-28 / 11:45:00	2022-04-01 / 11:00:00	PDC, Rheolab, Lund, Sweden, Tetra Pak

Viscosity	2022-03-11 / 09:00:00	2022-03-11 / 10:00:00	2022-03-25 / 14:00:00	2022-03-25 / 15:30:00	2022-04-01 / 13:00:00	Lund, Sweden, Tetra Pak
Emulsion stability (TurbiScan)	2022-03-11 / 13:30:00	2022-03-11 / 15:30:00	2022-03-29 / 07:30:00	2022-03-29 /09:00:00	2022-04-01 / 15:00:00	Kemicentrum, Lund University, Lund, Sweden
Lactulose concentration	2022-04-07 / duration of testing 2022-04-11 to 2022-04-22	2022-04-07 / duration of testing 2022-04-11 to 2022-04-22	2022-04-07 / duration of testing 2022-04-11 to 2022-04-22	2022-04-07 / duration of testing 2022-04-11 to 2022-04-22	2022-04-07 / duration of testing 2022-04-11 to 2022-04-22	Muva kempten GmbH, Ignaz-Kiechle-Straße, Germany
Red ink (cream+ package in contact 1 hour)	2022-03-15 / 09:00:00	2022-03-15 / 10:00:00	2022-03-29 / 10:00:00	2022-03-29 / 11:00:00	2022-03-30 / 16:00:00	Lund, Sweden, Tetra Pak
Red ink (tear packages to find spots)	2022-03-16 / 08:20:00	2022-03-16 / 09:20:00	2022-03-30 / 08:20:00	2022-03-30 / 10:20:00	2022-03-31 / 10:00:00	Lund, Sweden, Tetra Pak
Puncture tests send samples to Stuttgart (samples in refrigeration)	2022-03-14	2022-03-14	2022-03-30	2022-03-30	2022-03-30	D&T materials & package, Tetra holding GmbH, Stuttgart, Germany
Puncture test analysis	2022-03-18	2022-03-18	2022-03-31	2022-03-31	2022-03-31	D&T materials & package, Tetra holding GmbH, Stuttgart, Germany
Others						
Sample D1, Lactose free, 35% fat content	Expire date: 2022-05-03		shopping date at ICA supermarket, Malmö, Sweden 2022-03-21		Date of the analysis in Turbiscan: 2022-03-22	
Sample C1, 36% fat content	Expire date: 2022-04-24		shopping date at ICA supermarket, Malmö, Sweden 2022-03-21		Date of the analysis in Turbiscan: 2022-03-22	
Production of packaging	14:40:00					

Appendix E

- Technical information carrageenan

CPKelco
A HUBER COMPANY

APPLICATION NOTE 01400-01
Global

UHT WHIPPING CREAM – 35% OR 25% FAT
Using GENULACTA® carrageenan type LP-60 or SGI-3F

Whipping cream manufactured from high quality cream, may be added GENULACTA® carrageenan type LP-60 to prevent creaming-up during storage and to improve foam stability of the whipped cream or GENULACTA® carrageenan type SGI-3F, which gives a slightly more dense foam.

The below recipe serves as guideline. It is based on cream with 35% or 25% fat. The latter requires addition of a whipping emulsifier (see page 2).

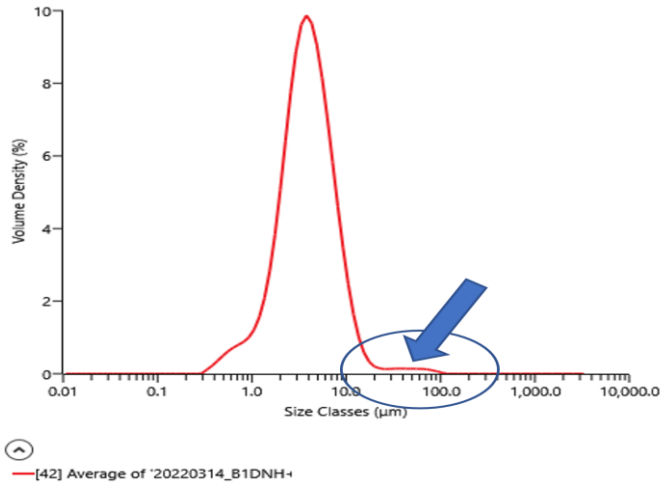
If the cream is produced from recombined cream, addition of emulsifiers may be required to prevent presence of free fat separation into fat and water phases.

Ingredients	35 % Whipping cream %	25% Whipping cream %
Cream 35% fat	99.85	-
Cream 25% fat	-	98.43
GENULACTA® carrageenan type LP-60 or SGI-3F	0.015	0.02
Sodium caseinate	-	0.50
Lactylated monoglycerides (E 472b)	-	1.00
Total ingredients	100.00	100.00

Appendix F

- Measurement data for batch B1 direct+ non-homogenization with addition of Sol A, where a second peak is visualized, it was concluded that it was not air bubbles due to size, and since air bubbles usually appear as a totally separate peak

D [4;3] (µm)	*D [5;3] (µm)*	*Dx (10) (µm)*	*Dx (50) (µm)*	*Dx (90) (µm)*	*Dx (100) (µm)*	*Weighted Residual (%)*	*Mode (µm)*
5	7.95	1.59	3.81	8.48	108	0.6	3.8



Appendix G

ANOVA and Tukey test

- With no sol A, statistics analysis with Minitab19

with No Sol A

Means

Dx (90) (µm)				
Factor	N	Mean	StDev	95% CI
B1 DNH	6	6,51	0,19	(6,3538; 6,6662)
B2 DDH	6	6,98	0,05	(6,8255; 7,1378)
B3 IDH	6	4,11	0,02	(3,95049; 4,26284)
B4 IUH	6	11,05	0,15	(10,8938; 11,2062)
B5 INH	6	6,83	0,33	(6,675; 6,988)
Pooled StDev = 0,185742				

	Dx (90) (µm)	StDev
B1 DNH	6,51	0,19
B2 DDH	6,98	0,05
B3 IDH	4,11	0,02
B4 IUH	11,05	0,15
B5 INH	6,83	0,33

Means

MODE				
Factor	N	Mean	StDev	95% CI
B1 DNH	6	3,665	0,0308	(3,5998; 3,7302)
B2 DDH	6	3,80667	0,01366	(3,74144; 3,87189)
B3 IDH	6	2,78167	0,01169	(2,71644; 2,84689)
B4 IUH	6	7,2767	0,1639	(7,2114; 7,3419)
B5 INH	6	4,005	0,0442	(3,9398; 4,0702)
Pooled StDev = 0,0775758				

	Mode (µm)	StDev
B1 DNH	3,67	0,03
B2 DDH	3,81	0,01
B3 IDH	2,78	0,01
B4 IUH	7,28	0,16
B5 INH	4,01	0,04

Dx (90) (µm)

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping			
B4 IUH	6	11,05	A			
B2 DDH	6	6,9817		B		
B5 INH	6	6,832		B		
B1 DNH	6	6,51			C	
B3 IDH	6	4,10667				D

Means that do not share a letter are significantly different.

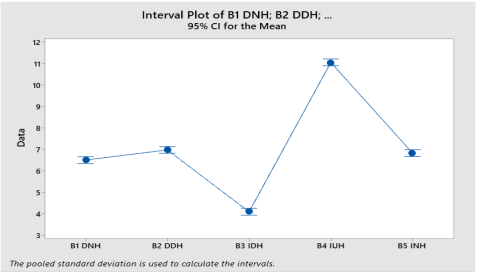
MODE

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping				
B4 IUH	6	7,2767	A				
B5 INH	6	4,005		B			
B2 DDH	6	3,80667			C		
B1 DNH	6	3,665				D	
B3 IDH	6	2,78167					E

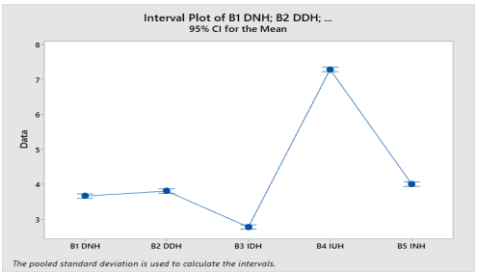
Means that do not share a letter are significantly different.

Dx (90) (µm)



B2 AND B5 ARE NO SIGNIFICANTLY DIFFERENT

MODE



With sol A

Means

Dx (90) (µm)				
Factor	N	Mean	StDev	95% CI
B1 DNH	6	8,08833	0,00408	(8,05867; 8,11800)
B2 DDH	6	3,075	0,02345	(3,04534; 3,10466)
B3 IDH_s	6	2,9467	0,028	(2,9170; 2,9763)
B4 IUH_s	6	3,345	0,00837	(3,31534; 3,37466)
B5 INH_s	6	6,8	0,0693	(6,7703; 6,8297)
Pooled StDev = 0,0352798				

	Dx (90) (µm)	StDev
B1 DNH	8,09	0,00
B2 DDH	3,08	0,02
B3 IDH_s	2,95	0,03
B4 IUH_s	3,35	0,01
B5 INH_s	6,80	0,07

Means

MODE				
Factor	N	Mean	StDev	95% CI
B1 DNH	6	3,8	0	(3,788; 3,812)
B2 DDH	6	1,9617	0,0286	(1,9493; 1,9740)
B3 IDH_s	6	1,86167	0,01169	(1,84935; 1,87399)
B4 IUH_s	6	2,14	0	(2,128; 2,152)
B5 INH_s	6	4,03	0,01095	(4,01768; 4,04232)
Pooled StDev = 0,0146515				

	Mode (µm)	StDev
B1 DNH	3,80	0,00
B2 DDH	1,96	0,03
B3 IDH_s	1,86	0,01
B4 IUH_s	2,14	0,00
B5 INH_s	4,03	0,01

Dx (90) (µm)

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping				
B1 DNH	6	8,08833	A				
B5 INH_s	6	6,8		B			
B4 IUH_s	6	3,345			C		
B2 DDH	6	3,075				D	
B3 IDH_s	6	2,9467					E

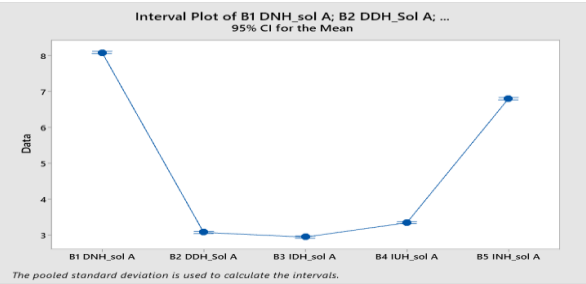
Means that do not share a letter are significantly different.

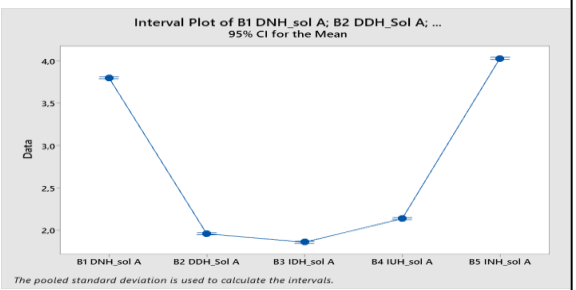
MODE

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping				
B5 INH_s	6	4,03	A				
B1 DNH	6	3,8		B			
B4 IUH_s	6	2,14			C		
B2 DDH	6	1,9617				D	
B3 IDH_s	6	1,86167					E

Means that do not share a letter are significantly different.





Appendix H

INTERVAL 3, TEMPERATURE 6 °C								INTERVAL 6 TEMPERATURE 20 °C							
B1 DNH															
Point No.	Shear Rate	Shear Stress	Viscosity	Temperature	Torque	Status	Time	Point No.	Shear Rat	Shear Str	Viscosity	Temperat	Torque	Status	Time
	[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]		[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]
1	20	0,68205	34,098	6,08	0,301		526	1	20	0,35764	17,882	19,87	0,15783	M-	856
2	25,8	0,74184	28,719	6,08	0,3274		532	2	25,8	0,54804	21,218	19,87	0,24185		862
3	33,4	0,94288	28,263	6,07	0,4161		538	3	33,4	0,5969	17,892	19,88	0,26341		868
4	43,1	1,1881	27,574	6,04	0,5243		544	4	43,1	0,79398	18,426	19,91	0,35039		874
5	55,7	1,5259	27,418	6,05	0,6734		550	5	55,6	0,96592	17,358	19,9	0,42627		880
6	71,9	1,9165	26,664	6,05	0,8458		556	6	71,9	1,2045	16,758	19,93	0,53154		886
7	92,8	2,4035	25,891	6,05	1,0607		562	7	92,8	1,555	16,75	19,93	0,68622		892
8	120	3,0098	25,104	6,04	1,3283		568	8	120	1,9423	16,2	19,93	0,85717		898
9	155	3,7612	24,29	6,05	1,6599		574	9	155	2,4314	15,701	19,96	1,073		904
10	200	4,7047	23,523	6,05	2,0762		580	10	200	3,0736	15,368	19,96	1,3564		910
B2 DDH															
Point No.	Shear Rate	Shear Stress	Viscosity	Temperature	Torque	Status	Time	Point No.	Shear Rat	Shear Str	Viscosity	Temperat	Torque	Status	Time
	[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]		[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]
1	20	1,523	76,154	6,05	0,6721		503	1	20	0,90986	45,48	19,89	0,40153		834
2	25,8	1,9141	74,1	6,06	0,8447		509	2	25,8	1,1043	42,753	19,89	0,48733		840
3	33,4	2,3889	71,602	6,05	1,0542		515	3	33,4	1,3733	41,164	19,92	0,60606		846
4	43,1	3,0452	70,681	6,06	1,3439		521	4	43,1	1,7147	39,794	19,93	0,7567		852
5	55,7	3,6758	66,051	6,06	1,6222		527	5	55,7	2,1549	38,722	19,95	0,95097		858
6	71,9	4,5819	63,745	6,04	2,022		533	6	71,9	2,6903	37,428	19,95	1,1873		864
7	92,8	5,649	60,848	6,04	2,4929		539	7	92,8	3,3093	35,649	19,96	1,4604		870
8	120	6,9198	57,713	6,05	3,0538		545	8	120	4,0869	34,086	19,97	1,8036		876
9	155	8,4416	54,51	6,04	3,7253		551	9	155	5,0597	32,675	19,98	2,2329		882
10	200	10,287	51,433	6,05	4,5397		557	10	200	6,2479	31,24	19,98	2,7573		888
B3 IDH															
Point No.	Shear Rate	Shear Stress	Viscosity	Temperature	Torque	Status	Time	Point No.	Shear Rat	Shear Str	Viscosity	Temperat	Torque	Status	Time
	[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]		[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]
1	20	2,1805	109,05	6,07	0,9623		554	1	20	1,3978	69,893	19,87	0,61684		884
2	25,8	2,807	108,67	6,07	1,2387		560	2	25,8	1,7447	67,541	19,88	0,76993		890
3	33,4	3,5164	105,4	6,07	1,5518		566	3	33,4	2,1947	65,779	19,9	0,96852		896
4	43,1	4,4733	103,81	6,05	1,9741		572	4	43,1	2,7766	64,441	19,89	1,2253		902
5	55,7	5,5679	100,04	6,05	2,4572		578	5	55,7	3,4042	61,165	19,92	1,5023		908
6	71,9	6,9325	96,447	6,03	3,0594		584	6	71,9	4,2297	58,846	19,91	1,8666		914
7	92,8	8,6771	93,467	6,06	3,8293		590	7	92,8	5,2718	56,79	19,94	2,3265		920
8	120	10,798	90,059	6,06	4,7652		596	8	120	6,5641	54,745	19,95	2,8968		926
9	155	13,394	86,49	6,04	5,911		602	9	155	8,1809	52,829	19,95	3,6103		932
10	200	16,566	82,823	6,03	7,3106		608	10	200	10,342	51,708	19,97	4,5638		938
B4 IUH															
Point No.	Shear Rate	Shear Stress	Viscosity	Temperature	Torque	Status	Time	Point No.	Shear Rat	Shear Str	Viscosity	Temperat	Torque	Status	Time
	[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]		[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]
1	20	5,012	250,63	6,07	2,2118		540	1	20	9,5021	475,49	19,88	4,1933		871
2	25,8	6,5531	253,75	6,08	2,8919		546	2	25,8	11,874	459,8	19,88	5,2402		877
3	33,4	8,3337	249,8	6,06	3,6777		552	3	33,4	13,866	415,59	19,9	6,1193		883
4	43,1	10,731	249,03	6,04	4,7356		558	4	43,1	15,82	367,14	19,9	6,9816		889
5	55,7	13,938	250,43	6,04	6,151		564	5	55,7	17,933	322,14	19,91	7,9138		895
6	71,9	18,032	250,87	6,04	7,9577		570	6	71,9	19,977	277,87	19,94	8,8161		901
7	92,8	22,946	247,17	6,06	10,126		576	7	92,9	22,33	240,49	19,93	9,8544		907
8	120	28,722	239,5	6,04	12,675		582	8	120	24,962	208,15	19,95	11,016		913
9	155	35,039	226,21	6,03	15,463		588	9	155	28,085	181,32	19,94	12,394		919
10	200	41,516	207,51	6,06	18,321		594	10	200	31,727	158,6	19,96	14,001		925
B5 INH															
Point No.	Shear Rate	Shear Stress	Viscosity	Temperature	Torque	Status	Time	Point No.	Shear Rat	Shear Str	Viscosity	Temperat	Torque	Status	Time
	[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]		[1/s]	[Pa]	[mPa·s]	[°C]	[mN·m]		[s]
1	20	0,82609	41,3	6,09	0,3646		544	1	20	0,52003	25,999	19,88	0,22949		876
2	25,8	1,0962	42,446	6,06	0,4838		550	2	25,8	0,63396	24,542	19,88	0,27977		882
3	33,4	1,3648	40,903	6,05	0,6023		556	3	33,4	0,80074	24,003	19,89	0,35337		888
4	43,1	1,6595	38,513	6,05	0,7324		562	4	43,1	1,0015	23,244	19,92	0,44199		894
5	55,7	2,0792	37,361	6,05	0,9176		568	5	55,7	1,259	22,623	19,91	0,55559		900
6	71,9	2,5846	35,959	6,04	1,1406		574	6	71,9	1,5911	22,136	19,93	0,70217		906
7	92,8	3,2014	34,485	6,03	1,4128		580	7	92,8	1,9662	21,18	19,94	0,8677		912
8	120	3,9501	32,946	6,04	1,7432		586	8	120	2,4457	20,399	19,94	1,0793		918
9	155	4,8402	31,256	6,05	2,136		592	9	155	3,0366	19,61	19,99	1,3401		924
10	200	5,8793	29,396	6,03	2,5946		598	10	200	3,7462	18,731	19,98	1,6532		930

Appendix I

- Regression results viscosity with Lactulose concentration

Minitab Summary , Regression and R-sq

Regression Analysis: Viscosity (mPa.s) 100-s, **6 °C** versus Lactulose in mg/kg

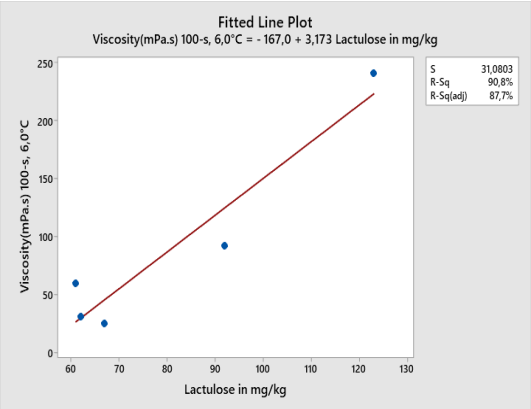
The regression equation is

Viscosity(mPa.s) 100-s, 6,0°C = - 167,0 + 3,173 Lactulose in mg/kg

Model Summary		
S	R-sq	R-sq(adj)
31,0803	90,80%	87,74%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regressio	1	28613,9	28613,9	29,62	0,012
Error	3	2897,9	966		
Total	4	31511,9			

Fitted Line: Viscosity (mPa.s) 100-s, 6 °C versus Lactulose in mg/kg



Minitab Summary , Regression and R-sq

Regression Analysis: Viscosity (mPa.s) 100-s, **20 °C** versus Lactulose in mg/kg

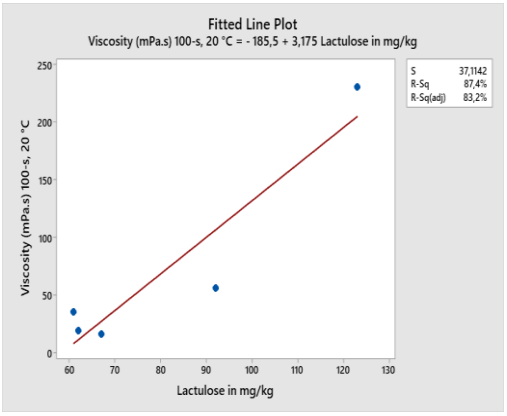
The regression equation is

Viscosity (mPa.s) 100-s, 20 °C = - 185,5 + 3,175 Lactulose in mg/kg

Model Summary		
S	R-sq	R-sq(adj)
37,1142	87,39%	83,19%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regressio	1	28641,8	28641,8	20,79	0,02
Error	3	4132,4	1377,5		
Total	4	32774,2			

Fitted Line: Viscosity (mPa.s) 100-s, 20 °C versus Lactulose in mg/kg



Appendix J

- Analysis of fat content one way ANOVA

One-way ANOVA: % Fat versus SAMPLE

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
SAMPLE	10	B1 DNH; B1 raw; B2 DDH; B2 raw; B3 IDH; B3 raw; B4 IUH; B4 raw; B5 INH; B5 raw

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE	9	18.3614	2.04016	527.86	0
Error	10	0.0387	0.00387		
Total	19	18.4001			

Model Summary

SAMPLE	N	Mean	StDev	95% CI
B1 DNH	2	33.32	0,156	(33.222; 33.418)
B1 raw	2	33.345	0,1202	(33.2471; 33.4429)
B2 DDH	2	32.97	0	(32.87; 33.07)
B2 raw	2	33.38	0	(33.28; 33.48)
B3 IDH	2	33.74	0	(33.64; 33.84)
B3 raw	2	33.34	0	(33.24; 33.44)
B4 IUH	2	35.19	0	(35.09; 35.29)
B4 raw	2	35.51	0	(35.41; 35.61)
B5 INH	2	35.26	0	(35.16; 35.36)
B5 raw	2	35.09	0	(34.99; 35.19)

Pooled StDev = 0,0621691

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

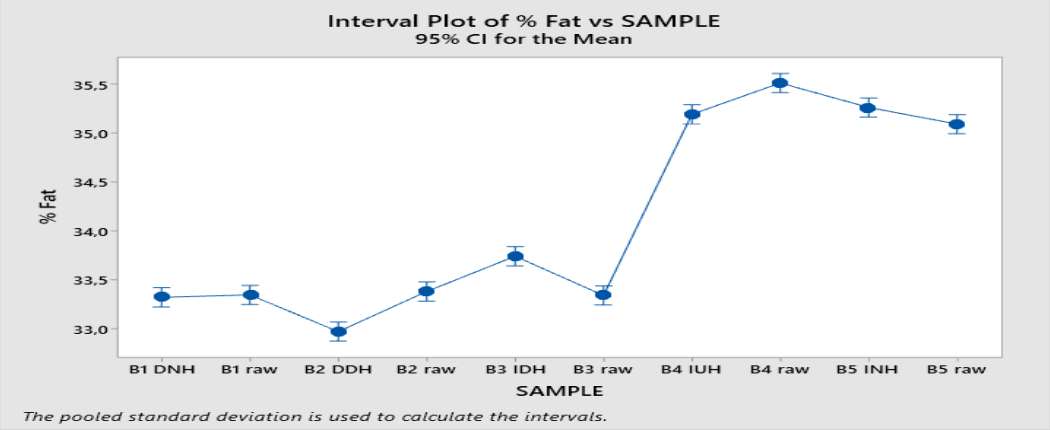
SAMPLE	N	Mean	Grouping				
B4 raw	2	35.51	A				
B5 INH	2	35.26		B			
B4 IUH	2	35.19		B			

B5 raw	2	35.09		B			
B3 IDH	2	33.74			C		
B2 raw	2	33.38				D	
B1 raw	2	33.345				D	
B3 raw	2	33.34				D	
B1 DNH	2	33.32				D	
B2 DDH	2	32.97					E

Means that do not share a letter are significantly different.

Tukey Simultaneous 95% CIs

Interval Plot of % Fat vs SAMPLE Residual Plots for % Fat



Appendix K

- Profile delta backscattering vs height all the samples

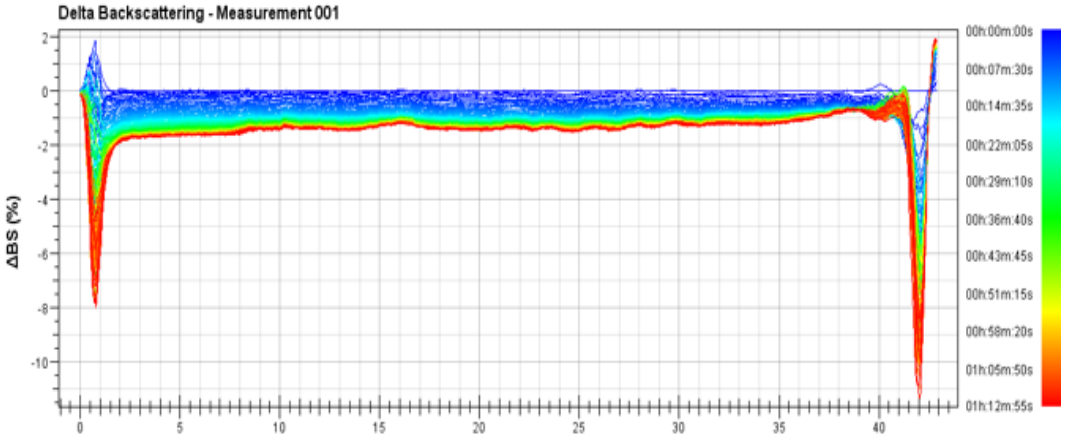


Figure 40 B1 Direct + No homogenization

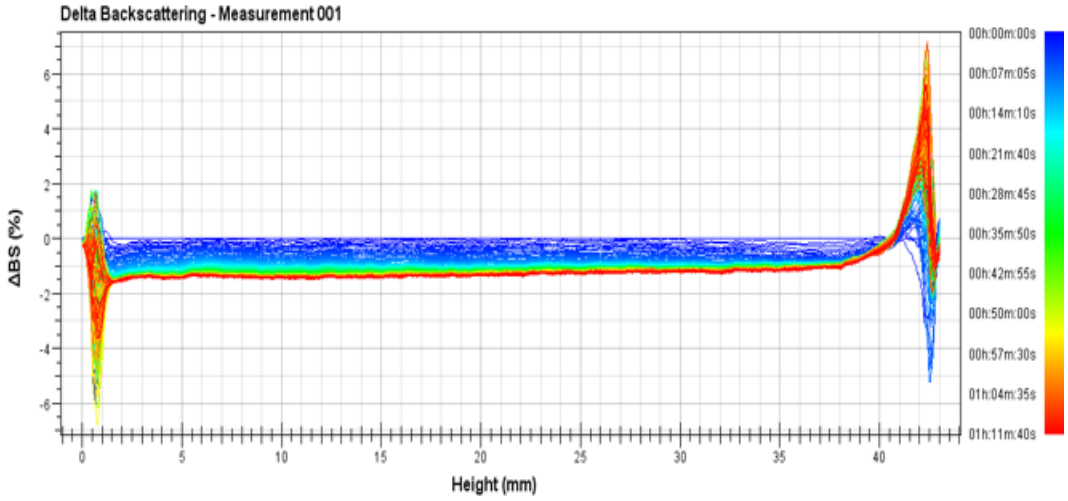


Figure 41 B2 Direct+ Downstream homogenization

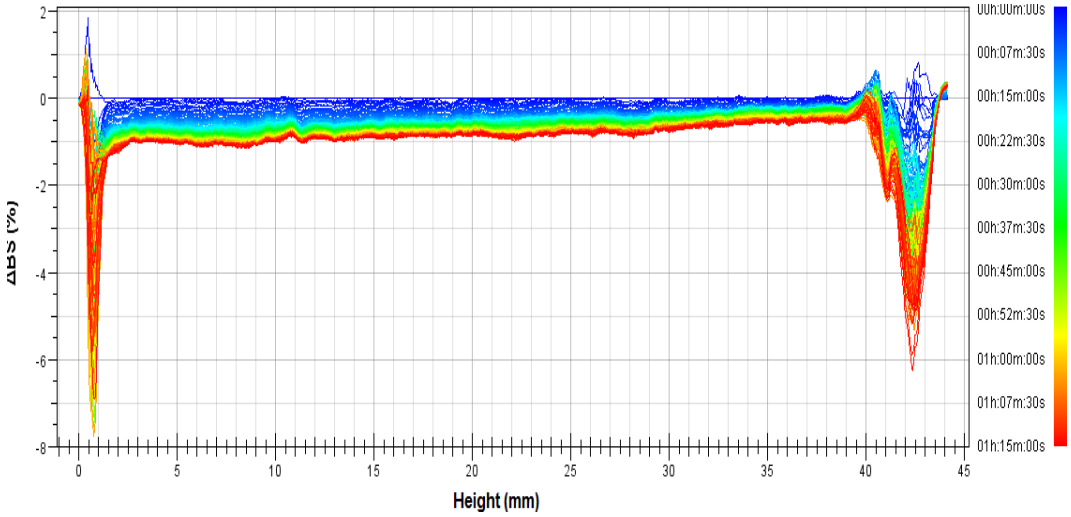


Figure 43 B3 Indirect + Downstream Homogenization

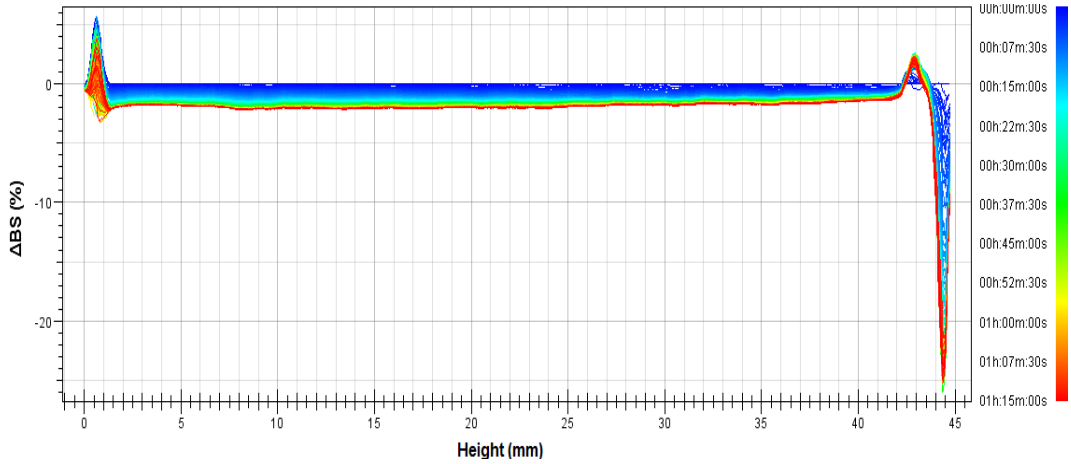


Figure 42 B4 Indirect + Upstream homogenization

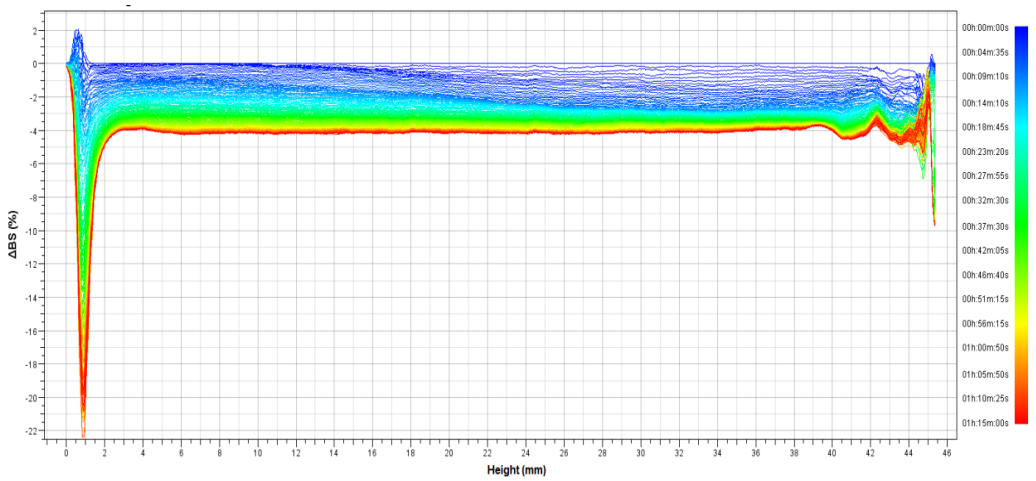


Figure 44 B5 Indirect+ No homogenization

- Two samples from supermarket

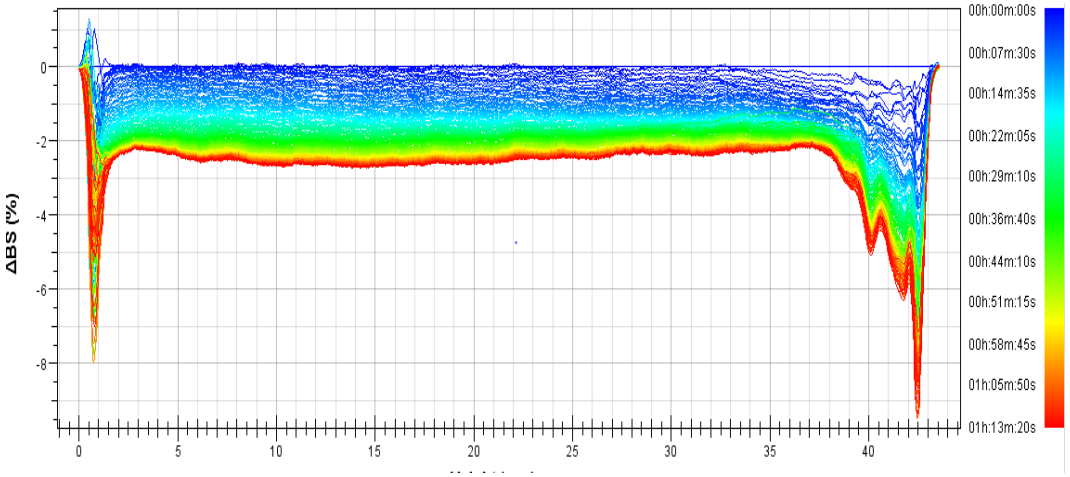


Figure 45 C1 Dairy cream 36% fat content, from C1 (sample from the local supermarket, Lund, Sweden)

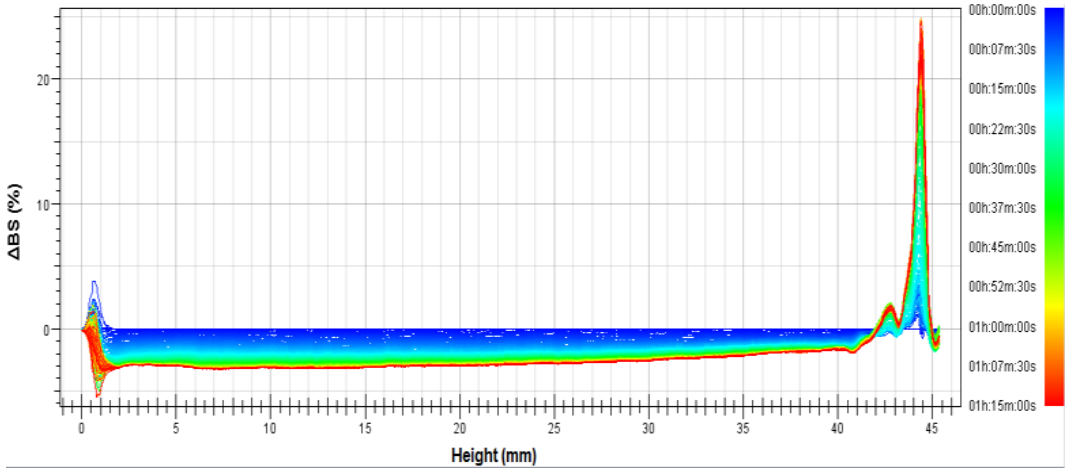


Figure 46 D1 Dairy cream 35% fat content, from D1, lactose free (sample from the local supermarket, Lund, Sweden)

Appendix L

Heat treatment and No homogenization process

The heat treatment and no homogenization probably will have the effect observed in Figure 47as the whey proteins will denature when heat treated and then form aggregates with themselves and attach themselves to the casein micelles.

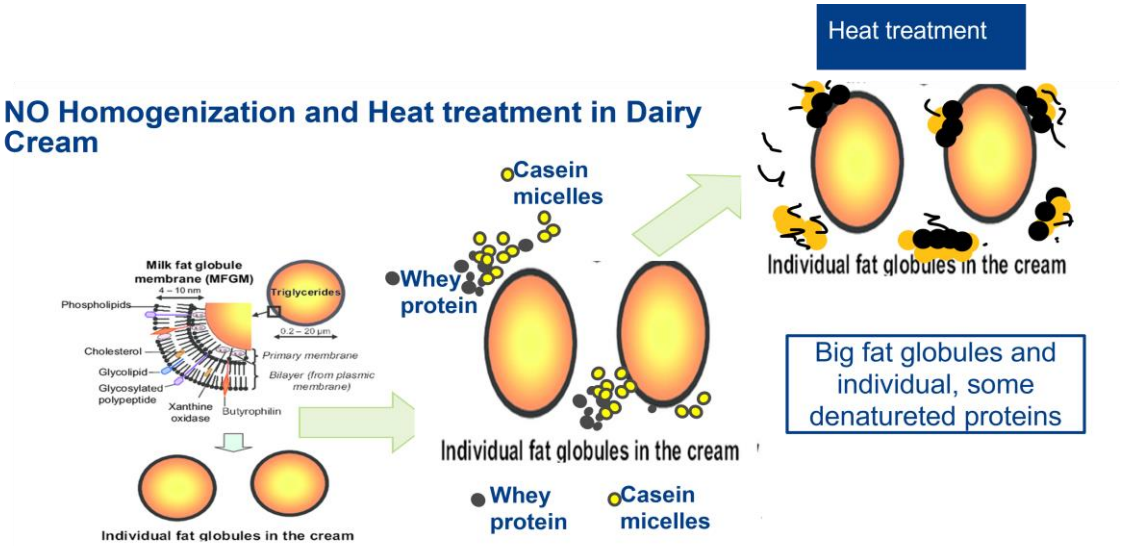


Figure 47 Schematic representation of the micro molecular re-organization of the dairy cream and changes after processing line with heat treatment and downstream homogenization, NOT TO SCALE source Gassi et al., 2008 and Lopez and Briard-Bion, 2007 with some editions by Alejandra Fernandez

Heat treatment and downstream homogenization process

In downstream homogenization the proteins will denature first and after the proteins will attach to the fat globules at homogenization process.

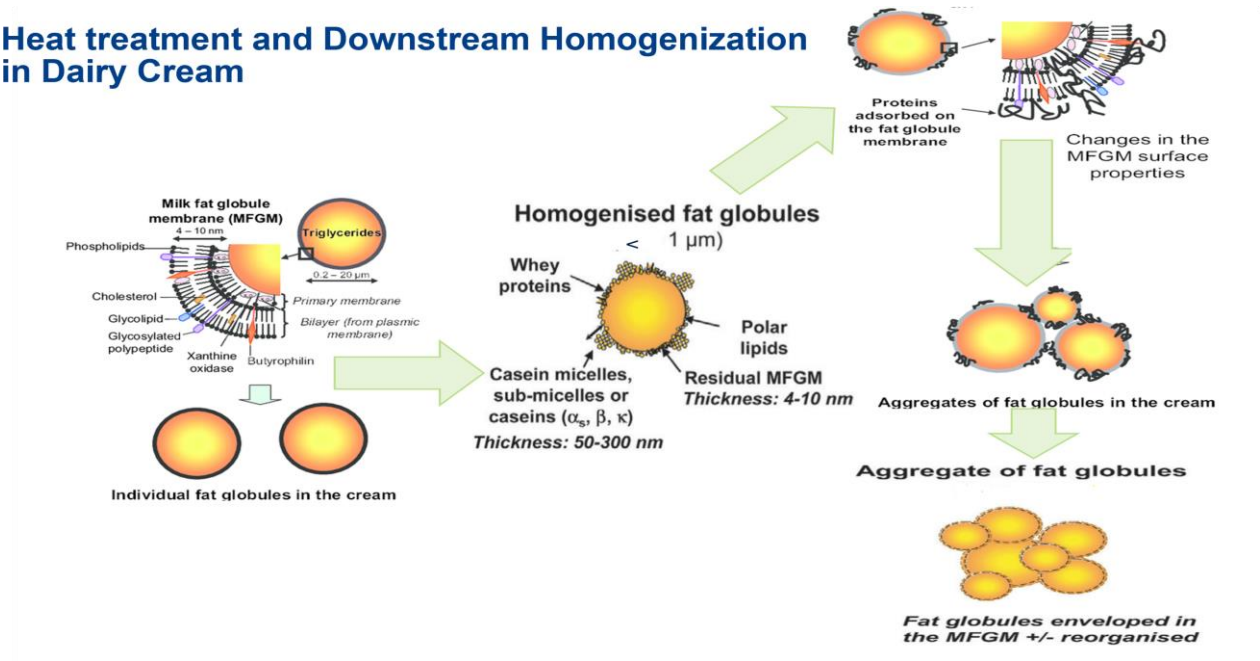


Figure 48 Schematic representation of the micro molecular re-organization of the dairy cream and changes after processing line with heat treatment and no homogenization, NOT TO SCALE source Gassi et al., 2008 and Lopez and Briard-Bion, 2007 with some editions by Alejandra Fernandez

Heat treatment and upstream homogenization process

With upstream homogenization the whey protein and the casein micelles separately will attach to the fat globule first, and then after it comes the heat treatment process, this means that the proteins on the surface of the fat droplets will be denatured by heat treatment and then begin to aggregate with proteins on other fat globules.

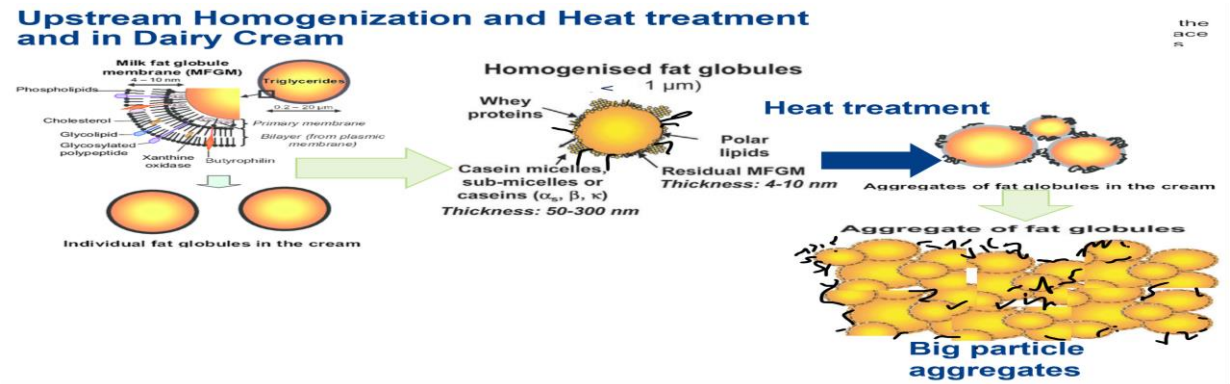


Figure 49Schematic representation of the micro molecular re-organization of the dairy cream and changes after processing line with heat treatment and upstream homogenization, NOT TO SCALE source Gassi et al., 2008 and Lopez and Briard-Bion, 2007 with some editions by Alejandra Fernandez