Wageningen University

Food Technology

Food Quality and Design

The influence of lactic acid bacteria on the cheese texture during ripening

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September 2018 – January 2019

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Abstract

The texture of cheese is a very important quality parameter. The texture is primary determined by the ratio moisture to intact casein, its pH the fat content. Cheese ripening is important to develop the texture characteristic of the cheese variety. Lactic acid bacteria (LAB) play a key role during ripening of cheese; the non-starter bacteria (NSLAB), which are present in raw milk, contains a lot of proteolytic enzymes and the starter bacteria (SLAB) is added to start the fermentation process. It is already known how the texture of cheese is developing during ripening, but not a lot of research has been done about the contribution of NSLAB and SLAB to the cheese texture during ripening. To research this, three different cheese samples were made. One was made from raw milk with SLAB, so it contains NSLAB and SLAB. The second was made from pasteurized milk with SLAB, so it only contains SLAB. And the third one was made from raw milk without a starter, so it only contains NSLAB. A chemical acidifier, GDL, was added to the third sample to replace the need of SLAB. The samples were stored in a 16°C stove for 47 days for ripening. At 8 different days samples were taken to measure the difference in texture, the pH, moisture content, protein content and proteolysis. The texture is analyzed with a texture analyzer and the proteolysis is measured with SDS-PAGE. The results show that cheese which only contain NSLAB, has the highest rate of proteolysis. Secondly, cheese which contain NSLAB and SLAB and thirdly cheese which only contain SLAB, shows the lowest rate of proteolysis. The rate of proteolysis corresponds with the softness; when the rate of proteolysis increases, the softness increases. The absolute softness of the cheese is highly dependent on the moisture content; the higher the moisture content, the softer the cheese. Overall, this research has shown that cheese with only NSLAB ensures the highest rate in proteolysis and thus in the highest rate of softening.

Keywords: LAB, texture, proteolysis, pH, casein, moisture

Contents

1 Introduction 6
1.1. Background 6
1.2. Problem definition 6
1.3. Objective 7
1.4. Research questions 7
1.5. Hypothesis 7
2. Theoretical background 8
2.1. Composition of milk 8
2.1.1. Casein 8
2.1.2. Whey proteins 8
2.1.3. Minerals 8
2.1.4. Milk lipids 8
2.2. Cheese manufacturing 9
2.2.1. Milk treatment 9
2.2.2. Acidification 9
2.2.3. Coagulation 9
2.2.4. Cutting the coagulum 10
2.2.5. Heating the curd and syneresis 10
2.2.6. Whey removal 10
2.2.7. Milling the curd 10
2.2.8. Ripening 10
2.3. Cheese ripening 10
2.3.1. Lactic acid bacteria 10
2.3.2. Glucono-δ-lactone 11
2.3.3. Rennet 11
2.3.4. Calcium chloride 11
2.3.5. Natrium chloride 12
2.3.6. Natamycin 12
2.4. Texture development during ripening 12
2.4.1. Proteolysis 12
2.4.2. pH 12
2.4.3. Fat content 13
2.4.4. Moisture content 13
3. Materials and method 14
3.1. Experimental overview 14
3.2. Cheese making 14

3.2.1. Sample preparation 14
3.2.2. Milli-cheese procedure 15
3.3. Analysis of the cheese samples 15
3.3.1. pH measurement 15
3.3.2. DUMAS 15
3.3.3. SDS-PAGE 16
3.3.4. Texture Analyzer 16
4. Results and discussion 17
4.1. Sample preparation 17
4.1.1. Cheese milk composition 17
4.2. Analysis of the cheese samples 17
4.2.1. Moisture content 17
4.2.2. Protein content 18
4.2.3. pH 18
4.2.4. Protein weight 19
4.2.5. Texture analyze 20
5. Conclusion 21
6. Recommendations 22
6.1. LAB isolation and identification 22
6.2. Texture analyzer 22
7. References 23
8. Appendix 25
8.1. Milk standardization 25
8.2. Layout of well plates 25
8.3. Milli-cheese procedure 25
8.4. MilkoScan 26
8.5. Texture analyzer 26
8.6. pH measurement 26
8.7. SDS-PAGE 27
8.8. Ethical appendix 28

List of abbreviations

LAB: lactic acid bacteria

SLAB: starter lactic acid bacteria

NSLAB: non-starter lactic acid bacteria

GDL: glucono-δ-lactone

RS: cheese from raw milk + starter culture

RG: cheese from raw milk + GDL

P: cheese from pasteurized milk + starter culture

1 Introduction

1.1. Background

In 2005, 'Boerenkaas' became a Guaranteed Traditional Specialty (GTS). The accompanying product specification states that the milk for 'Boerenkaas' must not have undergone a heat treatment higher than 40 °C. In practice this rule appears to be overridden. A large share of the 'Boerenkaas' that comes on the market, would be made from milk heated to above 40 °C (Zelfkazer, 2011). When heating the milk above 40 °C, non-starter lactic acid bacteria (NSLAB) are killed. This is favorable for the cheese-maker, because presence of NSLAB introduces variability into the ripening process that cannot be easily controlled (Settanni & Moschetti, 2010).

Cheese is a viscoelastic material that consists of a continuous network of casein in which fat globules and water are interspersed, which is formed during ripening (Gwartney et al., 2002). The ripening process of cheese is very complex and involves microbiological and biochemical changes to the curd resulting in the flavor and texture characteristic. Three primary events occur during ripening; glycolysis, proteolysis and lipolysis. Proteolysis is the most complex of three primary events during cheese ripening and possibly the most important for the development of texture (Fox & Law, 1991).

Cheese has a few main components: protein, fat, water, and sugar. Each of these can influence the final texture of cheese, but the main texture of cheese is the protein casein. The texture of cheese changes during ripening because of hydrolysis of the casein micelle by proteolysis and changes to the water-binding ability of the curd and changes in pH (Lawrence, Creamer, & Gilles, 1987). This texture development takes place in two distinct phases. The first phase takes place in the first 7 to 14 days of the process, in which a small fraction of the α_{s1}-casein is hydrolyzed. This results in a network of casein that is greatly weakened. In the second phase a relatively slow change in texture takes place due to the breakdown of the other α_{s1}-caseins, which is determined by the rate of proteolysis (Lawrence et al., 1987). This second phase can take ranging from 4 weeks to more than 2 years, dependent on the desired cheese type.

During cheese production, lactic acid bacteria (LAB) play an important role. First, the starter lactic acid bacteria (SLAB) contributes to the curd formation; it converts lactose into lactic acid. This ensures the correct pH for coagulation (Leroy & De Vuyst, 2004). Second, the non-starter lactic acid bacteria (NSLAB) contributes to the ripening process, since it possess a wide range of hydrolytic enzymes and therefore have the potential to contribute to cheese maturation (Settanni & Moschetti, 2010). However, still little is known about the influence of NSLAB and SLAB on the texture development of cheese.

1.2. Problem definition

It is already known how the texture of cheese is developing during ripening, but what is not known yet is what influence NSLAB and SLAB have on the cheese texture development during ripening. Therefore, it is interesting to research what influence the different LAB have on the cheese texture during ripening.

To answer this question three different cheeses are made. The first one is made of raw milk, which contains NSLAB. The second is made of raw milk, with added SLAB. The third one is made of pasteurized milk with SLAB added.

1.3. Objective

The aim of this research is to determine the difference between the influence of the NSLAB and/or SLAB on the texture of cheese during ripening.

1.4. Research questions

- What influence do the different LAB have on the ratio moisture to intact casein of the cheese during ripening?
- What influence do the different LAB have on the softness of the cheese during ripening?
- What is the correlation between the amount of intact casein and the change in softness of the cheese?

1.5. Hypothesis

In the raw milk cheese, a higher proteolytic activity is expected, as NSLAB contains a high number of proteolytic enzymes (Williams and Banks, 1997; McSweeney et al., 1993). The difference between the two raw milk cheeses is the mode of acidification added; GDL or SLAB. GDL will only contribute in reducing the pH to prevent spoilage (Fox & Law, 1991). The SLAB contain enzymes which are expected to contribute to the proteolysis in the ripening process. A higher amount of proteolysis result in an overall softer cheese (Bertola, Bevilacqua, & Zaritzky, 1991). The NSLAB cannot compete with the SLAB and therefore decrease in numbers (Mounier et al., 2008).
In conclusion, the cheese which only contains NSLAB is expected to have the highest proteolytic activity which will result in the softest cheese texture. Second, the cheese which contain NSLAB and SLAB and at last, the pasteurized milk cheese, is expected to have to lowest proteolysis and thus the least loss in firmness during ripening.

2. Theoretical background

2.1. Composition of milk

The milk that is used for this experiment is originating from the cows from the Carus farm on the WUR campus. The milk composition is constantly changing from day to day, due to different conditions, for example the temperature. But, cowmilk always consist of the main components; casein proteins, whey proteins, lipids, lactose, minerals, minor components (enzymes, free amino acids, peptides) and water. These components will be further discussed in this section.

2.1.1. Casein

Casein is the main structural protein of both rennet- and acid-induces milk gels. Casein in milk exist in the form of spherical-shaped colloid particles, known as micelles. The casein micelle is an associate of thousand small nanoclusters. These nanoclusters are the building block of the self-assembling casein micelles (Tuinier & De Kruif, 2002). The structure of a micelle is shown in image 1. The casein consists of four main types: α_{s1}, α_{s2}, β and κ (Hill, 2000). The casein micelle has κ-caseins at the surface. One part of the κ-casein is inside the micelle and one part is outside the micelle. The exterior part of the κ-casein provides steric stabilization of the casein micelles. In cheese-making the steric stabilization is removed enzymatically and that causes gelation into the cheese curd (Tuinier & De Kruif, 2002). During cheese ripening, the caseins are broken down into smaller proteins by proteolysis.

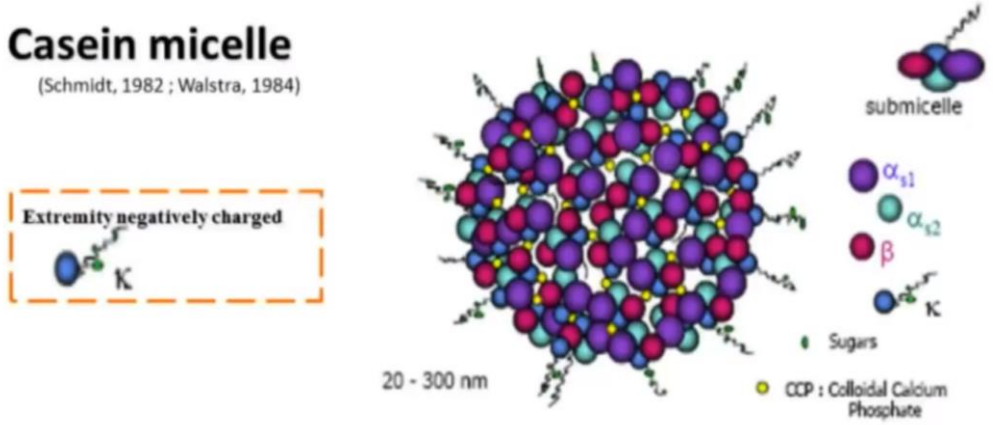

Figure 1 The structure of a casein micelle.

2.1.2. Whey proteins

Whey proteins in cows' milk consists of four main types; β-lactoglobulin, α-lactabumin, immunoglobulin and bovine serum albumin (BSA). In milk they exist as soluble globular proteins. Whey protein is a mixture of proteins isolated from whey, the liquid material created as a by-product of cheese production (Hill, 2000).

2.1.3. Minerals

Cows' milk contains K^{+}, Ca^{2+}, Cl^{-}, P^{5+}, Na^{+} and Mg^{2+}. These minerals are partitioned to varying degrees between the serum and the casein in native milk at room temperatures (Hill, 2000).

2.1.4. Milk lipids

Cows' milk contains lipids, the lipids can be divided into triacyclglycerols, denoted as milk fat, phospholipids, diacylglycerols, sterols and trace quantities of carotenoids, fat-soluble vitamins and traces of free fatty acids (FFA). The fat in milk exist in the form of dispersed globules,

surrounded by a lipoprotein membrane (milk fat globule membrane, MFGM). The MFGM stabilizes the enclosed fat against coalescence and fusion and access from lipases (Hill, 2000).
During cheese ripening, the lipids are broken down by lipolysis. The lipids undergo hydrolysis by the action of lipases, which result in the liberation of fatty acids in cheese during ripening (Susanto, 2017).

2.2. Cheese manufacturing

Cheese is a concentrated protein gel, which occludes fat and moisture. Its manufacture essentially involves gelation of cheese milk, dehydration of the gel to form a curd and treatment of the curd. Cheese can be produced in many ways, but the basic steps in any cheese processing are essentially the same. Figure 2 shows those basic steps of the cheese manufacturing.

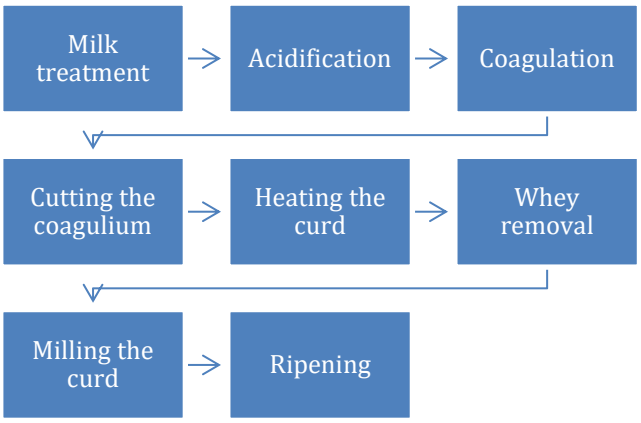

Figure 2. A flowchart of the cheese manufacturing process.

2.2.1. Milk treatment

In large scale processing, the milk is heat-treated at 73ºC for 15 seconds, to destroy pathogens and reduce microbial growth. Within the raw-milk cheese production the heat-treatment is not applied. Also, the milk can be standardized; the fat content or casein-to-fat ratio can be increased or reduced. The standardization is mostly done to have a uniform fat content in the finished dairy product (Marcelino, 2013).

2.2.2. Acidification

Before coagulation, the milk must be acidified, because the coagulants are active in a more acidic environment. The milk can be acidified by the addition of a starter-culture or the use of acidifying agents such as glucono-δ-lactone (GDL). The starter-culture is a mixture of different lactic acid bacteria (LAB), that convert lactose into lactate and thus make the milk acidic (Graaf & Franke, 2003). GDL is an acidifier that hydrolyses in water to gluconic acid (Lucey, Tamehana, Singh, & Munro, 2000).

2.2.3. Coagulation

During coagulation, casein micelles initially form a network of thin strands and small aggregates. The spaces between the strands are filled with whey. The network develops around the fat globules. Further in the process, the strands begin to form into larger, interconnecting aggregates. The pores between the aggregates become larger (Hill, 2000). Coagulants are available for this process, like lemon juice, plant rennet or more commonly a proteolytic enzyme such as chymosin. The enzymes are active in a slightly acidic environment. So, the LAB in this phase are crucial as they are required to produce enough acid, to lower to pH to around 6.2. Later on the LAB lower the pH to 4.5, to create an environment where unwanted bacteria cannot survive in. (Marcelino, 2013)

2.2.4. Cutting the coagulum

The coagulum formed during coagulation is cut. The cutting is a very important step in the manufacture, because it determined the rate of acid development and the texture of the cheese. (Marcelino, 2013)

2.2.5. Heating the curd and syneresis

The curd is heated to 37–45 ºC, depend on the type of cheese, to affect the rate at which whey is expelled from the curd particles and the growth of the starter microorganisms (Marcelino, 2013). During heating syneresis occurs; syneresis is the rearrangement of casein molecules, which results in a tightening of the casein network. The result is that moisture is squeezed out of the casein network. The most important factors influencing syneresis are: temperature, the drop in pH after the curd is cut (rate of acid development) and pressure. The greater the drop in pH after cutting the coagulum, the more moisture will be squeezed out of the curd. The higher the temperature used to heat the curd after the coagulum is cut, the lower the moisture in the curd. (Hill, 2000)

2.2.6. Whey removal

As soon as the required acidity and firmness of the curd have been attained, the residual whey is removed from the curd. The whey is removed to allow the curd particles to mat together. (Marcelino, 2013) The manner in which the whey and curd are separated can play a role in the texture of the cheese. (Hill, 2000).

2.2.7. Milling the curd

When the curd has the desired texture, the cheese is cut into small pieces to enable it to be salted evenly. Salt is added to the curd to enhance the taste of the curd and to increase the safety and shelf life. (Marcelino, 2013)

2.2.8. Ripening

Finally, the curd is put to ripening for periods that may vary from 15 days to years. Ripening is crucial for the development of aroma and flavor, brought about by the action of the many enzymes released by LAB. During ripening the protein in cheese is broken down from casein to low molecular weight peptides and amino acids. Proteolysis is the major and certainly the most complex of biochemical events that take place during ripening of most cheese varieties. LAB play an important role in ripening (Marcelino, 2013). In section 2.3., the cheese ripening process will be further discussed.

2.3. Cheese ripening

2.3.1. Lactic acid bacteria

A starter culture is a preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating its fermentation process. The group of LAB occupies a central role in these processes (Leroy & De Vuyst, 2004). The LAB play different roles in cheese-making; the SLAB participate in the fermentation process by rapidly ferment lactose producing high concentrations of lactic acid, the NSLAB are involved in the maturation process (Settanni & Moschetti, 2010). The LAB play an important role in the texture development of cheese, which will be discussed in section 2.4.

2.3.1.1. Starter lactic acid bacteria

The SLAB play an important role during cheesemaking and ripening. It converts lactose into lactic acid, this ensures the correct pH for coagulation, in the press and in the final cheese curd. It also

brings the cheese to the final moisture content. They also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes. These compounds are of importance to enhance shelf-life and microbial safety, improve texture and contribute to the pleasant sensory profile of the end product (Leroy & De Vuyst, 2004). SLAB are high in number (about 10^8 - 10^9 cfu/g) at the beginning of ripening and decrease regularly by two or more log cycles during ageing (Beuvier and Buchin, 2004; Franciosi et al., 2008).

The group of SLAB mainly includes Lactococcus lactis and Leuconostoc spp. among mesophilic species and Streptococcus thermophilus, Lactobacillus delbrueckii and Lactobacillus helveticus among their- mophilic species (Fox et al., 2004).

2.3.1.2. Non-starter lactic acid bacteria (NSLAB)

After curd formation enzymatical transformation of the curd takes place. A basic role in the transformation is played by microbial enzymes released by SLAB, NSLAB and/or other microorganism naturally present in milk or added by the cheese-maker. NSLAB plays an important role in cheese ripening, since they contain a wide range of hydrolytic enzymes (Williams and Banks, 1997). NSLAB are present at low concentrations after pressing and may increase of about four to five orders of magnitude within a few months of ripening (Fox et al., 2004). NSLAB is naturally present in raw milk, so it is not sure which bacteria the NSLAB contains. So, the presence of NSLAB introduces variability into the ripening process that cannot be easily controlled by the cheese-maker (Settanni & Moschetti, 2010).

The group of NSLAB is particularly heterogeneous with lactobacilli being mostly represented: Lactobacillus farciminis among obligately homofermentative species, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus curvatus and Lactobacillus rhamnosus among facultatively heterofermentative species and Lactobacillus fermentum, Lactobacillus buchneri, Lactobacillus parabuchneri and Lactobacillus brevis among obligately heterofermentative species (Settanni & Moschetti, 2010).

2.3.2. Glucono-δ-lactone

Raw milk cheese is cheese made milk that is not pasteurized and therefore contains NSLAB. During cheese manufacturing, the milk must be acidified, this normally happens by using a starter culture. But when only using raw milk, a starter culture is not present. This brings safety risks with it, like the development of Listeria monocytogenes. To reduce this safety risk, a chemically acidifier can be added to the milk. This acidifier can reduce the pH to a certain level, in which microorganisms cannot grow. GDL is such a chemical acidifier, it hydrolyses to gluconic acid and thereby reducing the pH (Lynch et al., 1997). 6.7g GLD/ kg curd was required to reduce the pH by 0.1 pH unit (Lynch, McSweeney, Fox, Cogan, & Drinan, 1997).

2.3.3. Rennet

Rennet is a preparation of proteolytic enzymes that influences the milk coagulation (Hill, 2000). Rennet coagulation consists of two phase; a primary step in which the casein micelle stabilizing component, κ-casein, is hydrolyzed to yield para-κ-casein micelles; a secondary step in which the para-κ-casein undergo limited aggregation. (Guinee & Wilkinson, 1992)

2.3.4. Calcium chloride

Addition of calcium chloride (CaCl2) to cheese milk decreases the pH, reduces clotting time and speeds up the curd formation. The effect is probably due to a combination of: calcium- binding to the casein micelles in such a way that it reduces the repulsive forces between them, thus enhancing hydrophobic interactions and a slight drop in pH promotes the action of the coagulant and increases the rate of aggregation. (Hill, 2000)

2.3.5. Natrium chloride

Addition of natrium chloride results in a salt gradient in the cheese. Moisture is pulled to the surface that is high in salt. (Hill, 2000)

2.3.6. Natamycin

Natamycin is an antifungal antibiotic produced by *Streptomyces natalensis*. It is used to control fungus growth in the surface of most cheese and is not effective against bacteria or viruses. The use of natamycin in allow by the European Community as an additive for food preservation. (Moreira De Oliveira et al., 2007)

2.4. Texture development during ripening

The texture of cheese is determined primarily by the ratio moisture to intact casein, its pH and the fat content. The amount of intact caseins depends on the rate of proteolysis (Lawrence, Creamer, & Gilles, 1987). The processes or characteristics that can have an influence on the cheese texture, will be discussed in the following section.

2.4.1. Proteolysis

Proteolysis is a set of processes that result in the hydrolysis of one or more of the peptide bonds in a protein. Proteolysis plays an important role in the texture development during cheese ripening. It contributes to the textural changes of cheese, due to breakdown of the protein network, decrease of aw through water binding by liberated carboxyl and amino groups and increase in pH (Sousa, Ardö, & McSweeney, 2001).

Proteolysis in cheese during ripening, is catalyzed by different enzymes. The process of proteolysis is illustrated in figure 2. The initial hydrolysis of caseins in caused by the coagulant and plasmin. This results in the formation of large and small-sized peptides which are degraded by the coagulant and enzymes from the SLAB and NSLAB of the cheese. The final products of proteolysis are free amino acids (Sousa et al., 2001).

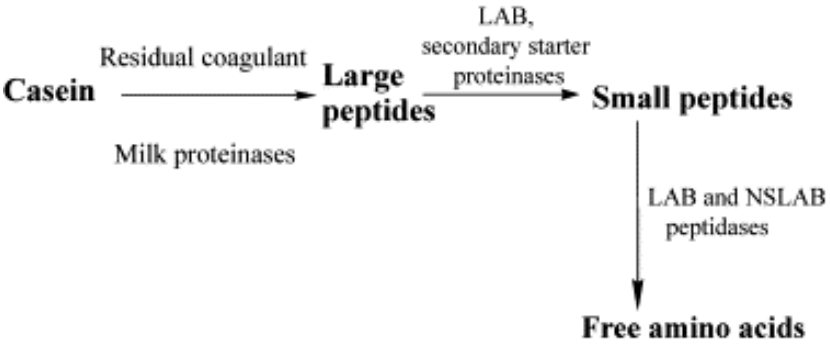

Figure 3 The breakdown process of casein with the corresponding proteolytic agents in cheese during ripening (Sousa et al., 2001).

2.4.2. pH

An influencing factor of the cheese texture is the pH. The basic reaction in cheese making is the production of lactic acid by starters. The lactic acid production makes the pH drop to a certain value which determines the future formation of the cheese. Lactic acid later serves as a substrate for surface flora, allowing the pH to rise to a level where enzymes become more active, leading to a highly-flavored product (Adda, Gripon, & Vassal, 1982).

2.4.3. Fat content

During cheesemaking, casein forms a protein matrix that includes fat and water. With less fat, the casein networks form a tighter matrix that results is a harder cheese (Creamer & Olson, 1982).

2.4.4. Moisture content

Water is trapped in the protein matrix of cheese. The lower the ratio of moisture to casein, the firmer the casein matrix of the cheese will be. Small changes in the moisture to casein ratio also result in relatively large changes in available moisture, since much of the moisture is bound to the caseins and their breakdown products (Lawrence et al., 1987). The moisture content in the cheese is determined by the pH during curd formation. The higher the pH at the end of curd formation, the less protein-protein interaction and therefore less syneresis. This will result in a higher moisture content in the cheese (Hill, 2000).

3. Materials and method

3.1. Experimental overview

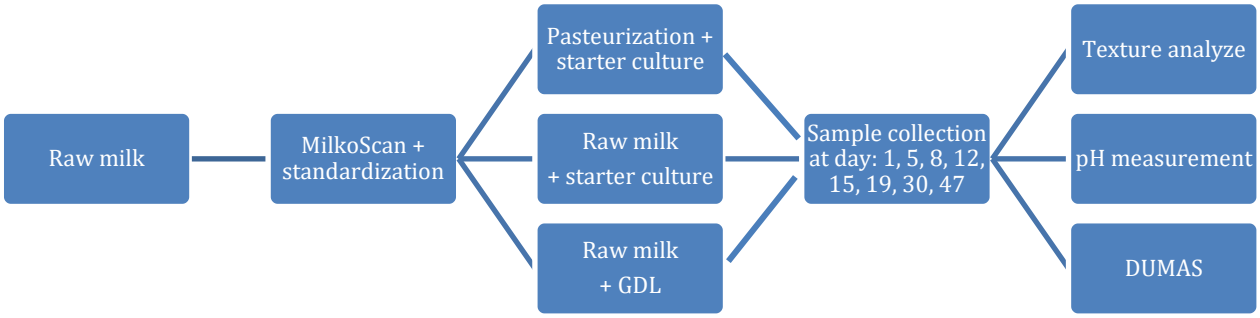

Figure 4 Experimental overview

3.2. Cheese making

3.2.1. Sample preparation

The micro-cheeses were made from raw milk originating from the cows at Carus Farm.
The LAB starter that is added to two out of three batches of milk is called D447.7 (CSK, the Netherlands). The starter D447.7 consist of several *lactococci* strains including; *lactococcus lactis* spp *cremoris* and *lactococcus lactis* subsp. *Lactis* biovar. *Diacetylactis* (Susanto, 2017).

The starter culture D447.7 is obtained in frozen liquid form which has been stored at -80°C. To activate the starter, make a reconstituted, sterilized skimmed milk consisting of 10% (w/v) NILAC low-heat skim-milk powder (NIZO, Ede, Netherlands) and milliQ water. The NILAC solution is stirred with a magnetic stirrer for 10 minutes and after that, sterilized at 121°C for 12 minutes. The milk is ready for pre-culturing after the milk reaches room temperature. For pre-culturing, approximately inoculate 10_8 CFU/mL of the starter culture in the sterile milk solution. Next, inoculation of 20h at 20°C is needed to activate the starter culture. After the inoculation, the starter is ready to use for fermentation of the milk.

The milk must be standardized. This is done by mixing low fat milk with raw milk. The fat is separated from the milk by putting milk in a 250 mL centrifuge tube and place in a centrifuge. The milk is centrifuged for 30 minutes at 4750 xg. The milk is first warmed up to 35°C, to homogenize the milk. Then it is sieved and then analyzed with the MilkoScan™. The ratio centrifuged milk to raw milk can be determined by the calculations in appendix 1.

Before the starter culture is added, the milk is pasteurized for 30 min at 63^0C. The pasteurized milk is then cooled down and then stored in the refrigerator. To start with cheese making, a 20% sodium chloride solution (w/v) with Sodium Chloride, 33% CaCl2 solution (w/v) with CaCl2, a 1% natamycin solution (w/v) with natamycin and 1.4% (w/v) GDL solution with GDL and sterile milliQ water (121^0C, 15 minutes) need to be made. Also, a renneting enzyme at a concentration of 200-300 µl/ L of milk is used. Also, pipettes of L-100, L-5000 were sterilized (121^0C, 15 minutes).

Greinertubes were filled with 150 mL of raw milk (2 tubes) and pasteurized milk (1 tube). First, 1% (w/v) of pre-cultured starter culture was added to one sample with raw milk and one sample with pasteurized milk. The starter-free sample was chemically-acidified with 1% (w/v) GDL (Ribeiro et al., 2016). The following solutions were subsequently added to all the three samples: 34.5 µL rennet, and 60 µL $CaCl_2$.

3.2.2. Milli-cheese procedure

Two deep-well plates were prepared; to do the analysis in duplicates. The two 24-deep well plates were filled with 8 mL of the mixtures per well. The layout of the well plates can be found in appendix 2. The plates were incubated at 30 °C for 60 min. Subsequently, the curd was cut with a custom-made sterile stirring device for 20 min (stirring for 20 s, resting for 3 min alternated) followed by 5 min of resting. The plate was sealed with an adhesive cover (Microseal®, Bio-Rad, USA) and centrifuged at 2176 rpm for 15 min at 22°C. 3.76 mL whey was removed and replaced by 2.9 mL sterile milliQ water of 55°C, which brought the temperature in the wells to around 36°C. The curd was cut for 40 min (stirring for 40 sec, resting for 6 min alternated) at in in a 35.5°C water bath and additionally rested for 20 min in the same water bath. The plate was sealed again and centrifuged for 75 min at 3600 rpm at 30 °C. The whey was removed by turning the plate upside down. After the whey was removed, the plate was sealed with a gas-permeable seal (BREATHseal™, Grainier Bio One, Germany) and placed in a 16°C stove. After overnight incubation, 80µL of a 20% (w/v) NaCl solution and 47µL of 1% natamycin was pipetted to each well to let the milli-cheeses absorb it. The plate was sealed with a gas-permeable seal and placed in a 16°C stove to let the cheese ripening. The cheeses were ripened for 30 days and at several days of ripening, a cheese sample is taken for measurements. The texture changes rapidly in the first 1 to 2 weeks (Gwartney, Larick, & Foegeding, 2002). So, measuring the texture in those two weeks is crucial. Samples were taken on the following days; 1, 5, 8, 12, 15, 19, 30 and 47.

3.3. Analysis of the cheese samples

3.3.1 MilkoScan™

Before cheese manufacturing, the milk samples must be standardized. For this purpose, the exact starting concentrations of fat and protein of the milk sample must be analysed. This analysis is carries out with the MilkoScan™. The MilkoScan™ analyses the main product components in milk, like protein, fat, and lactose content. Four measurements per sample were carried out. Every measurement took up about 20mL of sample.

3.3.1. pH measurement

The pH of the cheeses has been measured with a glass-calomel 3mm point-electrode. The cheeses has been kneaded into balls while wearing gloves. Thereafter the electrode is pressed into the cheese. The pH is measured in duplicates, as acidity is not homogeneous throughout the cheese.

3.3.2. DUMAS

The amount of proteolysis is found by measuring the soluble nitrogen with DUMAS, as is the moisture content. To prepare the dry samples, the tin weighing cups need to be cleaned properly with ethanol to avoid deviations in the measurements. Wet samples are measured by weighing a tin cup, pipetting 200µL into the cup with the sample and make a note of the weight. This is done for each sample and placed on a rack. The rack is placed in the 60^0C stove to dry overnight. Next day, the tin cups are closed, using a tweezer and a closing device. While wearing gloves, the closed thin cups are shaped into small tin balls rubbing between thumb and pointing finger. The samples are put back in the rack and one only one sample tray on the system is used (position 0 to 31). The sample tray must be refilled if there are more than 32 samples. A blank sample with known nitrogen content is necessary to make in order to stabilize the equipment. For the blank sample, cellulose is being used. 10-15 mg cellulose in tin cups are weighed, noted and closed as mentioned for the dry samples. Standard samples are made by weighing approximately 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg of D-Methionine tin cups. The procedure of weighing, noting the weight and closing the cups are again done the same way as with the dry samples. The order of analysis: 1 Blank sample, 6 standard samples, 1 Blank sample, 10 samples, 1 Blank sample, 10 samples etc. In the end of the series, add a methionine sample (as unknown) and the last sample should be a Blank sample

3.3.3. SDS-PAGE

For the SDS-page, solid sample preparation was carried out. 0.5mg of every cheese sample of day 1, 20, and 41 was weighed, to which 250 µl sample buffer (4 x concentrated) and 650 µl water was added. The samples were centrifuged for 1 minute at 2000 rpm and then heated in a heating block for 10 minutes at 70°C. After heating, the samples were again centrifuged for 1 minute at 2000 rpm to get all the solution to the bottom of the tube. Meanwhile, the running- and washing buffer were prepared by diluting 40mL MOPS running buffer 20 times to 800 mL, and by preparing 10% ethanol and 7.5% acetic acid in MilliQ water for the washing buffer.

After the preparation of all samples, electrophoresis was carried out according to the SDS-PAGE protocol (SDS-PAGE, NUPAGE® NOVEX® Bis-Tris Gels, Fahui Liu, version 2014). The first step of electrophoresis was removing the block tape from the gel. Assemble the gel tank in a proper way (Figure 14, Appendix 8.1), and make sure that the short plate of the gel faces the upper buffer chamber. Then, the comb was removed from the gel and 200 mL running buffer was added to the upper buffer chamber. The sample wells needed to be rinsed very carefully when adding the running buffer. After adding 200mL running buffer to the upper buffer chamber, 600 mL of this running buffer was added to the lower buffer chamber. Then, 5 µL of protein marker was loaded to the wells (one or two maker lane per gel). This was followed by loading 10 µL of sample to the wells. In the next step, the electrophoresis could run at 120 V for 90 minutes with an expected current of 100-125 mA at the start to 60-8- mA at the end. If the front of the samples (the blue colour) did not run far enough, the running time can be extended by a few minutes. After electrophoresis, the gel was separated from the plastic plate and three times rinsed with water. Then, the gel was stained with 100mL Coomassie Brilliant Blue R-250 Staining Solution for 1 hour at room temperature under slowly shaking, and afterwards washed again with water for three times. At last, the gel was washed overnight with washing buffer at room temperature under slowly shaking to get rid of the background colour.

3.3.4. Texture Analyzer

The texture of the three types of cheeses is measured using a TA.TX Plus Texture analyzer. The puncture probe of 4mm is used to calibrate and measure the cheeses. A load cell of 5 kg is connected to the texture analyzer to calibrate the force with the 2kg weight. Two measurements for each type of cheese are measured in order to obtain an average and to exclude systematic deviations. Macro's for the micro-cheeses are made, the maximum force and the amount of force needed to go to a 40% strain deformation of the cheeses are measured. This macro is called micro-cheese and can be found on the computer of TA.TX plus Texture analyzer 1 in the physics Lab.

4. Results and discussion

In this chapter the results are shown and discussed. First the sample preparation and second the analysis of the cheese samples will be discussed. The analysis of the samples contain; the determination of the moisture and protein content, pH measurement, SDS-PAGE and texture analysis.

4.1. Sample preparation

4.1.1. Cheese milk composition

Before milli-cheese production, the milk must be standardized. To standardize the milk, the exact composition of the milk must be analyzed. The milk composition analysis was carried out by MilkoScan™. The exact fat and protein percentages for the different samples can be found in Appendix 8.4. The formulas to calculate are stated in Appendix 8.1.

4.2. Analysis of the cheese samples

4.2.1. Moisture content

The moisture content of the samples is measured by drying the samples in a 60°C stove overnight. The samples were weighed before and after drying. From this data, the moisture content was calculated, which can be found in Table 1. The cheese samples were ripened in a closed system. This means that no moisture could be lost from the cheeses, as well as no moisture could have been absorbed from the air outside. So, the obtained contents are assumed to be constant over the entire ripening period.

Table 1 The moisture content in %(w/w) of the cheeses made from; raw milk with starter (RS), pasteurized cheese with starter (P), raw milk without starter (RG) .

Sample	Moisture (w/w)
RS	42.13
P	46.16
RG	54.39

In literature can be found that the moisture content of Gouda cheese must be in the range of 36-44% (Bertola, Califano, Bevilacqua, & Zaritzky, 2000). RG has a moisture content of 54.39%, this is more than 10% above the upper limit. This difference in moisture content can be caused by the different acidifiers used for the cheese production, namely GDL for RG and SLAB for RS and P. Acid production is the main factor in the expulsion of moisture from the cheese curd, and it determines the final moisture content of the cheese. This is related to the phenomena syneresis, explained in section 2.2.6. The higher the pH at the end of curd formation, the less protein-protein interaction and therefore less syneresis (Hill, 2000). This will result in a higher moisture content in the cheese.

So, the higher moisture content in RG can be a result of less acid formation in the curd with GDL than in the curd with SLAB. This can be caused by the fact that too little GDL has been added to the milk.

4.2.2. Protein content

After drying, the protein content of the cheese samples was analyzed. The protein content was analyzed with DUMAS, with DUMAS the total nitrogen content can be determined. The protein content is assumed to be constant during the entire ripening period, because during ripening casein will be broken down into smaller peptides and amino-acids, but will not be lost, so the total nitrogen content will be equal. With the conversion factor, the total protein content in the samples can be calculated, which can be found in table 2. The conversion factor for milk protein is 6.38. The protein content is used for loading amount of protein for the SDS-PAGE.

Table 2 The protein content in % (w/w) of the cheeses made from; raw milk with starter (RS), pasteurized cheese with starter (P), raw milk without starter (RG).

Sample	Protein (w/w)
RS	52.34
P	47.89
RG	54.80

4.2.3. pH

The pH of the samples is measured in duplicates with a point-electrode. This electrode is suitable for solid products. The measured pH of the cheese samples is plotted against the days of ripening. The results can be found in figure 3.

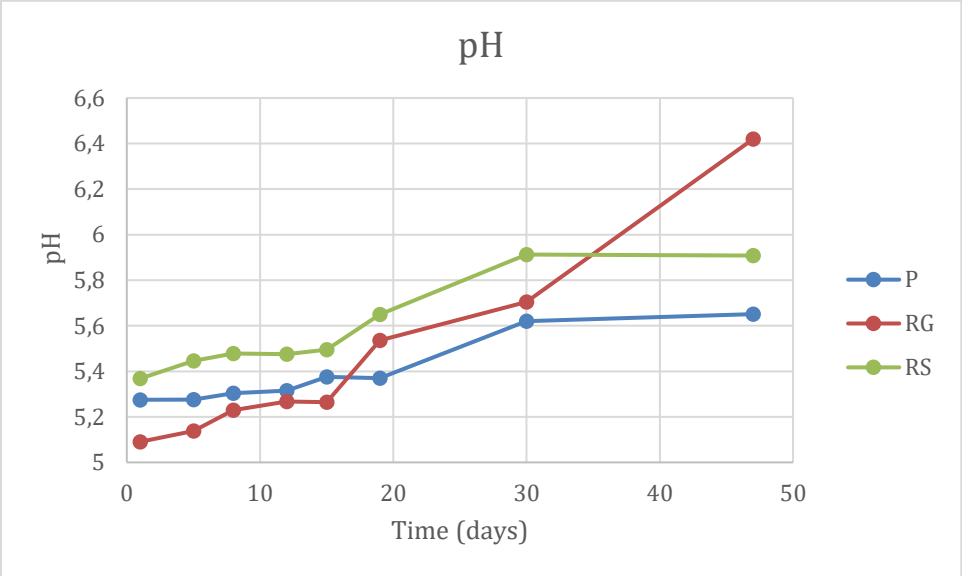

Figure 5 The measured pH against the days of ripening of the cheeses made from; raw milk with starter (RS), pasteurized cheese with starter (P), raw milk without starter (RG).

The pH of the three samples is increasing as the maturation time increases. Formation of amino acids and other nitrogenated compounds results in an increase in pH during cheese ripening (Furtado, 1979). In literature can be found that the pH ranges from 5.2-5.4 during ripening (Düsterhöft, Engels, & van den Berg, 2016). The cheeses all exceed this range during ripening.

Although LAB can grow in a wide pH range from 3.2 to 9.6, cheese pH (5.2 - 5.6) is not optimal for their growth. Lactobacilli compared to other LAB have the closest optimal pH (5.5 - 6.2) to that in cheese (Adamberg, 2015). NSLAB consist of a lot of lactobacilli. The pH of RG after 15 days is above 5.5, so there can be assumed that the amount of NSLAB increases and this results in more

proteolysis. This high pH is also a good environment for unwanted bacteria to grown in, these bacteria can cause spoilage. There were no visual appearances of spoilage on the samples.

4.2.4. Protein weight

With SDS-PAGE the molecular weight of the proteins is measured. The proteins will be separated on their molecular weight. The results can be found in figure 5.

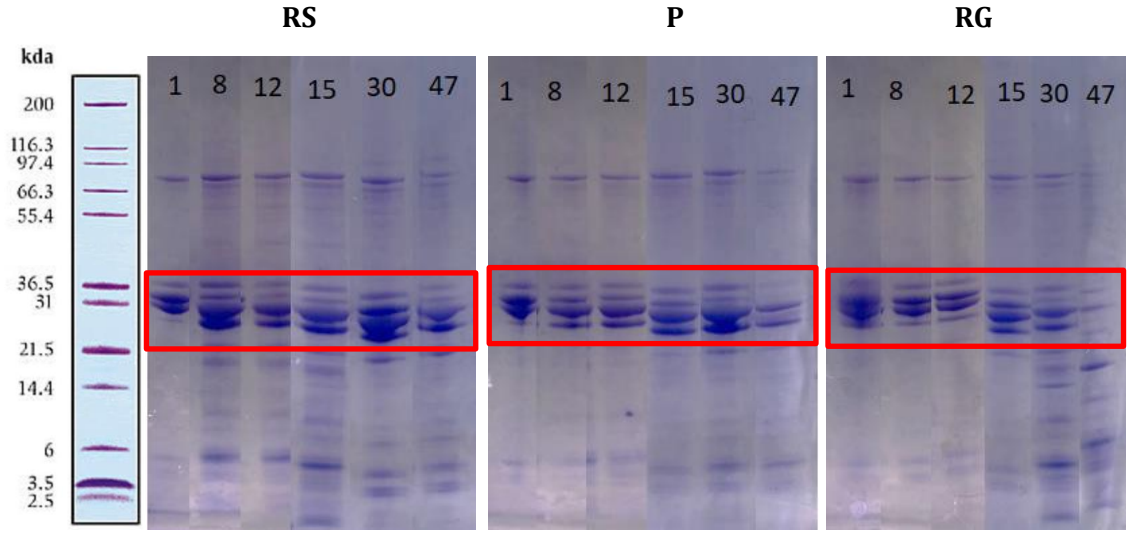

Figure 6 The image on the left represent the molecular weight ladder. The other three images are the SDS-PAGE gel of the cheeses made from; raw milk with starter (RS), pasteurized cheese with starter (P), raw milk without starter (RG) respectively. The red boxes illustrate were the intact casein proteins are located.

In figure 5, the SDS-PAGE gel is shown. For every sample 6 lanes are illustrated, these show the protein distribution at day 1, 8, 12, 15, 30 and 47. The lanes can be compared with the lane on the left, which represents the molecular weight ladder. The intact caseins have a weight of 25-35 kDa, these proteins are in figure 6 indicated with a red box. The lower in the gel, the lower the weight of the proteins, these are the breakdown products of proteolysis. The less intact caseins, the higher the rate of proteolysis.

At day 1, all the samples contain mostly intact casein. Between day 1 and 15, for RS there were a lot of peptides in comparison to RG and P. Between day 15 and 47 there were also a lot of peptides for RG and P. At day 47, in P and RS there were still some intact caseins, but in RG almost all the intact casein were broken down. The difference during ripening was that, a higher rate of proteolysis takes place in RS in the beginning of ripening then for RG and P. For RG and P the rate of proteolysis increases after day 15.
This increase in rate of proteolysis may be due to the high pH. The pH of RS after day 1 and the pH of P and RG after 15 days is higher than 5.5, this is favorable for the NSLAB and therefore will grow and produce more proteolytic enzymes.

At the end, the most proteolysis has taken place in RG, second for RS and third for P. This is since the NSLAB cannot compete with the SLAB and therefore decrease in numbers (Mounier et al., 2008). NSLAB contain the most proteolytic enzymes and thus the cheese with the most NSLAB has the highest rate of proteolysis (Williams and Banks, 1997; McSweeney et al., 1993). At last P has the lowest rate of proteolysis. P only contains SLAB and thus no NSLAB in contrast with the other two cheeses.

4.2.5. Texture analyze

The three different samples were measured in duplicate with the texture analyzer. The force that is needed to obtain a 40% strain deformation is plotted against the day of ripening. The force that is needed can be used to compare the softness of the cheese; the less force needed, the softer the cheese. The results can be found in figure 4.

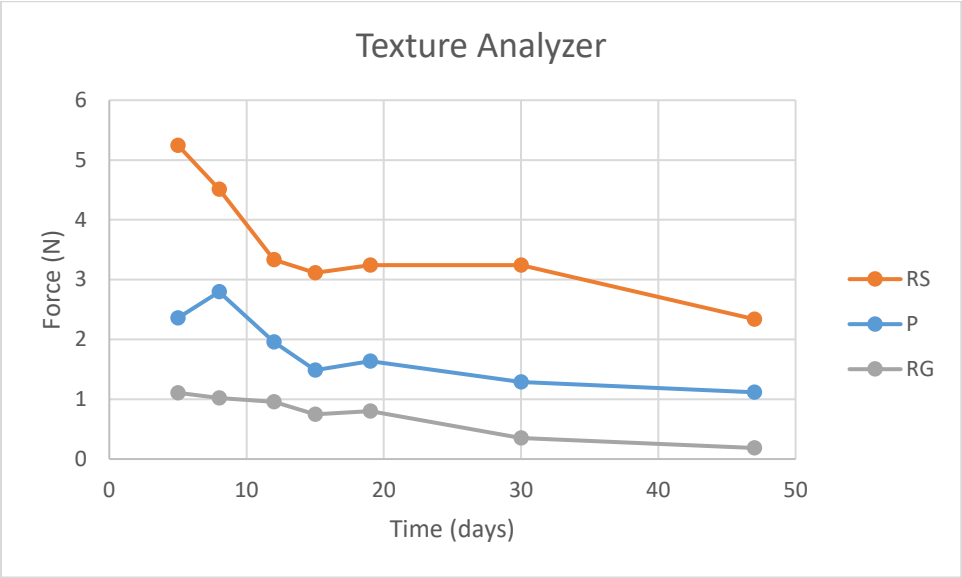

Figure 7 The force that is needed to obtain a 40% strain formation against the days of ripening of the cheeses made from; raw milk with starter (RS), pasteurized cheese with starter (P), raw milk without starter (RG).

Looking at the absolute softness of the cheeses; RG is the softest and RS is the hardest cheese. This is probably because of its moisture content, found in 4.2.1. RG has the highest and RS the lowest moisture content; the higher moisture content, the softer the texture of the cheese (Reinhardt, Vahl, & Jeuken, 1984).

The softness is increasing during ripening for all the cheese samples. This is because, the higher the moisture to intact casein ratio, the softer the matrix. In this research it is assumed that the moisture content during ripening remains the same. As explained in section 2.4.1., the intact casein content is decreasing during ripening, this is caused by proteolysis. This decrease results in an increase in moisture to casein ratio and thus an increase in softness. During ripening, the softness of RG decreased the most, second RS and at last P. This decrease in softness corresponds with the rate of proteolysis. This ranking in rate of proteolysis corresponds with the results found in 4.2.4.

In the first 15 days, for RS the softness is increasing fast in comparison to the other 15-47 days. The rapid increase can be explained by primary proteolysis, the casein network is greatly weakened when a single bond in about 20% of the αl-casein is hydrolyzed to give the peptide α1-I. The second phase involves a more gradual change in texture, as the rest of the αsl casein and the other caseins are broken down (Lawrence et al., 1987). After 15 days, for RG the softness is increasing fast in comparison to the first 15 days. This increase can be a result of the high pH found in 4.2.3. The increase in softness found with the texture analyzer corresponds to the rate of breakdown of the caseins that is found in 4.2.4.; when rate of breakdown of the caseins is increasing, the softness is also increasing.

5. Conclusion

The texture of cheese is primary dependent on the ratio moisture to intact casein, its pH and fat content. In this research the moisture content remains the same during ripening, so the ratio moisture to intact casein is during ripening only dependent on the decrease of intact casein, which correlates with proteolysis. Raw milk contains NSLAB, which contain a lot of proteolytic enzymes. So, a high rate of proteolysis is expected in raw milk. But when adding SLAB to the raw milk, the bacteria start to compete. The NSLAB cannot compete with the SLAB and therefore decrease in numbers. So, the SLAB in RS suppress the growth of NSLAB and therefore RS contains less NSLAB then RG and thus RG has a higher rate of proteolysis then RS. So, cheese with only NSLAB, has the highest rate of proteolysis, second cheese with NSLAB and SLAB and third cheese with only SLAB. A high rate of proteolysis will result in softening of the cheese, because proteolysis ensures the breakdown of intact casein into smaller proteins. The cheese with only NSLAB has the softest absolute texture, followed by cheese with only SLAB and at last cheese with NSLAB and SLAB. These differences in absolute softness, were depended on the moisture content. The higher the moisture content, the softer the cheese. The differences in moisture content is determined by the pH at the end of curd formation; the higher the pH, the higher the moisture content. So, to properly compare the influence of the different LAB on the softness of the cheeses, the moisture must be the same in the samples. The high rate of proteolysis in the cheese with only NSLAB can be a result of the high pH. The high pH is favorable for some of the bacteria in NSLAB, which results in an increase of growth of these bacteria. Because NSLAB contain proteolytic enzymes, a high pH will result in an increase in rate of proteolysis

To conclude, the rate of softening depends on the rate of proteolysis; the higher the rate of proteolysis, the higher the rate of softening. The rate of softening is the highest in the cheese with only NSLAB, second in the cheese with NSLAB and SLAB and third in the cheese with only SLAB.

6. Recommendations

6.1. LAB isolation and identification

In order link the experiments to the type LAB, isolation and identification of the LAB needs to be done. As explained by Susanto (2017), LAB identification can be performed to characterize the LAB in cheese. In this way, the NSLAB and SLAB can be identified separately and more precise rather than LAB counts method. Serial dilutions of sample are prepared, spread plate method is then implemented. All plates then incubated at 25?C for 24 to 48 hours. Afterwards, colonies that grew in plates are transferred in MRS broth medium and incubated at 25?C for 24 hours. Several characterization analyses on the strains should be performed to obtain accurate result of LAB identification.

- Microscopy
- Growth at different temperatures (15, 25, 35 and 45?C)
- Salt resistance (4% NACl)
- Casein breakdown / milk fermentation (using milk agar plates or sterile milk)
- CH50 API for identification of LAB strains

6.2. Texture analyzer

In order to measure the influence of LAB on the softness, the moisture content must be the same in the different samples. The difference in softening is better to compare, when the moisture contents are the same. To achieve this, the pH of the samples must be the same during curd formation.

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8. Appendix

8.1. Milk standardization

With the following formulas, the ratio raw-milk to centrifuged-milk for the cheese milk can be determined. (Posthumus, Booy & Klijn, 1964)

Formula 1:
vkm = e*r + q
r: 0.91
q: 0.197
e: protein content in the raw milk (measured by MilkoScan)
vkm: fat content in the cheese milk

Formula 2:
vrm*X+(0,01-X).vom=vkm*quantity cheese milk
vrm: fat content raw milk
X: quantity raw milk
Vom; fat content centrifuged milk
Vkm: fat content cheese milk

Formula 1 is used to calculate the fat content in cheese milk. This outcome can be used for formula 2. With this formula the quantity raw milk can be calculated. The quantity cheese milk is determined on the amount of milk needed for cheese production.

8.2. Layout of well plates

RS 1	RS 5	P 1	P 5	RG 1	RG 5
RS 8	RS 12	P 8	P 12	RG 8	RG 12
RS 15	RS 19	P 15	P 19	RG 15	RG 19
RS 30	RS 47	P 30	P 47	RG 30	RG 47

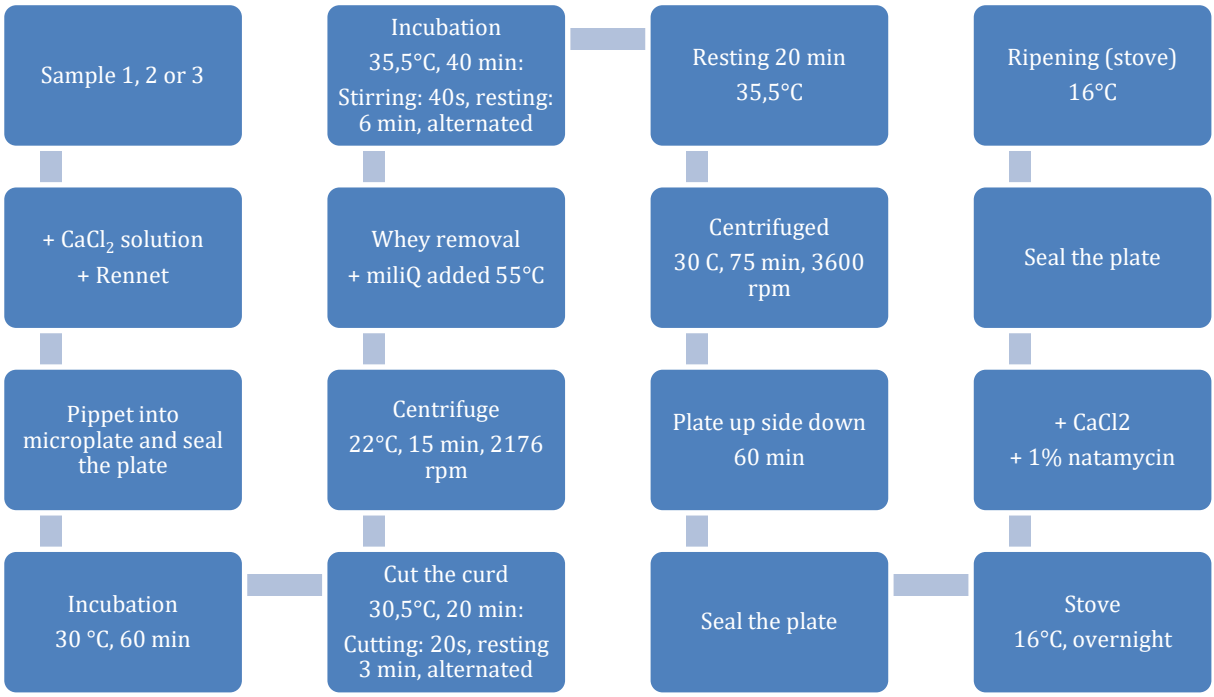
RS 1	RS 5	P 1	P 5	RG 1	RG 5
RS 8	RS 12	P 8	P 12	RG 8	RG 12
RS 15	RS 19	P 15	P 19	RG 15	RG 19
RS 30	RS 47	P 30	P 47	RG 30	RG 47

8.3. Milli-cheese procedure

Sample 1:
Pasteurized milk (63°C, 30 min)
+ Culture (1%)

Sample 2:
Raw milk
+ Culture (1%)

Sample 3:
Raw milk
+ GDL



8.4. MilkoScan

	Raw milk	**Centrifuged milk**	**Cheese-milk**
Protein (%)	3.725	3.665	3.705
Fat (%)	4.20	0.315	3.665

Amount cheese milk: 600 mL
X (raw milk): 514.6 mL
Centrifuged milk: 85.4 mL

8.5. Texture analyzer

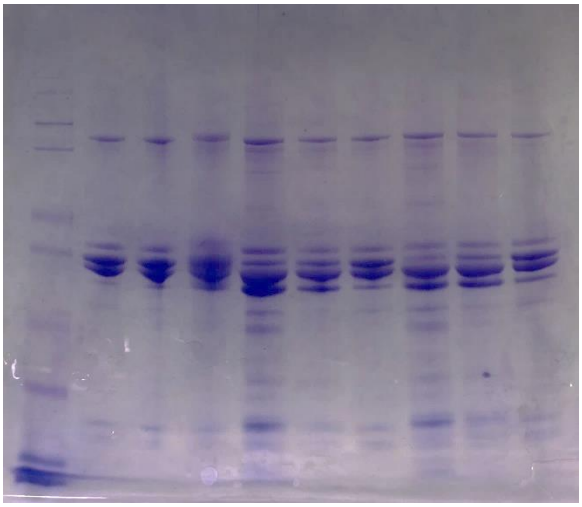
Day\sample	**P1**	**P2**	**RG1**	**RG2**	**RS1**	**RS2**
1	0.3134	0.2915	0.154	0.1092	0.3242	0.5126
5	2.87	1.8446	1.7795	0.4356	5.2916	5.201
8	2.2368	3.3532	1.3204	0.7175	4.2662	4.7522
12	1.4814	2.4313	0.6569	1.258	3.3001	3.3667
15	1.1198	1.8548	0.6032	0.8946	3.2682	2.9556
19	1.4966	1.7754	0.7096	0.8882	2.8211	3.6655
30	1.013	1.5625	0.35	0.3571	2.5756	3.911
47	0.8922	1.3394	0.1835	0.1854	1.9355	2.7414

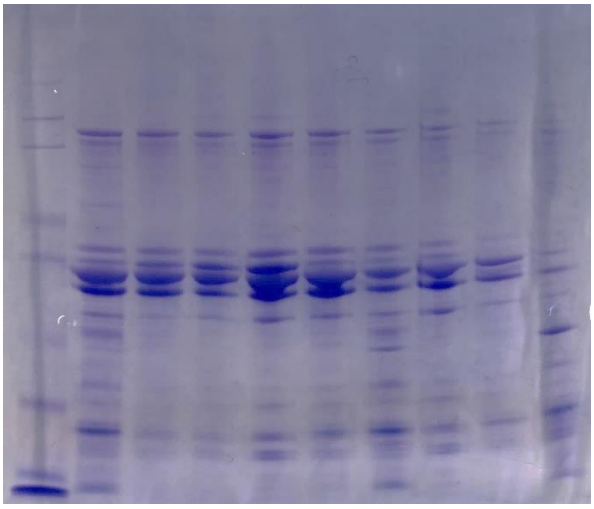
8.6. pH measurement

Day\sample	**P**	**RG**	**RS**
1	5.275	5.0905	5.3679

5	5.2755	5.138	5.446
8	5.304	5.229	5.4775
12	5.3145	5.2675	5.475
15	5.376	5.265	5.495
19	5.37	5.536	5.6495
30	5.6205	5.705	5.9125
47	5.651	6.4195	5.9075

8.7. SDS-PAGE





8.8. Ethical appendix

Gebruik je gezond verstand

Voor mijn Bachelor thesis ben ik bezig met het onderzoek naar de invloed van verschillende melkzuur bacteriën op de structuur van kaas tijdens het rijpen. Hiervoor maak ik kaasjes op kleine schaal en voeg hier verschillende soorten melkzuur bacteriën aan toe. Om de kaas te maken, maak ik gebruik van verse melk van de Carus boerderij op de campus. Hier wordt er gezegd dat ik de melk drie dagen kan bewaren in de koelkast en daarna weg moet gooien. Aangezien ik elke week nieuwe kaasjes maak, kan ik de melk van die week ervoor niet meer gebruiken en moet ik deze weg gooien. Vaak is de melk na drie dagen nog prima te consumeren, maar voor gezondheidsredenen gooi ik het toch weg. Ik merk dat dit vaker in de praktijk voorkomt, niet alleen bij melk, maar bij meerdere voedingsmiddelen. We moeten ons altijd houden aan de houdbaarheidsdatum van voedsel, maar hierdoor gaat er een hoop 'goed' voedsel verloren. Zouden we niet de houdbaarheidsdatum af kunnen schaffen, zelf kunnen nagaan of het product nog veilig is om te consumeren en hierdoor de voedselverspilling verminderen?

De houdbaarheidsdatum is een wettelijk voorgeschreven vermelding op een levensmiddel die de klant informatie biedt over de uiterste datum waarop het product kan worden geconsumeerd of hoelang het kan worden bewaard (Holthuysen, Kremer & Bos-Brouwers, 2016). Zolang de verpakking niet geopend is, kun je het product bewaren tot de houdbaarheidsdatum. Het probleem met een houdbaarheidsdatum is dat er vaak een ruime marge wordt genomen, dus dat de datum onnodig vroeg wordt gekozen. Dit kan er toe leiden dat voedsel wordt verspild, doordat goed voedsel uit de handel wordt genomen of door consumenten wordt weggegooid. Door het afschaffen van een houdbaarheidsdatum zal de voedselverspilling omlaag gaan.

In het eenvoudigste geval zou er hiervoor een besluit worden genomen door alleen rekening te houden met de belangen van het milieu en de consumenten. Maar in dit scenario zijn de effecten op en de belangen van supermarkten en producenten ook belangrijke stakeholders die moeten worden meegenomen in etische besluitvorming (Mepham, 2013). Om het besluitingsprocess te vergemakkelijken is de etische matrix geintroduceerd. Hierin worden alle belangensgroepen geanalyseerd op drie morele waarden; recht, autonomie en eerlijkheid. Respect voor welzijn vertegenwoordigt het belangrijkste utilitaire principe; respect voor autonomie vertegenwoordigt het belangrijkste deontologische principe; en respect voor eerlijkheid is belangrijk voor zowel de utilitaire als deontologische tradities. Het utilitaire principe handelt naar 'het recht' en 'wat gedaan moet worden' en het deontologische principe handelt naar wat de consequenties zijn (Van der belt, 2016).

Aangezien ik een levensmiddelentechnologie student ben, heb ik meer kennis over voeding dan de gemiddelde mens. Hierdoor kan ik redelijk goed inschatten of voedsel bedorven is of niet. Maar niet iedereen heeft deze kennis paraat en kan inschatten of voedsel nog veilig is om te consumeren. Daardoor zal de kans groter worden dat consumenten bedorven voedsel consumeren. Hierdoor zal de gezondheid van mensen achteruit gaan. Een ander nadeel aan het afschaffen van de houdbaarheidsdatum voor de consumenten is dat ze nu meer moeite moeten doen voor hun producten. Ze kunnen niet meer klakkeloos de houdbaarheidsdatum op het etiket volgen, maar moeten dit zelf gaan inschatten. Maar doordat ze nu hun voedsel niet meteen weggooien als hij over de houdbaarheidsdatum zit, kunnen ze in de meeste gevallen hun voedsel langer bewaren. Dit is dus voordeliger voor de portemonnee.

Supermarkten laten dagelijks hun personeel het assortiment doorspitten of er producten tussen zitten die aan hun houdbaarheidsdatum zitten. Dit is de enige indicatie die supermarkten hebben om te zien of ze het product nog mogen verkopen. Zij kunnen namelijk niet zonder de

verpakking te openen, zien of het product bedorven is of niet. Als er geen houdbaarheidsdatum op het product staat, zouden ze dus bedorven producten kunnen verkopen. Een oplossing hiervoor zou een 'ten minste te verkopen tot'-datum zijn. Deze datum is dus alleen bestemd voor de supermarkten, niet voor de consumenten.

Voor de producenten zou het nadelig zijn om de houdbaarheidsdatum af te schaffen, als de consumenten namelijk minder gaan kopen, zullen zij minder verkopen. Ook is het vermelden van een houdbaarheidsdatum voor de producent een verplichting van Europeesrechtelijke aard. Het is dus niet mogelijk om dit 'zomaar' op nationaal niveau te wijzigen. De wet verplicht de producent een tenminste houdbaar tot-datum of een tenminste gebruikt tot-datum op de verpakking te zetten. Ook gebruik van beide, de één bedoeld als kwaliteitsgarantie, de ander als veiligheidswaarborg, is dus niet geoorloofd. Een andere benaming, bijvoorbeeld 'bij voorkeur gebruiken tot'-datum, is ook niet toegestaan. (Soethoudt, Van der Sluis, Waards & Tromp, 2012)

Een optie is om de consument op te leiden tot ze zelf na kunnen gaan of een product bedorven is. Hierbij kun je denken aan een uitleg op de verpakking van het product, met wanneer je het product niet meer kunt consumeren en hoe je dat kunt zien en/of ruiken. Bij producten zoals melk is het vaak goed te zien of het product bedorven is. Je ziet klontering ontstaan en de melk begint zuur te ruiken. Maar het is ook niet bij elk product zo goed te zien of het bedorven is. Daardoor is het niet mogelijk om bij elk product de houdbaarheidsdatum af te schaffen.

Het beter begrijpen van verspillingsgedrag bij consumenten vormt een belangrijke sleutel tot het verminderen van voedselverspilling. Uit eerder onderzoek blijkt dat het niet juist interpreteren van houdbaarheidsdata door consumenten met grote regelmatigheid wordt aangegeven als een van de belangrijkste oorzaken van voedselverspilling. Consumenten meer algemene uitleg geven over de houdbaarheidsdatum, ook na opening van de verpakking, zou een groot deel van de voedselverspilling kunnen voorkomen. Meer informatie geven in de krant, televisie, radio etc. zou al een groot verschil kunnen maken. Door op juiste wijze om te gaan met houdbaarheidsdata, kan er volgens het Centraal Bureau Levensmiddelenhandel (CBL) tot wel 14 kilo verspilling per persoon worden voorkomen (EuroCommerce, 2015).

Ik vind dat het afschaffen van de houdbaarheidsdatum en dit geheel over te laten aan de consumenten teveel risico's met zich mee neemt. Maar de optie om informatie op de verpakking te plaatsen met hoe je kunt zien of je het product nog kunt consumeren, zal al veel voedselverspilling tegen kunnen gaan. Hierdoor zullen consumenten alsnog minder snel een goed product weggooien, maar zullen mensen die hier niet toe in staat zijn gewoon de 'tenminste houdbaar tot'-datum kunnen volgen. Ook het geven van algemene informatie over het juist interpreteren van de houdbaarheidsdatum zal al veel verspilling kunnen tegengaan.

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