Understanding product build up at packaging material surface to address food waste

Ashri Nugrahini and Sonam Lhamo

DIVISION OF PACKAGING LOGISTICS | DEPARTMENT OF DESIGN SCIENCES
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Abstract

Difficulty to empty the package is one of the main reason of food waste in yoghurt at consumer level with an estimated 5-10% of product residue being left on the packaging material. The phenomenon of product build up is explained by numerous hypothesis. However, these studies are empirical in nature. The purpose of this study is to develop a tool box to address the link between the physicochemical product properties and its macroscopic build up at a polymer surface. Build up was quantified using the gravimetric dip test in natural yoghurts with 0.5% and 3% fat content and vanilla yoghurts with 0.5% and 2.5% fat content. A theoretical build up value was calculated from the yield stress obtained from rotational rheometer and texture analysis. Limitations was observed in the yield stress values obtained from texture analysis, thus the values were not used for predicting the build up. Visualization of the morphology of protein content in yoghurt and build up on packaging material was done by fluorescence microscopy using Rhodamine B. In the gravimetric method the fat content was directly proportional to the build up and the effect was statistically significant. The theoretical assumption is that yield stress value obtained from rheometer is directly proportional to build up. Higher degree of correlation between yield stress value and product build up was observed in the low fat vanilla and natural yoghurt. In the high fat yoghurt, the build up could be an influence of other factors besides yield stress. Build up measurements with similar order of magnitude were seen in the experimental values from gravimetric test and theoretical values obtained from the rheometer. Good correlation was also obtained between rheometer measurements and images from the fluorescence microscope. Accumulation of aggregated protein flocs along the packaging material was observed with hollow structure of what appeared to be swollen starch granule. Clear distinction between protein and fat was not seen due to the fat globules being entrapped within the protein network.

Keywords: Yoghurt, Packaging material, Build up, Yield stress, gravimetric dip test
Executive Summary

Introduction

One of the reasons for wasted yoghurt at consumer level is due to difficulty in getting all the yoghurt out from the packaging material (William et al, 2012). Around 10% of fermented milk product stick on the inner surface of the packaging material (Hansson, Andersson & Skepö, 2012). Product build up is residual amount of product layer (yoghurt) on the packaging material surface. It is attributed to the adhesion of the product to the packaging material. In emulsions such as yoghurt, fat, protein and hydrocolloid stabilizers are thought to adsorb to the packaging material surface. Rheological properties are also considered to have some impact on the sticking phenomenon (Hansson et al., 2012). It is often difficult to establish the effect of a single ingredient and property independently because yoghurt is presented as a complex system. Further the rheological property of yoghurt is dynamic and changes with time and shear history of the product.

Objective

This thesis focuses to address food waste by getting an understanding of macroscopic product build-up on the inside of the packaging material.

Therefore, the objectives of the thesis are to:

1) Develop a tool box to address the link between the physicochemical product properties and its macroscopic build up at a polymer surface.
2) Evaluate if the yield stress value obtained from rheometer and texture analysis could be used to predict product build up.
3) Understand the microstructure of yoghurt and how it affects the phenomenon of product build up.

Hypothesis

The intrinsic property of yoghurt affects the quantity and morphology of product build up on packaging material. This hypothesis covers the following points:

1) The product build up can be quantified by gravimetric method
2) Yield stress and other texture parameters could be used to predict the product build up.

3) Distribution of fat and protein could influence the product build up on the polymer surface.

This hypothesis is tested by comparing influences of intrinsic properties on build up in different yoghurts (low and high fat natural yoghurt, low and high fat vanilla yoghurt).

Materials

Four different stirred yoghurt types were used in this study. The yoghurts were purchased from supermarkets in Lund and Malmö in Sweden and then kept in cold storage room at 5°C prior to the experiment. The yoghurt types used were vanilla yoghurt (0.5% and 2.5% fat content) and natural yoghurt (0.5% and 3% fat content).

Packaging material samples and thin polyethylene films were provided by Tetra Pak Packaging Solutions AB. Packaging material used in this study was Tetra Brik Aseptic. The other materials used were Rhodamine B ([9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride) as fluorescent probe and were prepared by dilution with Acetone. The dye solutions were kept at 4°C in the bottle glass wrapped with aluminium foil.

Methods

Quantification of product build up by Gravimetric Dip Test method

The thickness of product build up on the inside of packaging material surface was evaluated by gravimetric force measurement using Instron Tensile Tester (Instron 5565) with 100N load, which was calibrated to 2N. The force exerted by the remaining weight of yoghurt is measured as function of hanging time after packaging material was pulled out from yoghurt container. The force then calculated into weight per area to quantify product build up. Total of six packaging material samples were used in this study. The prepared packaging material with the food contact surface exposed on both sides was incubated for 10 minutes in the sample before being pulled up and left hanging for 5 minutes to record the weight. In addition, the thickness of product build up on packaging material was also measured using Laser Scanner (Scan control 2950-100 by Micro-epsilon).
Yield stress measurement by Linear Shear Stress Ramp test

Linear shear stress ramp method was used to measure yield stress on a controlled stress rheometer by applying increasing stress into yoghurt samples from 1 to 10 Pa for 20 minutes at 23°C with sampling interval 20 s. The yield stress was measured using Rheometer (Malvern Kinexus Rheometer) with a bob geometry (C25 SC0053SS, diameter = 250mm) and a conical cylinder (PC250086AL, diameter = 250mm). Yoghurt samples were kept in a refrigerator for 1h then shaken 2 times at an angle of 90° and stirred once prior to pouring in the conical cylinder. Yield stress was measured using tangent method. The measurement was carried out in triplicate and the data were presented in averaged value.

Texture Profile Analysis

The cohesiveness and firmness of yoghurt samples were measured in Texture Analyzer TA-XT2i (Stable Micro Systems, UK) using cylinder probe (d = 35mm) with back extrusion method. The test was carried out at 1 mm/s speed for pre-testing, during testing and post-testing. The probe was held for 10 s inside the container (d = 57mm) containing 50g of yoghurt and immersed at distance of 10mm. All the measurements were carried out in triplicate and the data were presented in averaged value.

Fluorescence microscope

Yoghurt samples were diluted with whey to the ratio 1:10. Rhodamine B (excitation and emission wavelengths of 543 and 625 nm, respectively) was used to stain the yoghurt samples. Delaminated polyethylene film was folded with the food contact side exposed on the outside and mounted on the glass slide. A piece of folded polyethylene film was mounted on the glass slide with cover glass and few drops of specimen were added through the space between cover glass and slide. Then, the prepared glass slide was observed under TRITC filter. The microscope analysis was performed using Nikon Eclipse Ti-U with x 20 magnification objective with suitable filters and dilution to obtain a clear image.

Susceptibility to Syneresis

The drainage method whereby 200 g of yoghurt was placed on a funnel containing an ordinary coffee filter paper was kept undisturbed for 2hr at 5°C in a refrigerator. The amount of whey expelled was weighed at the end of 2 hours.
Results and Discussion

Quantification of product build up

Both high fat yoghurts (vanilla 2.5% and natural 3%) had significant greater build-up than the low fat yoghurts (vanilla 0.5% and natural 0.5%) \((P < 0.05)\) as per a T-test. During the gravimetric dip test, it was observed that formation of canal occurred on the film of build up on the packaging material. The canal formation could be correlated to syneresis. The susceptibility to syneresis (STS) in natural yoghurts are higher and these are more prone to canal formation as observed in picture compared in vanilla yoghurt.

Thickness of product build up measured by Laser Scanner

The thickness of product build up were 1-1.5mm for vanilla 0.5%, vanilla 2.5%, and natural 3%. Meanwhile, natural 0.5% had the lowest thickness value, which was less than 1mm. It was observed that the thickness of product build up decreased over 5 minutes in all yoghurt types. The results were comparable to the thickness obtained from gravimetric dip test and rheometer. However, the method is limited because the method cannot be performed on the same sample to quantify build up in gravimetric dip test because of test conditions.

Yield stress measurements

The yield stress values obtained from the rotational rheometer showed a clear difference in the yield stress between the vanilla and natural yoghurt. Presence of stabilizer in the vanilla sample might be a contributing factor. However, small differences are observed between the yoghurt with high and low fat content both for the vanilla and natural yoghurts. Yield stress values obtained for texture analysis were four times higher than the values from rheometer. Because of limitation due to geometrical factors the results from texture analysis would not be considered for this work.

Texture Analysis

The result obtained from texture analysis showed that the firmness and cohesiveness values of different types of yoghurt does not seem very different from each other. This might possibly be due to processing parameters to keep the eating quality of the yoghurt similar irrespective of fat content.
Comparison of experimental and theoretical build up

The measurement from the gravimetric dip test and the rheometric test shows the same order of magnitude and agrees reasonably. The predicted build up value from yield stress in the low fat yoghurt are closer to the measured value of build up. Hence, yield stress does not fully explains the build up in the high fat yoghurt suggesting that there is an additional factor responsible for the product build up in the high fat systems.

Characterization of Fat and Protein Distribution in Yoghurt using Fluorescence Microscope

Visualization of fat and protein component separately was not achieved by the fluorescence microscope. This is attributed to the smaller size of the fat globule in comparison to the aggregated protein network and also because in the emulsion fat globule is entrapped within the protein network. However, aggregated floc of protein network along the packaging material was seen and also presence of swollen starch globules were detected in the vanilla yoghurt. The thickness of build up was approximated to be < 0.3mm which gives reasonable agreement with the thickness value from gravimetric and rheometric measurement considering the fact that the samples in the fluorescence microscope were diluted.
Conclusion and Future recommendations

Gravimetric dip test and laser measurement were the two methods used quantify experimental build up. The gravimetric dip test gave reproducible results making it a viable method while further improvements needs to be done on the laser measurement such as measurement on more than one position and better data handling process to obtain robust results. Fat content of the yoghurt does seem to have significant influence on the build up. The prediction model using yield stress value fully explains build up in low fat yoghurt while it only gives a partial explanation for the high fat yoghurt. Clear distinction between fat and protein component was not seen in the fluorescence microscope. It was observed that there was not much difference in the microscopic images of the different yoghurt. More work on yoghurt with obvious difference might give noticeable difference. Nonetheless, it would be interesting to study in the future the build up of other food products in the same category or on other packaging material surfaces using the proposed methods.

Figure 2 Representative of yoghurt microstructure obtained from Fluorescence Microscope
Acknowledgments

This master’s thesis was part of project carried out at Tetra Pak Packaging Solutions AB and Faculty of Engineering Lund University. We learned a lot of new knowledge and experiences during this project. We sincerely thank those who have given their time and support for us.

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Lund, May 2019

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<th>Description</th>
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<tbody>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GVM</td>
<td>gravimetric measurement</td>
</tr>
<tr>
<td>LB</td>
<td><em>Lactobacillus bulgaricus</em></td>
</tr>
<tr>
<td>MSNF</td>
<td>milk solid not fat</td>
</tr>
<tr>
<td>PE</td>
<td>polyethylene</td>
</tr>
<tr>
<td>PM</td>
<td>packaging material</td>
</tr>
<tr>
<td>ST</td>
<td><em>Streptococcus thermophilus</em></td>
</tr>
<tr>
<td>STS</td>
<td>susceptibility to syneresis</td>
</tr>
<tr>
<td>TBA</td>
<td>Tetra Brik Aseptic</td>
</tr>
<tr>
<td>TPA</td>
<td>texture profile analysis</td>
</tr>
<tr>
<td>TRITC</td>
<td>tetramethylrhodamine isothiocyanate</td>
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1 Introduction

1.1 Project Background

Yoghurt and sour milk products are one of the widely consumed processed dairy product in most part of the world. Yoghurt and sour milk product in the year 2018 is estimated at a retail value of 180 billion SEK in Western Europe (Statista, 2018) and around 4 billion SEK for yoghurt in Sweden (Euromonitor, 2018). With commercialization of yoghurt production came the need to deliver the product in packages accessible to the consumer.

According to Paine & Paine (2012) the functional role of packaging is safe delivery of the product in sound condition to the consumer at minimum cost. A rationale behind this function is to prevent food wastage. However, when it comes to consumption of the product especially in case of yoghurt “difficulty to empty” is one of the identified reasons for food waste in yoghurt as per the study conducted by Williams et al (2012). The product loss brings negative economic aspect for consumers, since they pay for the product but not all the product can be consumed. The negative impact of this issue is food waste, economical loss for consumer and reduced recyclability of the packaging (Hansson, 2011, Saikhwan et al., 2006). Consumers also feel that it is difficult to dispose off the packaging. They have to wash the packaging before put it to trash bin. If the packaging is not clean from organic material, it can reduce the recycling efficiency. This issue challenges the sustainable development goal 2: Zero hunger and goal 12: Responsible production and consumption. It becomes imperative to understand if the intrinsic properties of the product have an impact on the build up of yoghurt occurring on the packaging material.

Therefore, the need to establish an understanding and method of how intrinsic properties of yoghurt affect the build up arises. This work attempts to develop the method to study the possible correlation between how intrinsic properties and morphology of yoghurt affects the product build up on the packaging material. The input from this work could in the future be used in developing compatible product-packaging combination to prevent or reduce the phenomenon of build up and help address the issue of food waste at consumer level and recyclability of the discarded packaging material after consumption.
1.2 Research problem and Question

One of the reason of wasted yoghurt at consumer level is due difficulty in getting all the yoghurt out from the packaging material. Around 10% of fermented milk product stick on the inner surface of the packaging material. Product build up in food product such as yoghurt is attributed to the adhesion of the product to the packaging material. In emulsions such as yoghurt, fat, protein and hydrocolloid stabilizers are thought to adsorb to the packaging material surface. Rheological properties are also considered to have some impact on the sticking phenomenon (Hansson & Skepö, 2012). It is often difficult to establish the effect of a single ingredient and property independently because yoghurt is presented as a complex system. Further the rheological property of yoghurt is dynamic and changes with time and shear history of the product. Thus, this study will attempt to find out if the intrinsic property of different yoghurts affects the build up, and if it does affect then to what extent can we predict product build up through characterization of the yoghurt. The finding could then be useful in the future to find the best-fit combination of package and product to reduce food waste.

1.3 Objective

This thesis focuses to address food waste by getting an understanding of macroscopic product build-up on the inside of the packaging material.

Therefore, the objective of the thesis are to:

1) Develop a tool box to address the link between the physicochemical product properties and it’s macroscopic build up at a polymer surface.
2) Evaluate if the yield stress value obtained from rheometer and texture analysis could be used to predict product build up.
3) Understand the microstructure of yoghurt and how it affects the phenomenon of product build up.

1.4 Hypothesis

The intrinsic property of yoghurt affects the quantity and morphology of product build up on packaging material. This hypothesis covers the following points:

1) The product build up can be quantified by gravimetric method
2) Yield stress and other texture parameters could be used to predict the product build up.
3) Distribution of fat and protein could influence the product build up on the polymer surface.

This hypothesis is tested by comparing influences of intrinsic properties on build up in different yoghurts (low and high fat natural yoghurt, low and high fat vanilla yoghurt).

1.5 Limitations

This thesis is part of a joint project with Tetra Pak Packaging Solutions AB aimed to characterize the product build up layer from commercial stirred yoghurt in Sweden. This thesis will look primarily at quantification of product build up using different methods, visualization of yoghurt gel network, and determination of yoghurt intrinsic properties (yield stress, firmness, and cohesiveness) on different commercial yoghurt types. However, this study will not address the specific mechanism of how yoghurt adheres to the packaging material and the effect of packaging material properties in relation to the product residual build up. Also due to limitation of time, the gravimetric dip test is performed with a contact duration of the yoghurt samples with the packaging material in the range of minutes.
2 Literature review

2.1 Food waste

Approximately one third of the food equivalent to 1.3 billion tonnes produced for human consumption in the world is lost and or wasted per year (Schanes, Doberner & Gözet, 2018). As per Stenmarck et al (2016) food loss occurs throughout the supply chain and in Europe particularly food waste at household level is the highest, contributing 53% to a total of 87.6 million tonnes in 2012. Moreover, household waste contributes to 2/3rd of the cost associated with food waste in EU because high amount of household food waste is still fit for consumption.

Packaging plays an important role in maintaining the quality and shelf life of a product (Paine & Paine, 2012), however in some cases packaging could be a reason for food waste especially during and after consumption. In Sweden, per capita consumption of fermented milk product inclusive of yoghurt is 29 kilograms adding up to an annual consumption of 296 million kilogram in the year 2018 (Euromonitor, 2018). One of the most common cause of food waste in yoghurt at consumer level is the difficulty of emptying from the package. With an estimated 5-10% loss as residue on packaging material (Hansson, 2011) in Sweden alone, this loss adds up to million liters of yoghurt being lost every year. Besides the obvious loss of still viable for consumption food product, there is an equally important issue of diminished recyclability and quality of recycled polymer (Al-Salem et al., 2009).

2.2 Yoghurt

The history of fermented milk was started from nomadic people to preserve their milk in containers made from animal stomach that resulted in thick and acidic food (Baglio, 2014). There is still no evidence about the exact origins of yoghurt, but it could be from 10000 to 15000 years ago in different parts of the world, from China, Middle East and Eastern Africa (Baglio, 2004; Tamime and Robinson, 1999).

At present time, yoghurt is manufactured in all countries with different name depending on the country. According to Codex Alimentarius CXS 243-2003, yoghurt is a milk product fermented by *Streptococcus thermophilus* and
Lactobacillus delbrueckii subsp. bulgaricus with or without compositional modification (FAO/WHO, 2003). Yoghurt can be classified depending on their textures (stirred, set-type, frozen, drinking), flavors (sweetened, flavored), and nutritional content such as fat and lactose content (Corrieu and Béal, 2016). The physical and sensory properties of yoghurt depend on the composition of raw milk, addition of ingredients (flavoring agents, sweeteners, stabilizers), starter cultures, and manufacturing process design (Magdaleno, 2016; Lee and Lucey, 2010; Corrieu and Béal, 2016).

The synergetic effect between Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus during milk fermentation modifies the texture, nutritional value, and flavor of yoghurt by producing metabolites (lactic acid, exopolysaccharides, and aroma compound) (Corrieu and Béal, 2016). ST produces lactase and β-galactosidase enzyme which convert lactose into glucose and galactose. Lactic dehydrogenase convert glucose to pyruvate which is metabolized to lactic acid (Sfakianakis and Tzia, 2014). Lactic acid bacteria then break caseins into peptides and free amino acids that gives flavor characteristic of yoghurt and stimulate the growth of LB. The major flavor compound of yoghurt, acetaldehyde, is derived from pyruvate through the action of pyruvate dehydrogenase and aldehyde dehydrogenase (Corrieu and Béal, 2016). At pH 5, the growth of ST starts to decrease while LB starts to dominate the fermentation process until pH 4.6 is obtained. The fermentation will be stopped through cooling process to reduce further acid development and to control the flavor of yoghurt (Lee and Lucey, 2010).

The dropping pH or acidification contributes to the coagulation as the result of disruption of casein micelles. At pH close to the isoelectric point of casein (pH 4.6), the electrostatic repulsion between casein molecules decrease due to reducing of the net negative charge. The colloidal calcium phosphate (CCP) neutralizes this negative charge. Then, electrostatic and casein molecules interaction increase due to hydrophobic interaction and form three-dimensional network consisting of small casein chains, which is responsible for yoghurt gel formation (Lee and Lucey, 2010; Corrieu and Béal, 2016; Sfakianakis and Tzia, 2014).

Exopolysaccharides (EPS) polymers are synthesized by some lactic acid bacteria that contribute to physical properties of stirred yoghurt (Corrieu and Béal, 2016). EPS can increase the thickness, ropiness and creaminess but resulting lower gel firmness in stirred yoghurt (Magdaleno, 2016). The mechanism of yoghurt gel network formation is shown in Figure 2.1 below.
2.2.1 Yoghurt Processing

There are many different varieties of yoghurt in the market, from flavored yoghurt to different fat content and additional fortification. The interest in yoghurt manufacture has increased to produce additional health functionality, improved sensory as well as textural properties (Sfakianakis and Tzia, 2014). The variation of yoghurt types can require different processing or addition of additives to improve the sensory and textural properties. The changes on the yoghurt production must be aligned with consumer acceptance. Consumer acceptance not only depends on the nutritional value but also the sensory characteristic (Magdaleno, 2016). Therefore, the wider market demand of yoghurt results in the use of stabilizing agents, functional ingredients, sweetening agents, and flavors to bring desirable consistency and taste to the customers (Tamime and Robinson, 1975).

The composition of milk plays important role to the nutritional value and the physicochemical properties of yoghurt. The fat content of yoghurt should be at least 3% and maximum 0.5% for low fat yoghurt (Tamime and Robinson, 1975). For low fat yoghurt, the milk-solid-non-fat (MSNF) content is increased to obtain high viscosity product as well as to improve nutritional and functional properties. This process can be done by adding non-fat dairy powder and other concentration methods (Fernandes, 2009; Torres et al., 2017). The milk ingredients can be added as individual ingredients or mixtures. The common milk ingredients used are skim milk powder, whey protein concentrate, whey protein isolate, micro-particulate
whey protein, and caseinates (Torres et al., 2017). Those milk powders might give different effect on the textural properties depending on the type and concentration (Tamime, 2007; Karam et al., 2013).

The textural properties of yoghurt are affected by its microstructure and physicochemical interaction between structural elements (fat globules, colloidal protein aggregates, water and additives). Stabilizers can reduce syneresis by binding water or interacting with protein. Its main function is to stabilize protein network and prevent movement of other components in yoghurt (Tamime, 2007; Temesgen & Yetneberk, 2015). Addition of starch as stabilizers resulted in increasing fibers and sheets, which at the free end of the fibers are connected to casein micelles (Tamime, 2007). Starch will absorb water then swell by many times of its original size, resulting in increasing viscosity of yoghurt and preventing syneresis (Temesgen & Yetneberk, 2015).

Syneresis is a phenomenon of leakage of whey from yoghurt. It is more likely to happen in flavored yoghurt. Some of the reason of syneresis are excessive whey to protein ratio, low solid contents, mishandling of product during distribution and storage, also type and concentration of flavoring agent. Therefore, addition of stabilizers can help to improve the stability of yoghurt (Temesgen & Yetneberk, 2015).

2.2.2 Product build up

Product build up in yoghurt packages arise due to the sticking of product to the packaging material. Numerous theories are proposed to explain the mechanism of how and why the sticking phenomenon to solid surface occurs. Adhesive forces, combination of adhesive and cohesive force, viscosity and viscoelasticity are some of the proposed factors affecting this phenomenon (Adhikari et al., 2001). Other studies propose that rheological and surface material properties are influential factors (Michalski et al., 1998). However, all the studies are empirical, and the attributable factors may depend on the specific food material being studied (Adhikari et al., 2001; Cragnell et al., 2014; Michalski et al., 1998).

Hansson, Andersson & Skepö (2012), hypothesized that in fermented dairy product, initially smaller fat molecules adhere first to the packaging material which later with larger time scale of incubation are replaced with bigger protein molecules due to the Vroman effect. Further, the author concludes that the adhesion process is independent of the material polarity as both fat and protein are amphiphilic in nature and can interact with hydrophilic and hydrophobic surfaces.

The build up of different yoghurts on the packaging material were quantified experimentally. It was assumed that the gravitational force acting per area of a packaging material sample after being taken up from yoghurt and hanging for 5
minutes is equal to the yield stress value. Thus, the theoretical build up values were calculated from the yield stress obtained from the rheometer and texture analysis. The details of the calculations are mentioned in section 3.2.2 and 3.2.3.

### 2.2.3 Intrinsic properties

#### 2.2.3.1 Rheology and yield stress

The rheology of yoghurt is known to be dynamic and changes depending on the shear history (Domagala et al., 2005). Yoghurt are known to exhibit pseudoplastic behavior and are said to be viscoelastic fluid or solid depending on the type. Stirred or drinking yoghurt can be classified as a viscoelastic fluid meaning it has the elastic properties of solid and flow properties of a viscous liquid (Lee & Lucey, 2010). It is also a shear thinning material (increasing shear rate results in decrease in the viscosity) which is characteristic of thixotropic material, but the shear induced structural breakdown is not completely reversible hence it is not a true thixotropic material. (Domagala et al., 2005, Lee & Lucey, 2010). As is with most shear thinning pseudoplastic material, yoghurt is known to have a critical yield stress which is defined as the applied stress required to initiate flow (Malvern Instruments Limited, 2015). The relevance of yield stress is applicable in yoghurt, when the yoghurt material flows out from the packaging material. Yield stress is listed as one of the key factors affecting drain off in emulsions (Schmidt, 2012).

Theoretically, when emptying the yoghurt from the packaging material the yoghurt flows when the gravitational force exceeds the yield stress of the yoghurt. Towards the end of finishing the yoghurt, the mass of the yoghurt in the package decreases and so does the gravitational force applied on this mass. The force thus becomes lesser than the yield stress and as a result the yoghurt would not flow out of the package.

#### 2.2.3.2 Texture profile

Conventionally texture analysis is done to assess the sensory perception of a product. Texture analysis is also paramount in determining the rheological attributes of the product. Firmness and cohesiveness are the two parameters that are measured in this study. Firmness would be recorded in this study as maximum force required by the probe to penetrate the sample and cohesiveness as the maximum force required to withdraw the probe from the sample, adapted from the method used by Joon & et al. (2017), with slight modifications.

Firmness could be an important parameter to understand if the strength of the gel network has any bearing on the product build up and run off on the packaging material. Cohesiveness has been used as a representation of how particles stick forming agglomeration thus reducing its ability to flow. (Peleg, 1976, Bhandari & Howes, 2005). Furthermore, Hanssson, Andersson & Skepö (2012) emphasized on
the cohesive strength of yoghurts and the possibility that it could determine the quantity of adhered product.

2.2.4 Fluorescence microscope

The absorption of light and re-radiation by organic and inorganic substances are the result of fluorescence or phosphorescence. (Spring, 2003). The working principle of fluorescent microscope is based on this phenomenon. Such substance with the ability to fluoresce by absorbing energy from photons in the excited state and subsequently losing the energy by emission are called “fluorophores” (Ogundele et al., 2013). The molecular transition of how fluorophores fluorescence is explained by Jablonski diagram depicted in Figure 2.2.

![Jablonski diagram](retrieved from Lleres, Swift & Lamond, 2007)

The fluorophores are absorbed and bound randomly to the specimens. Rhodamine B and Nile red are used to stain protein and fat, respectively, in yoghurt in this study. Both Rhodamine B and Nile red interacts via non-covalent bond with protein and fat, respectively. (Sozer, 2016; Mercadé-Prieto et al, 2017). Rhodamine B contains a reactive Sulfonyl Halide group that is reactive to the primary amine group in protein. As a result, a sulfonamide bond is formed between the dye and protein via conjugation (Holmes & Lantz, 2001). This allows for quick labeling of protein with the dye (Hermanson, 2013). Nile red binds to fat globule forming a dye-lipid complex that can absorb and emit photons thus causing the fat globule to fluorescence. (Halim & Webley, 2015).
The fluorophores in the stained specimens are then excited by light of certain wavelength that enable the electron to absorb a photon and jump to the excited state (as shown in figure 2.2.4.1). After vibrational relaxation the electron fall back to the ground state by emission of fluorescence photon. Due to loss of some energy in the process the emitted lights are of lower energy and longer wavelength compared to the excitation light that is absorbed by the fluorophores. The change in the energy and wavelength is called the Stokes shift (Sanderson et al., 2014). The colored arrow represents the energy of the excitation photon (blue arrow) and emission photon (green arrow) in the Figure 2.2.

The operating principle of Fluorescence microscope is to irradiate the stained sample with the suitable excitation wavelength of light by specific filters then separate the much weaker and longer wavelength of emitted light with a second filter and project the image from the fluorescence obtained on a dark background with a camera.

### 2.3 Tetra Pak®

Tetra Pak® is one of three companies of Tetra Laval Group founded by Ruben Rausing in Lund, Sweden. As a world’s leading company in processing and packaging solutions, Tetra Pak® is committed to provide safe, innovative and environmentally sound products to the customers. The company’s motto “Protect What’s Good” is reflected by committing to protect food, people and futures as the core value. Through the innovative processing equipment and packaging solutions, safe, nutritious, and flavorful food can be accessible to the customers. Reducing food loss and waste is one of the business principles. The packaging solutions developed by Tetra Pak® can prolong the shelf life while maintaining the nutrition and taste of the product through laminated paperboard. The multi-layer packaging can provide good barrier solution the protect the food inside as shown in Figure 2.3.
Tetra Brik Aseptic® (TBA) is one of laminated paperboard product from Tetra Pak that commonly used as yoghurt container. TBA consists of a multi layered packaging material with paperboard, polyethylene layers, and aluminium foil (Figure 2.3.1). Paperboard is the main material (around 65%). The function of paperboard is to provide stability, strength, and smoothness to the printing surface. Polyethylene has some functions depending on the position, as the outer part, PE can protect the paperboard from moisture ingress from the environment, PE also works as glue so that the aluminium foil can stick to the paperboard, PE also has function to protect the product to not penetrate to the paperboard. Finally, aluminium foil is the thinnest layer, which works as the main barrier against oxygen and light as well as maintain the nutritional value and flavors of the product inside (Tetra Pak, n.d.).
3 Methodology

3.1 Materials

Four different stirred yoghurt types were used in this study (shown in Figure 3.1). Stirred yoghurts are incubated and cooled in the processing tank, the coagulum is gently broken down to be less firm than the set yoghurt yet thicker consistency than the drinking yoghurt. (Tetra Pak 2015, Yildiz 2010). The yoghurts were purchased from supermarkets in Lund and Malmö in Sweden. The yoghurt types used were:

1. Low fat Vanilla Yoghurt 0.5% (Lätt Vaniljyoghurt 0.5% 1000 g) from Skånemejerier
2. High fat Vanilla Yoghurt 2.5% (Vaniljyoghurt 2.5% 1000g) from Skånemejerier
3. Low fat Natural Yoghurt 0.5% (Hjordnära Ekologisk Mild Naturell Lätt Yoghurt 0.5% 1000g) from Skånemejerier
4. High fat Natural Yoghurt 3% (Hjordnära Ekologisk Mild Naturell Yoghurt 3% 1000g) from Skånemejerier

![Figure 3.1 Types of yoghurt used in this study](image)

Table 3.1 shows comparison of components of four different types of yoghurt used in this study. The information obtained from the company’s (Skånemejerier) website.
Table 3.1 Comparison of components of four different types of yoghurt

<table>
<thead>
<tr>
<th>Ingredient lists</th>
<th>Vanilla 0.5%</th>
<th>Vanilla 2.5%</th>
<th>Natural 0.5%</th>
<th>Natural 3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>0.5 g</td>
<td>2.5 g</td>
<td>0.5 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>9 g</td>
<td>10.6 g</td>
<td>4.2 g</td>
<td>3.8 g</td>
</tr>
<tr>
<td>Of which sugar</td>
<td>8.9 g</td>
<td>10.4 g</td>
<td>4.2 g</td>
<td>3.8 g</td>
</tr>
<tr>
<td>Protein</td>
<td>4 g</td>
<td>3.7 g</td>
<td>4.1 g</td>
<td>3.6 g</td>
</tr>
<tr>
<td>Added sugar</td>
<td>Yes (5%)</td>
<td>Yes (6.5%)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Added aroma and flavoring agent</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Added pectin</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Added modified corn starch</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Added milk protein</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Packaging material samples and thin polyethylene films were provided by Tetra Pak Packaging Solutions AB. The other materials used were fluorescent probes as fat and protein dyes. Rhodamine B ([9-(2-carboxyphenyl)-6-diethylamino-3-xanthenyliidine]-diethylammonium chloride) and Nile Red ([9-diethylamino-5-benzo[α]phenoxazinone]) procured from Sigma Aldrich) were prepared by dilution with Acetone (Sigma Aldrich). The dye solutions were kept at 4℃ in the bottle glass wrapped with aluminium foil.

3.2 Methods

3.2.1 Quantification of product build up by Gravimetric Dip Test method

The thickness of product build up on the inside of packaging material surface was evaluated by gravimetric force measurement using Instron Tensile Tester (Instron 5565) with 100N load, which was calibrated to 2N. The principle test method was acquired from Svensson, A. (personal communication, January 30, 2019). This test method was further developed to define the parameters suited to the scope of this
work. The process of method development is further described in Appendix B. The force exerted by the remaining weight of yoghurt is measured and calculated into weight as a function of hanging time after PM was pulled out from yoghurt container.

The packaging material (TBA) was cut and folded into size of 15 x 10 cm in machine direction (MD) with the inner polyethylene surfaces exposed to yoghurt. The folded PM was attached with double sided scotch tape. PM was weighed using Mettler Toledo d=0.1mg then incubated for 10 minutes in the plastic container (2 L volume) containing yoghurt until covering half of its height (approximately 5 cm).

Two different types of yoghurt with high and low fat content were used on this test (Skånemejeriers Vanilla 2.5% and 0.5% and Skånemejerier Hjordnära Eko Naturell 3% and 0.5% fat). Yoghurt sample taken out from cold storage was shaken at 90° angle for 10 times before pouring to the container and then stirred once in a circular manner from side to the middle of the container with a spoon. Six PM were incubated simultaneously in two containers with suitable time intervals to allow an incubation period of 10 minutes. The incubation process was done in the cold storage room (5°C) for approximately 8 minutes and thereafter taken out for 2 minutes at ambient temperature during which the PM was clamped to the Instron tensile tester. After 10 minutes of incubation the PM was pulled out from yoghurt sample at a speed of 1000mm/min and left hanging 2 cm above yoghurt for a period of 5 minutes to record the weight of the PM with yoghurt as shown in the Figure 3.2. Pictures were taken to analyze the behavior of the flow of yoghurt on the PM. At the end of the 5 minutes of hanging process, the build up was calculated.

Temperature of the yoghurt was measured before and after incubation using Thermometer Testo 925 by Testo AG Germany. Temperature measurements were taken at four different positions: near the sidewall of the container which is 1 cm below top level of yoghurt and 1 cm above bottom side of the container on the front and back side of container.
The thickness of product build up on PM was also measured using Laser Scanner (Scan control 2950-100 by Micro-epsilon). The laser scanner was placed approximately 30 cm in front of the yoghurt container. The scanner is connected to a computer laptop with Baseline software (micro epsilon) to scan the distance between the PM and the laser scanner. The set-up of laser scanner is as seen in Figure 3.3.

The measurement starts when PM is hanging and lasts for 5 minutes. The data obtained from the software is analyzed using MATLAB to obtain distance vs thickness graph. In Figure 3.4, the Y-axis represents the height of the PM and the X-axis represents thickness calculated by subtracting the distance of PM with
sample from the distance of PM without yoghurt build up on it. On Y-axis the points from -10 to -60 mm corresponds to the height of PM that is dipped in the yoghurt during incubation. The graph gives the thickness measurement during 0 min, 2.5 mins and 5 mins of hanging.

Figure 3.4 Representation of data points of position on the graph to the PM in Natural yoghurt 0.5% fat content

### 3.2.2 Yield stress measurement by Linear Shear Stress Ramp test

Linear shear stress ramp method was used to measure yield stress on a controlled stress rheometer by applying increasing stress into yoghurt samples from 1 to 10 Pa for 20 minutes at 23°C with sampling interval 20 s. (Sun and Gunasekaran, 2009; TA Instruments, n.d; Malvern Instruments, 2015). The yield stress was measured using Rheometer (Malvern Kinexus Rheometer) with a bob geometry (C25 SC0053SS, diameter = 250mm) and a conical cylinder (PC25086AL, diameter = 250mm). Yoghurt samples were kept in a refrigerator for 1h then shaken 2 times at an angle of 90° and stirred once prior to pouring in the conical cylinder. Tangent analysis method was used to find the yield stress from the flow curves of viscosity versus shear stress in logarithmic scale. Tangents are applied to the linear viscoelastic and the flow region to find the stress value at which two tangents cross which is defined as yield stress (Malvern Instruments, 2015). The corresponding value of shear stress to the intersection is given by the intercept of the red dotted line on x-axis, as shown in Figure 3.5.
With the assumption that gravitational force per area of PM is equal to the yield stress value, product build up was calculated as per Equation 1. Thickness of product build up could also be calculated using Equation 2 and Equation 3.

Product build up (kg/m²) = \( \frac{\tau_0}{g} \) \hspace{1cm} (1)

Thickness of product build up (m) = \( \frac{\text{product build up}}{\rho} \) \hspace{1cm} (2)

Thickness of product build up (mm) = \( \text{thickness of product build up} \times 1000 \) \hspace{1cm} (3)

Where: \( \tau_0 \) is yield stress (Pa or kg/ms²)

\( g \) is acceleration due to gravity (m/s²)

\( \rho \) is density (kg/m³). Assumption: \( \rho \) yoghurt = \( \rho \) water = 1000 kg/m³

The unit in kg/m² could be converted to g/dm² by multiplying with a conversion factor of 10. To express the product build up in thickness (mm), multiplication with conversion factor of one can be done.

Figure 3.5 Representative graph for tangent method for finding estimated yield stress value in Vanilla yoghurt 2.5% fat content
3.2.3 Texture Profile Analysis

The cohesiveness and firmness of yoghurt samples were measured in Texture Analyzer TA-XT2i (Stable Micro Systems, UK) using cylinder probe (d = 35mm) with back extrusion method (Figure 3.6). The test was carried out at 1 mm/s speed for pre-testing, during testing and post-testing. The probe was held for 10 s inside the container (d = 57mm) containing 50g of yoghurt and immersed at distance of 10mm. All the measurements were carried out in triplicate and the data were presented in averaged value.

![Figure 3.6 Picture of texture profile set up with cylinder probe](image)

The textural properties values were calculated using Exponent 6.1.15.0 software provided by Stable Micro Systems. The firmness measurement value was obtained from the highest force while the cohesiveness value was obtained from the maximum negative force from the Force (Newton) versus time (second) graph in Figure 3.7.
The yield stress was also assessed from the texture analysis measurement. This derivation is based on the principle of thixotropic fluid behaviors and was developed with Bergenståhl, B. (personal communication, March 14, 2019). For derivation of theoretical yield stress value, the yield stress was assumed to correspond to the frictional force per total wetted area of the probe. The frictional force here is assumed to be the tangential force between the product and the surface of the probe by considering the subtraction and addition of the buoyant force of water to the penetrating and retreating force respectively. The calculation also considers various correctional factors and geometrical scaling factors. A detailed mathematical script of the calculation is attached as Appendix C.

3.2.4 Fluorescence microscope

The fluorescence microscopy method was used to evaluate the distribution of fat globule and protein network in yoghurt. After reviewing some literatures, Nile Red and Rhodamine B were selected as dyes for fat and protein, respectively. The selected excitation and absorption spectra of the fluorophores were found compatible with the spectral profile of the available filter in the Emerging Lab in Food Technology Department, LTH, which are Fluorescein isothiocyanate filter (FITC) and Tetramethylrhodamine Isothiocyanate filter (TRITC). In this study, the sample preparation method was developed to be able to visualize the gel network of four yoghurt types and packaging layer (refers to Appendix D). A dilution factor of 1:10 of sample to whey was used for sample preparation. The whey used was obtained by filtration from the same variety of yoghurt. During the initial phase of
method development, Rhodamine B and Nile red dye were used to mark protein and fat respectively. However, both dyes produced same effect of fluorescence in the sample which was evident in the images obtained. In the yoghurt, milk protein aggregates build a gel network where fat globules are entrapped (Skytte et al, 2015), this could make it difficult to stain the protein and fat separately. Nile red dyes are also known to bind with very low density protein hence limiting their specificity to bind fat. (Rumin et al., 2015). Moreover, it was also observed that Rhodamine B seems to give images with better signal in both the high fat and low fat yoghurt. Therefore, it was decided to label the samples with only Rhodamine B

For the final test, four yoghurt samples were diluted with whey to the ratio 1:10. Rhodamine B (excitation and emission wavelengths of 543 and 625 nm, respectively) was used to stain the yoghurt samples. Delaminated polyethylene film was folded with the food contact side exposed on the outside and mounted on the glass slide as shown in figure 3.8. A piece of folded polyethylene film was mounted on the glass slide with cover glass and few drops of specimen were added through the space between cover glass and slide. The prepared glass slide was observed under TRITC filter. The microscope analysis was performed using Nikon Eclipse Ti-U with x 20 magnification objective with suitable filters and dilution to obtain a clear image. Pictures were taken along the interface of PE and yoghurt using Nikon software then analyzed with Image J software.

![Figure 3.8 Glass slide mounted with the packaging material (a), Schematic representation of sample on the glass slide (b)](#)

### 3.2.4 Susceptibility to Syneresis

The drainage method whereby 200 gm of yoghurt was placed on a funnel containing an ordinary coffee filter paper was kept undisturbed for 2hr at 5°C in a refrigerator. The amount of whey expelled was weighted at the end of 2hr. This method was
adapted from Hassan et al (1996) with slight modifications. The STS% was calculated by the equation:

\[
STS \ (%) = \frac{W_1}{W_2} \times 100\%
\]

Where, W1 is the whey expelled and W2 is the amount of yoghurt sample for the experiment.
4 Result

4.1 Characterization of product build-up through Gravimetric Dip Test Method

4.1.1 Quantification of product build up

The force (N) versus time (s) graph obtained from average of six replicates per yoghurt is shown in Figure 4.1. The graph shows the force when the PM is pulled up from the yoghurt sample at 1000mm/min after an incubation time of 10 minutes and is left hanging 2 cm above the yoghurt for 5 minutes.

Figure 4.1 Average of Time vs Force graph during pulling out and hanging of packaging material (PM) (six replicates per yoghurt)

The force required to pull out PM from yoghurt after incubation were different between high fat yoghurts and low fat yoghurts. The high fat yoghurt (Vanilla 2.5% and Natural 3%) required higher force during pulling out stage compared to low fat
yoghurt (Vanilla 0.5% and Natural 0.5%). The experimental Force value as a function of time was converted into product buildup (kg/m²) to compare the amount of build-up from four different types of yoghurt. At the end of hanging process (stage 2), the average of product build-up was calculated and presented in Figure 4.2.

The product build-up on the packaging surface was higher for the higher fat content. The product build-up of Vanilla 2.5% was higher than that of Vanilla 0.5% (0.74 kg/m² and 0.42 kg/m², respectively). Similar result was also shown in Natural yoghurt. The product build-up of Natural 3% (0.67kg/m²) was higher than Natural 0.5% (0.26kg/m²).

Table 4.1 Product build-up of four type of yoghurts on packaging material surface at 5 minutes of hanging (six replicates per yoghurt). The depicted values refer to the average±standard error of mean and the P values

<table>
<thead>
<tr>
<th></th>
<th>product build-up (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla low fat</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>Vanilla high fat</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>Natural low fat</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Natural high fat</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td>Vanilla 0.5% vs</td>
<td>P = 2.65E-05</td>
</tr>
<tr>
<td>Vanilla 2.5%</td>
<td></td>
</tr>
<tr>
<td>Natural 0.5% vs</td>
<td>P = 1.22E-05</td>
</tr>
<tr>
<td>Natural 3%</td>
<td></td>
</tr>
<tr>
<td>Vanilla 2.5% vs</td>
<td>P = 0.56</td>
</tr>
<tr>
<td>Natural 3%</td>
<td></td>
</tr>
<tr>
<td>Vanilla 0.5% vs</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>Natural 0.5%</td>
<td></td>
</tr>
</tbody>
</table>
A t-test utilizing Excel was performed to compare the differences between two types of yoghurt (Table 4.1). Both high fat yoghurts (Vanilla 2.5% and Natural 3%) had significant greater build-up than the low fat yoghurts (Vanilla 0.5% and Natural 0.5%) \((P < 0.05)\). The results showed that the amount of fat globules might influence the amount of product build-up on PM surface. Comparing the high fat yoghurts, the amount of product build-up was not significantly different \((P > 0.05)\). On the other hand, the amount of product build-up was significantly different among low fat yoghurts; vanilla low fat had higher product build-up than that of natural low fat yoghurt \((P < 0.05)\). The difference could be due to the formulation of low fat yoghurt. Low fat yoghurt is produced from partially skim milk or skim milk (Modhu, 2016). Milk protein is added to both natural and vanilla low fat yoghurt as it is stated in the packaging label. In the formulation of vanilla yoghurt, modified corn starch, pectin and sugar are added to increase the thickness, viscosity, and gelation of yoghurt (Magdaleno, 2016).

During the gravimetric dip test, it was observed that formation of canal occurred on the film of build up on the packaging material originating from the boundary between the clean surface and the surface immersed in yoghurt during incubation. The length of canal seems to progress with the hanging time. The canal formation is observed in all the four yoghurts in some but not all the replications during the test (Figure 5.1). However, the frequency of occurrence is more in the natural yoghurt with highest number of canals per replication in the high fat natural yoghurt. Canal on the low fat natural yoghurt shows the highest progressive increase in length as compared to the other samples.

The canal formation could be correlated to syneresis. The susceptibility to syneresis (STS) results are given in Table 4.2. The value was average of two replications of each yoghurt sample±standard error of mean.

<table>
<thead>
<tr>
<th>Sample</th>
<th>STS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla 0.5% fat</td>
<td>38.20±0.50</td>
</tr>
<tr>
<td>Vanilla 2.5% fat</td>
<td>37.93±1.31</td>
</tr>
<tr>
<td>Natural 0.5% fat</td>
<td>46.31±1.28</td>
</tr>
<tr>
<td>Natural 3% fat</td>
<td>41.83±3.00</td>
</tr>
</tbody>
</table>
Natural yoghurts both with high and low fat content had higher STS% as compared to the vanilla yoghurts. Low fat yoghurts (in both vanilla and natural) had higher STS% value compared to the high fat yoghurts.

4.1.2 Thickness of product build up measured by Laser Scanner

Figure 4.3 shows the representative thickness of product build up in one of the replicate for all four types of yoghurt during hanging period (5 minutes). The graphs from the remaining two replicates are presented in Appendix B.6. The thickness of product build up at 0min, 2.5min, and 5min of hanging corresponds to blue, red, and orange line, respectively. The thickness of product build up were 1-1.5mm for Vanilla 0.5%, Vanilla 2.5%, and Natural 3%. Meanwhile, Natural 0.5% got the lowest thickness value, which was less than 1mm. It was observed that the thickness of product build up decreased over 5 minutes in all yoghurt types.

Figure 4.3 Thickness of product build up of (a) vanilla 0.5%, (b) vanilla 2.5%, (c) Natural 0.5% and (d) Natural 3%
4.2 Yield stress measurement

Estimated values of yield stress from the rheometric method and texture analysis are given in the Table 4.3 below.

Table 4.3 Estimated value of yield stress (Pascal) from rheometer and texture analysis of four different yoghurt: standard error of mean

<table>
<thead>
<tr>
<th>Sample</th>
<th>Estimated yield stress obtained from Rheometer (Pa)</th>
<th>Calculated yield stress obtained from Texture Analyzer (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla 0.5% fat</td>
<td>5.03±0.10</td>
<td>20±0</td>
</tr>
<tr>
<td>Vanilla 2.5% fat</td>
<td>4.73±0.55</td>
<td>20±0</td>
</tr>
<tr>
<td>Natural 0.5% fat</td>
<td>3.24±0.22</td>
<td>20±0</td>
</tr>
<tr>
<td>Natural 3% fat</td>
<td>3.69±0.28</td>
<td>18.33±1.67</td>
</tr>
</tbody>
</table>

The result from the rheometric measurement shows high yield stress value for the vanilla yoghurt. Within the natural yoghurt category, the high fat yoghurt shows slightly higher value than the low fat yoghurt and vice versa for vanilla yoghurt. The result from texture analysis shows same value for all the yoghurt except the high fat natural yoghurt. The yield stress values from both the method do not have the same order of magnitude.

4.3 Texture Analysis

The firmness and cohesiveness of the yoghurt samples were measured for triplicates for each yoghurt. Average values with standard error are given for comparison in Table 4.4.

Table 4.4 Firmness and cohesiveness (Newton) of the yoghurt samples: standard error of mean

<table>
<thead>
<tr>
<th>Sample</th>
<th>Firmness (N)</th>
<th>Cohesiveness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla 0.5% fat</td>
<td>0.51±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Vanilla 2.5% fat</td>
<td>0.55±0.01</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>Natural 0.5% fat</td>
<td>0.57±0.00</td>
<td>0.13±0.00</td>
</tr>
<tr>
<td>Natural 3% fat</td>
<td>0.55±0.02</td>
<td>0.12±0.01</td>
</tr>
</tbody>
</table>
Comparing the vanilla samples, the higher fat yoghurt has somewhat higher value of both parameters. In the case of the natural yoghurt it is vice versa. Comparing natural and vanilla yoghurts, the high fat samples have similar values but the low fat vanilla yoghurt with added stabilizer seems to have lower value than the natural low fat yoghurt. Though different all the yoghurts are in the same range of measurement for firmness and cohesiveness.

4.4 Characterization of fat and protein distribution in yoghurt using Fluorescence Microscope

A fluorescence microscope is one of the techniques to characterize the morphology of yoghurt. The casein network in yoghurt can be visualized after staining with protein-specific fluorescent probes (Sozer, 2016). The results from method development is described in Appendix D.

Figure 4.4 shows the visualization of yoghurt gel network along packaging (Polyethylene) layer. The images shown below are representative of 3 sample replications with 10 images taken at different position along the packaging material in the prepared sample slides. There is a build up of aggregates along the packaging material in the diluted yoghurt. The picture shows the build-up as attached flocs of what appears to be the protein network. Presence of globular hollow granules (circled pictures) are also seen in the microscopic image of vanilla samples, which may be attributed to swollen starch granules. The thickness of the build-up in all yoghurt samples can be approximated to be around <300 micrometer.
Figure 4.4 Fluorescence microscope pictures of (a) vanilla 0.5%, (b) vanilla 2.5%, (c) Natural 0.5% and (d) Natural 3% at x20 magnification. Samples were diluted with whey in 1:10 ratio and stained with Rhodamine B
5 Discussion

5.1 Characterization of Product Build-up through Gravimetric Dip Test Method

5.1.1 Quantification of Build up

The gravimetric dip test can be used as one of methods to quantify product build-up on PM surface. During the test, temperature of yoghurt should be monitored to control the system, i.e. no viscosity changes due to higher temperature. The experimental product build-up obtained from this method agrees with the order of magnitude measured with rheometer as per the comparison in section 5.4 and estimated by fluorescence microscopy. In this method, fat content and stabilizers had effect on product build-up. High fat yoghurts of Vanilla and Natural had higher build up than that of Vanilla and Natural low fat yoghurts. In low fat yoghurt, vanilla had more build up compared to natural yoghurt. Similar effect of fat content on build up at short incubation time (<20 h) was noticed in the study carried out by Hansson & Skepö (2012). However, this effect is said to be short lived and disappear at longer incubation time.

The canal formation (as shown in Figure 5.1) observed during this test may be attributed to dewetting phenomenon. Dewetting phenomenon results in a product run off in a plug like feature as reported by Cragnell et al. (2014). This canal formation on the regular run offs are attributed to imbalance in the cohesive forces within the product and the adhesive forces between the product and the surface. Since the cohesive forces in the yoghurt types are not that different, this could point towards difference in adhesive forces in the yoghurt. Another possible explanation is the formation of thin serum phase due to syneresis at the product surface interface during incubation. This theory is supported by the observation that higher the STS value more is the canal formation. The lower value of STS in the vanilla yoghurt could be due to the presence of stabilizers and reduced syneresis as compared to the natural yoghurt.
The correlation of the canal formation with the product build up is only seen in the low fat natural yoghurt. According to visual observation, it is assumed that greater the number of canals and more developed the dimension are, lower the amount of build up. Though canal formation is more pronounced in the high fat natural yoghurt as compared to the vanilla yoghurt, it does not correlate to the gravimetric build up. A possible explanation is that the canals were not developed until the end of the PM to make a significant difference in the build up for the high fat natural yoghurt.

![Figure 5.1 Example of canal formation at 5 mins of hanging of (a) vanilla 0.5%, (b) vanilla 2.5%, (c) Natural 0.5% and (d) Natural 3%](image)

5.1.2 Thickness of product build measurement using laser scanner

The thickness of product build up measured by laser scanner was approximately 1-1.5 mm for Vanilla 05%, Vanilla 2.5%, and Natural 3% and less than 1 mm for Natural 0.5%. The results were comparable to the thickness obtained from GVM and rheometer as explained in section 5.4. The thickness value obtained from laser scanner was slightly higher because the measurement was taken only along one position. However, this methodology reasonably agrees with the thickness measurement from GVM dip test and rheometer.
5.2 Yield stress measurements

Based on the calculated yield stress values performed by rheometric and texture analysis methods, it can be said that the different methods give different results. The yield stress values obtained from the rotational rheometer showed a clear difference in the yield stress between the Vanilla and Natural yoghurt. However, small differences are observed between the yoghurt with high and low fat content both for the vanilla and natural yoghurts. As per Yu, Wang & McCarthy (2016), addition of low fat yoghurt with MSNF give higher value of yield stress than the high fat yoghurt. This confirmed that added protein and stabilizers have more influence on the yield stress than the fat content as is seen in this study.

Yield stress measurements done by Ostréus & Williamson (2019) gave values with same order of magnitudes as obtained in this study. However, they reported natural yoghurt 0.5% fat to have higher yield stress as compared to vanilla yoghurt with 2.5% fat. Regarding the influence of protein, since all the yoghurt samples have around the same value of protein content (3.6 to 4.1 gm/100gm), comparison cannot be made in the influence of protein content.

A yield stress value was also obtained from texture analysis. The yield stress value obtained for the texture analysis are higher than the value obtained from the rheometric analysis. In the test method using the texture analyzer the probe is not completely immersed in the sample and the calculations are also affected by the geometrical factors such as the diameter of probe and the container. These could be possible reason for the difference in yield stress value from the rheometric method. Due to these limitations, the yield stress value from this method will not be used to predict the build up. However, this method has potential but it need more time to be further developed.

5.3 Texture Analysis

The result obtained from texture analysis showed that the firmness and cohesiveness values of different types of yoghurt does not seem very different from each other. As per Yu, Wang & McCarthy (2016), the fat content of the yoghurt does not have any significant influence on the firmness nor the cohesiveness. However, addition of MSNF have shown to increase the parameter based on the explanation that texture in yoghurt is more dependent on the strength of the casein micelle network. Both low fat yoghurt have milk protein added to it and the Natural low fat yoghurt seem to have the highest firmness and cohesiveness. Thus, the hypothesis seems to stand true for the low fat Natural yoghurt but not for the low fat vanilla yoghurt.
Also, it seems that the added stabilizer does not seem to influence this measurement. It can be speculated that maybe not enough amount is added to have a significant influence. Modified starch increases firmness by binding and aligning the water molecule increasing the viscosity but only until a certain level. At higher concentration swelling of the starch granules hinder formation of casein chain which makes up the protein network as reported by various researchers. (Sandoval-Castilla et al., 2004; Radi & Amiri, 2009).

5.4 Comparison of experimental and theoretical build up

Possible correlation between yield stress value and product build up can be made based on the samples starting to flow off from the packaging material during gravimetric drip test when the gravitational force exerted exceeds the yield stress. Comparison could then be drawn between the experimental build up and the theoretical build up obtained from the yield stress measurement to see how comparable the results are. The conversion of yield stress to build up value was described in Equation (1) in Section 3.2.2. The correlation between the experimental and the predicted values are shown in the Figure 5.2. A table with compilation of the values with standard error of mean is given in Appendix E.1

The measurement from the gravimetric dip test and the rheometric test shows the same order of magnitude and agrees reasonably. Both low fat natural and vanilla yoghurt shows higher degree of correlation compared to the high fat yoghurt. The diagram seems to show that product build up is not fully explained by the yield stress value in the high fat yoghurts. Possible explanation could be due to the structure at the material/product interface and the fat content.

![Figure 5.2 Correlation between measured and predicted product build up in kg/m²](image-url)
5.5 Characterization of Fat and Protein Distribution in Yoghurt using Fluorescence Microscope

From the images obtained in the fluorescence microscope, build up appears along the packaging material in the form of aggregated flocs. The observed flocs comprise of the aggregated protein network with fat globules. This method can characterize the fat and protein distribution in diluted yoghurt. The limitation of this method is that the yoghurt must be diluted to obtain a clear image. The image obtained from undiluted yoghurt showed dense dyed aggregates of yoghurt, thus, it was difficult to observe the build up along the packaging material (Figure in Appendix D.1). Both fluorescent probes used in this study (Nile Red and Rhodamine B) could stain the aggregates in the yoghurt but could not distinguish the casein micelles and fat globules. This could be due to that the fat globules are embedded in the protein gel network (Skytte et al, 2015). The capillary movement of diluted yoghurt towards underneath the PE layer could affect the accumulation of flocs along the PE layer.

Presence of distinctively globular masses with the protein flocs in the vanilla sample point towards the role of stabilizers in the build up. The assumption that these granules could be protein from the skim milk powder was dismissed because these globules were also observed in the high fat vanilla samples which do not contain skim milk powder. The size of these globular structures is approximated to be 50 micrometer, which corresponds to 5-micrometer starch granules that can swell up in presence of water and increase 10 times the original diameter due to starch gelatinization (Gryszkin et al., 2017). The approximated thickness of the build-up is less than 0.3 millimeter (300 µm) which corresponds to a build up of less than 0.3 kg/m². This agrees with the order of magnitude of theoretical and experimental build up value as presented in section 5.4.
6 Conclusion and suggestion for future actions

6.1 Conclusion

Measurement of product build-up on packaging material could be affected by several factors, i.e. fat content, addition of stabilizers and method of quantification. Fat content and stabilizers had effect on product build quantified by gravimetric dip test. The effect of fat content is more pronounced while stabilizer seems to have some influence when yoghurt of similar fat content is compared. The influence of protein content has not been evaluated, since all the yoghurt samples have around the same value of protein content. The phenomenon of syneresis results in formation of thin serum phase. This serum phase causes dewetting on the PM leading to canal formation. This canal formation to some degree influences the build up especially in natural low fat yoghurt.

Product build-up can be measured using experimental and calculation approach by gravimetric dip test and calculated yield value from rheometer. These experimental and theoretical values have the same order of magnitude thus showing reasonable agreement between the yield stress value and the build up. There is a stronger correlation between the yield stress and the build up in the low fat vanilla and natural yoghurt than the high fat yoghurt. This correlation is based on the comparison of measured and predicted build up value.

There does not seem to be a direct correlation between the textural parameters and the product build up. The values for such measurement are similar for all the yoghurt. These parameters are perhaps maintained and or modified to be similar when processing to give the desired textural attributes to the consumer irrespective of the fat content.

Fluorescence microscope is one of method to visualize the yoghurt components along the polymer surface. The image from the fluorescent microscope shows what might appear to be aggregated protein flocs with swollen starch granules in the build up. However, visualization of fat globule is difficult because the fat globules are
entrapped within the protein network and thus the whole system gets labelled as one by the rhodamine marker.

To conclude, the methods investigated in this work can be used to evaluate the yoghurt product build up on packaging material surfaces. However, the products investigated did not give large differences. Moreover, a stronger correlation between the yield stress property and amount of product build up was seen in the low fat yoghurts suggesting other factors influencing the amount of build up in the high fat yoghurts. Further investigations could involve products with larger differences in character.

6.2 Suggestion for future actions

Some of the methods developed in this study can be implemented to measure product build up of yoghurt on packaging material surface. The proposed method to quantify product build up experimentally and theoretically are gravimetric dip test and yield stress measurement using rheometer, respectively. Calculating the thickness of product build up can be done also by using laser scanner. Further development of laser scanner method, i.e. determining the position of laser scanner and data processing could be improved to obtain the true representative thickness of product build up.

Measuring the product build up with different methods is a challenging work. Some improvement would be needed to obtain robust data from different methods. The yoghurt sample should have the same date of production and same handling during distribution and transportation to prevent any external influences. It would be good to have information about the condition of yoghurt before purchasing.

Nonetheless, it would be interesting to study in the future the build up of other food products in the same category or on other packaging material surfaces using the proposed methods.
References


https://www.tandfonline.com/doi/abs/10.1081/JFP-20005949 on 7th March 2019


Stenmarck, Å., Jensen, C., Quested, T., Moates, G., Buksti, M., Cseh, B., ... & Scherhaufer, S. (2016). Estimates of European food waste levels. IVL Swedish Environmental Research Institute.


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Appendix A. Work distribution and time plan

A.1 Work distribution

All of the experiment was conducted in equal part by two members with same background of study. The preliminary experiments done with in depth discussion and consensus of both members to finalize the parameters that would be used in the final experiments. The main experiments entailed the joint effort from both the members. Accordingly, equal work distribution was done for successful completion of experiments. The writing part was also distributed equally between the member. However, the member extensively reviewed each other’s work and engaged in discussion when required. The final editing and formatting was done together.

A.2 Project plan and outcome

A project timeline was made during the initial project proposal to estimate the time and to keep track of the progress. Minor changes were made to adjust the availability of the equipment and to ensure the completion of the thesis on time. Also, two more experiments, i.e. the STS test and the laser experiments were added. The initial and actual time plan is presented in Figure A.1 and Figure A.2.
Figure A.1. Initial time plan
Figure A.2 Actual time plan
Appendix B. Method development for Gravimetric Dip Test

Preliminary tests were conducted to identify, define & optimize the parameters that might affect the product build up during the gravimetric dip test. The parameters were:

1. Process set up
2. PM preparation
3. Mode of hanging of PM
4. Speed of pulling up of PM with the Instron tensile tester

B.1 Process set up

Objective - To determine an optimal set up with a time-temperature combination for the experiment.

Table B.1. Design setup of experiment

<table>
<thead>
<tr>
<th>Set up 1: One Packaging Material (PM) in one yoghurt container</th>
<th>Set up 2: Multiple PM(three) in one yoghurt container</th>
</tr>
</thead>
<tbody>
<tr>
<td>● The whole test is done in the laboratory at ambient temperature.</td>
<td>● PM was manually inserted in the yoghurt and left to incubate for 8min for 4 °C in the cold store.</td>
</tr>
<tr>
<td>● Sequence 1(Approx. 20 seconds): Dipping process. Clamp Set speed at 200mm/min and 60mm as the distance when the load moves from zero position/starting point.</td>
<td>● At the 8th minute, the yoghurt with PM was carried to the instron and clamped with initiation of sequence 1 after the 10th minute.</td>
</tr>
<tr>
<td>● Sequence 2: Incubation process. PM is incubated for 10mins</td>
<td>● Sequence 1 (Approx. 20 secs): Pulling out process. Speed: 200mm/min.</td>
</tr>
</tbody>
</table>
**B.2 PM preparation**

**Objective** - To determine if differently prepared PM had any effect on product build up.

Differently prepared PM as shown in Figure B.1 was used for the test to see if PM preparation had any effect on the build up. All the PM were prepared from the TBA with the same dimension, specific treatments differed in the PM preparation. The tests were performed in vanilla yoghurt with 2.5% fat content with 2 replicates each. The treatments were:

- **Treatment 1** - Pressed gently with hand.
- **Treatment 2** - Pressed firmly.
- **Treatment 3** - Pressed firmly and creased.
- **Treatment 4** - Pressed firmly and zig zag pattern.
- **Treatment 5** - Pressed gently and creased.

Result - It was observed that set up 2 with single sample instead of multiple sample in the same container gave better control over temperature. Temperature measurement recorded 7°C to 10°C in the natural yoghurt samples and 7°C to 9°C in the vanilla samples right before incubation with an increase of 1°C after incubation for 10 minutes in all the samples). This setup was also more time effective and gave better reproducibility in the result. Furthermore, the same yoghurt sample in the container could be used thrice by incubating the PM in different position in the sample.

<table>
<thead>
<tr>
<th>Sequence 3 (Approx. 20 secs): Pulling out process. Speed: 200mm/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 4: Hanging process. PM is hanging out for 5 mins.</td>
</tr>
<tr>
<td>Sequence 2: Hanging process. PM is hanging out for 5 mins.</td>
</tr>
</tbody>
</table>
Result - The assumption was that the creased sample would have more build up and the irregular zig zag cut sample at the edge would have less build up. For the later reasons for less build up would be the prevention of accumulation at the bottom edge of the PM. However as per the result shown in Figure B.2 as accumulation in mass (gm) during the five minute hanging time, the un-creased sample had the highest build-up followed by the zig zag cut samples. It was thus concluded that the PM preparation performed was of low importance for the build up. For the final test, treatment 2 was selected as the mode of packaging material preparation.
B.3 Mode of hanging of PM

Objective - To determine if hanging the PM inside the yoghurt would prevent accumulation at the edge of the PM.

Test with PM hanging 5mm inside and 20mm outside the yoghurt was done to compare if having the PM inside the yoghurt could help in understanding the effect of accumulation at the end of the PM. The test was performed in Natural yoghurt 0.5% and 3% fat content with two replicates each.

Result - The build up in both the test in low and high fat natural yoghurt is recorded as mass build up in gram within the 5 minutes (shown in Figure B.3). As per the hypothesis with prevention of accumulation, the yoghurt should flow into the container without clumping at the edge of the PM, but as per the result, there was not any distinct pattern or difference in the measurement. It was concluded that this procedure did not give difference in the product build up.
B.4 Speed of pulling up of PM with the Instron tensile tester

Objective - To see the influence of speed of pulling up the PM on the product build up.

Experiment was performed with 200, 500 and 1000 mm/min speed of taking out the sample from vanilla yoghurt 2.5% with 3 replicates. Time required for the operation of pulling out the PM was 20, 5 and 4 seconds respectively.

Result – In Figure B.4, the highest speed of 1000mm/min had more product build up as compared to 200 & 500 mm/min speed. This effect was observed in all the three replications of the 1000mm/min speed. Between the 200 and 500 mm/min speed no distinct difference is observed. Since the operation of emptying yoghurt from the PM at consumer is thought to be a quick process, the speed with lowest withdrawal time i.e. the highest speed 1000 mm/min was selected as the speed for the final tests.
Figure B.4. Mass versus time curve during five-minutes hanging time of PM pulled out at 200, 500 and 1000 mm/min speed in vanilla yoghurt 2.5% fat

B.5 Final test to characterize build up in all the yoghurts

With the parameters defined from all the prior mentioned test, the final quantification experiments were conducted with 6 replicates for each yoghurt type. An average representation from the six replicates are taken with standard error for the final result in Figure B.5. Temperature measurement are taken at 1 cm above the bottom of the container and below the surface of the yoghurt at the front and back of the container with sample.
Temperature measurement were taken at 1 cm above the bottom of the container and below the surface of the yoghurt at the front and back of the container with sample. Temperature measurement were taken before and after incubation of the PM in the sample and an average with standard error is taken as the representative temperature from the 4 measured points (Figure B.6). The temperature differences were within 2-4°C per yoghurt.
Figure B.6. Temperature measurement prior and after incubation in Vanilla 0.5%, Vanilla 2.5%, Natural 0.5% and Natural 3% from top left to right and bottom left to right.
B.6 Laser scanner results

Figure B.7. Laser scanner results of all replicates of Vanilla 0.5% fat (the file of first replicate was corrupted and could not be accessed)

Figure B.8. Laser scanner results of all replicates of Vanilla 2.5% fat
Figure B.9. Laser scanner results of all replicates of Natural 0.5% fat
Figure B.10. Laser scanner results and pictures of PM at 5 mins of hanging of all replicates of Natural 3% fat
Appendix C Calculation of yield stress from TPA

The tangential force between the product and the surface of the probe is assumed to the frictional force. The product yields to the penetration and retracting force only when the frictional force per area is overcome. Thus, frictional force per area corresponds to the yield stress in this calculation.

\[
\frac{F_f}{A} = \tau_0
\]  \hspace{1cm} (4)

Where:
\( F_f \) = Frictional Force  
\( A \) = Total wetted area of the probe  
\( \tau_0 \) = Yield stress

Geometrical measurements are:
Diameter of probe = 0.035m and Diameter of container = 0.057m.

Physical constraints are:
Acceleration due to gravity (\( g \)) = 9.81ms\(^{-2}\) and density of the yoghurt (\( \rho \)) = 1000kgm\(^{-3}\).

\( X \) will be the corrected total contact length by considering the rise of liquid, \( x \) the penetration distance obtained from the instrument and \( x' \) the rise of surrounding sample in the cup when probe is immersed in it.

Figure C.1 is a diagrammatic representation of geometrical factors considered in the calculation.
The calculations were made in the following steps toward finding the value of the frictional force per area.

**Step 1**- Calculation of geometrical scaling between penetration and rising liquid

\[ A_{\text{probe}} \cdot x = (A_{\text{container}} - A_{\text{probe}}) \cdot x' \]  
(5)

\[ X = x + x' \]  
(6)

Solving equation 5

\[ x' = \frac{A_{\text{probe}} \cdot x}{A_{\text{container}} - A_{\text{probe}}} \]  
(7)

Substitution \( x' \) equation 6

\[ A_{\text{probe}} \cdot x = (A_{\text{container}} - A_{\text{probe}}) \cdot x' \]  
(8)

\[ X = x + A_{\text{probe}} \cdot \frac{x}{A_{\text{container}} - A_{\text{probe}}} \]  
(9)

\[ A_{\text{probe}} = \frac{\pi \cdot (d_{\text{probe}})^2}{4} \]  
(10)

\[ A_{\text{container}} = \frac{\pi \cdot (d_{\text{container}})^2}{4} \]  
(11)

Where:

\[ A_{\text{probe}} = 0.00096m^2, A_{\text{container}} = 0.00255m^2 \]

Using this values in equation (9), we get
$X = 1.60x$, this is the geometrical scaling between penetration and rising liquid. Therefore, corrected value of $X$ (total contact length) is obtained by multiplying the value of $x$ with 1.60.

**Step 2** – Determine the slope $d\text{Force}/dx$ for sample and water from the raw data and the $x'$ value.

**Step 3** – Obtain $d\text{Ff}/dx$.

As per the direction of the force, when the probe is penetrating in the sample the direction force is downward. This force is the force required to overcome the buoyance force ($Fb$) of the water in the sample and the frictional force ($Ff$) both in the opposing direction. When the probe is withdrawn, the force required is the $Fb$ minus the $Ff$. A schematic representation is given in Figure C.2.

![Schematic representation of the forces involved](image)

**Figure C.2. Schematic representation of the forces involved**

A mathematical representation given below:

\[ Ff = F_{down} - Fb \] 
(10)

When the probe penetrates in the sample

\[ Ff = Fb - F_{up} \] 
(11)

Thus, \( dFf/dx = F_{down}/dx - dFb/dx \) 
(12)

And \( dFf/dx = Fb/dx - dF_{up}/dx \) 
(13)

**Step 4** – Estimation of buoyancy force ($Fb$) from the liquid acting on the probe

\[ Fb = X, A_{probe}, \rho, g = 9.438 \times X \] 
(14)
\( X.A probes \) is the wetted part of the probe

The actual numbers obtained for water was compared with estimated buoyance and the magnitude differs with 0.015m penetration giving 0.26 N while it should be 0.142. The experimental slope is 14.427 while theoretically it should be 9.43.

Thus, a correction factor \( (cs) \) was estimated.

\[
\text{cs} = \frac{\text{experimental slope}}{\text{theoretical slope}}
\]

\( cs = 1.568 \), this value would be multiplied to the \( dFf/dx \) values to obtain a corrected slope.

**Step 5** – Obtain the corrected value of \( dFf/dx \) by multiplying with 1.56.

**Step 6** – Obtain the value of \( dFf/dA \) from \( dFf/dx \) using the chain rule

\[
dFf/dx = dFf/dx - dA/dx
\]

\[
dA/dx = \pi(dprobe + dcontainer(1 - x/X))
\]

Thus, series of data set for \( dFf/dA \) can be obtained using this calculation on the raw data.

**Step 7** – Obtain a \( dFf/dA \) versus \( X \) graph from the treated data. Figure C.3a and C.3b shows a sample of before and after image of how the graph looks after the raw data have been treated and calculated to give value of \( dFf/dA \). The \( dFf/dA \) value corresponding to the force upward line was taken as the yield stress value in Pascal.

![Figure C.3a. Force versus distance curve in Natural yoghurt 0.5% fat content](image)
Figure C.3b. Frictional force per area versus distance curve after treatment of the raw data
Appendix D Method development for characterization of fat and protein distribution and interaction with packaging material using Fluorescence Microscope

The method development was aimed to identify, define & optimize the parameters to be able to observe the interaction of yoghurt with packaging material.

The parameters were:

D.1 Concentration of yoghurt used as specimen on glass slide

The first preliminary was done to find the best yoghurt concentration to obtain a clear image. Different dilution factors were proposed, ranging from 1 to 100 dilution factors. Each yoghurt was filtered using filter paper to obtain whey which is used as the solvent. Figure D.1 below shows the observation of yoghurt stained with Rhodamine B at x20 magnification with different dilution factor. The undiluted yoghurt showed dense network all over the picture with no clear contrast with the background. Dilution ratio 1:10 showed clear image and finally used as the dilution factor to compare fat and protein distribution in other yoghurt types. Samples were also prepared with higher dilution of 1:20 to see if it’s any different from the 1:10 dilution factor. In this preparation, the yoghurt component was too diluted and very less amount was visible on the pictures.
Figure D.1 Different dilution factor of Natural yoghurt 3% with Rhodamine B at x20 magnification
D.2 Placing PE layer on the glass slide

Different glass slide preparation aimed to prevent capillary movement of whey towards underneath PE layer. Folded PE layer attached on the glass slide was chosen due to no different result obtained from different sample preparation and it is the simplest way of PE layer preparation.

D.3 Determining the fluorescent probes to stain the specimen

There are many fluorescent probes used to stain yoghurt and each probe require different wavelength to emit the colour. TRITC and FITC are the available filter cubes in the microscope used during this experiment (Nikon Eclipse Ti-U). According to the available filter in microscope used during this experiment, only Rhodamine B and Nile Red that can be used as the markers. The excitation/emission wavelength of Rhodamine B and Nile Red is 540/625 nm and 485/525 nm, respectively (shown in Figure D.2 and D.3). Both dyes used in this study (Nile Red and Rhodamine B) could stain the aggregates in the yoghurt but could not distinguish the casein micelles and fat globules (Figure D.4). Rhodamine B seems to give images with better signal in both the high fat and low fat yoghurt. Therefore, it was decided to label the samples with only Rhodamine B.

Figure D.2. Fluorescence and Absorption of Rhodamine B (top) (Ioannides et al, 2014). Bandpass filter cubes of TRITC (bottom) (Retrieved from Nikon.com, 2019)
Figure D.3. Fluorescence and Absorption of Nile Red (Greenspan & Fowler, 1985) Bandpass filter cubes of FITC (Nikon.com)

Figure D.4. Diluted Vanilla 0.5%:whey = 1:10 stained with Nile red (left) and Rhodamine B (right) at x20 magnification
Appendix E Table of measured and predicted product build up value

Table E.1 Measured and Predicted product build up in four yoghurts with 6 replications in GVM thickness and 3 replications in rheological measurement in average±standard error of mean

<table>
<thead>
<tr>
<th></th>
<th>Vanilla 0.5%</th>
<th>Vanilla 2.5%</th>
<th>Natural 0.5%</th>
<th>Natural 3%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GVM thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kg/m²) (measured build up)</td>
<td>0.42±0.03</td>
<td>0.74±0.04</td>
<td>0.26±0.03</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td><strong>Rheometer (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(predicted build up)</td>
<td>0.51±0.01</td>
<td>0.48±0.01</td>
<td>0.33±0.02</td>
<td>0.38±0.03</td>
</tr>
</tbody>
</table>

Conversion to g/dm² and mm could be achieved by multiplying the value with conversion factor of 10 and 1, respectively.