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# Quality of fermented dairy products beyond best before date

*Fermenterade mejeriprodukters kvalitet efter bäst före-datum*

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## Abstract

Date labelling is one factor influencing food waste in consumer households and evaluation of optimum shelf life is a tool food industry can use to contribute to diminished food waste. This explorative pilot study aimed to investigate how quality of sour milk and yogurt changed beyond best before date. Sour milk and yogurt were stored in 8°C and 15°C for a total of 34 and 56 days, respectively. Instrumental measurements of pH, water holding capacity (WHC) and rheological behaviour were performed along with microbiological and sensory analysis. Lowest pH was observed at best before date and WHC was stable through storage. The solid-like properties of sour milk were temperature dependent and decreased during storage. The solid-like properties of yogurt increased during storage. Viability of *Streptococcus* spp. was more apparent in yogurt while other species of lactic acid bacteria were dominant in sour milk. Storage temperature appeared to be more influential on microbiological viability in yogurt. Yogurt maintained its microbiological quality beyond best before date but the observed fungal growth in sour milk, beginning at best before date, needs to be further investigated. The sensory evaluation showed that both products were associated with creaminess at best before date and associated with the attribute thin thereafter. Sour milk was associated with musty after best before date, which might relate to fungal growth. Further research is required to determine if the observed changes in sensory attributes have a significant influence on consumer acceptability.

*Keywords:* Yogurt, sour milk, date labelling, rheology, microbiological plating method, Pivot Profile

## Sammanfattning

Datummärkning är en faktor som påverkar mängden matsvinn som uppstår i konsumentled. Att utvärdera livsmedels optimala hållbarhet är ett verktyg livsmedelsindustrin kan använda för att bidra till minskat matsvinn. Denna pilotstudie syftade till att undersöka hur kvaliteten på filmjolk och yoghurt förändrades efter bäst före-datum. Filmjolk och yoghurt lagrades i 8°C och 15°C under totalt 34, respektive 56, dagar. Instrumentella mätningar av pH, vattenhållande kapacitet och reologiska egenskaper utfördes tillsammans med mikrobiologisk och sensorisk analys. Det lägsta pH-värdet uppmättes på bäst före datum och den vattenhållande kapaciteten var stabil under lagringstiden. Fastheten i filmjolk var temperaturberoende och minskade under lagring. Fastheten i yoghurt ökade under lagring. *Streptococcus* spp. dominerade i yoghurt medan andra arter av mjölksyrabakterier var dominerande i filmjolk. I yoghurt tenderade lagringstemperatur att i högre grad påverka det totala antalet mjölksyrabakterier. Yoghurt upprätthöll mikrobiologisk kvalitet efter bäst före datum men för filmjolk behöver den observerade svamptillväxten, som uppkom vid bäst före datum, undersökas ytterligare. Den sensoriska analysen visade att båda produkterna var associerade med krämighet vid bäst före-datum och därefter associerades med attributet tunn. Filmjolk associerades med attributet unken efter bäst före datum, vilket möjligen kan relatera till svamptillväxten. Ytterligare forskning krävs för att avgöra om de observerade förändringarna i sensoriska attribut har en betydande påverkan på konsumentacceptans.

*Nyckelord:* Yoghurt, filmjolk, datummärkning, reologi, mikrobiologisk odling, Pivot Profile

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## Abbreviations

EPS	Exopolysaccharide
LAB	Lactic acid bacteria
LVR	Linear viscoelastic region
MRS	De Man, Rogosa and Sharpe
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PP	Pivot Profile
VRB	Violet Red Bile
WHC	Water holding capacity

# 1 Introduction

Food production has a large environmental impact globally and working towards a sustainable food system is one of the greatest challenges of today. In Sweden, the food supply chain contributes to 20-25% the national climate impact (Naturvårdsverket 2019b). Depending on product, the total food waste along the food supply chain has been estimated to between 10-50%. The major part takes place on the consumer level, about 97 kilo food per person and year. At retail level, a Swedish case study showed that dairy products were the perishable product category with the least proportion of food waste (Eriksson & Strid 2011). Low-fat sour milk has been mentioned as the most discarded product among the fermented dairy products (Eriksson 2012).

The understanding of food waste and its causes is complex, since the system set-up in one part of the supply chain influence other parts (Toma *et al.* 2017). Date labelling is one example, which is set on the manufacturer or retail level, but likely influences the amount of food waste produced at consumer level. It has been suggested that food waste produced at consumer level can be considerably diminished merely with minor increases in labelled shelf life. Therefore, it may be argued that earlier levels in the food supply chain share responsibility for the reduction of food waste at consumer level.

The decision-making at consumer level is influenced by date labelling, both at time of purchase and when handling food in the household. Today, consumers rely less on their own judgement of edibility (Toma *et al.* 2017). Marklinder & Eriksson (2015) showed in a study of Swedish consumers that date labelling had the largest influence on perceived edibility of food products. Similarly, other studies showed that knowledge of the best before date of a product significantly influenced the rated acceptability among consumers (Majchrzak & Rudolph 2015).

In Sweden, about 34 kg fermented dairy products are consumed per person and year (Holmström 2017). About 6.5 kg of the liquid fermented dairy products are discarded per person and year (Naturvårdsverket 2019a). A survey conducted by

Axfood (2019) reports that 11% of consumers that were asked had discarded yogurt at some point during the previous month. Crème fraiche, yogurt and sour cream are among the twenty most discarded food products. Inadequate storage temperature is one factor that contributes to higher food waste. Marklinder & Eriksson (2015) showed that almost one fifth of yogurt and sour milk in consumer households were stored in temperatures over the recommended 8°C.

Inherently, fermented dairy products are considered safe due to their low pH and high numbers of lactic acid bacteria (LAB). However, parameters related to sensory quality and growth of spoilage organisms are also crucial for shelf life. In this study, several quality parameters of yogurt and sour milk were investigated in relation to storage time and temperature. Ideally, the knowledge obtained from this study may provide insights of possible improvements related to date labelling at manufacturer and retail level.

## 1.1 Aim

The aim of this study was to investigate how the quality of fermented dairy products changes after the labelled best before date. Natural yogurt and sour milk were chosen as test objects and several quality parameters that may be of relevance for the shelf life of these particular products were evaluated. More specifically, the aim was to investigate the influence of storage time and temperature on physicochemical, rheological, microbiological and sensory qualities. The hypothesis was that the products would have an acceptable quality for consumption after the labelled best before date.

## 1.2 Delimitations

This project was designed as an explorative pilot study and, as such, the aim was to target product quality in a comprehensive approach. The experimental design was limited to a single batch of each product, which limits the possibility to generalise the results beyond this particular batch. The study focuses on finding interesting quality aspects that may be further investigated to determine if it would be possible to extend the best before date with maintained quality.

## 2 Background

### 2.1 Shelf life

Shelf life may be defined as the time period after production when the product meets the expectations of the consumer (Muir & Banks 2000). As a consequence, shelf life is a dynamic concept that is somewhat difficult to define. Shelf life may be limited by food safety or food quality reasons depending on the type of product. Food safety aspects are related to the presence of pathogenic microorganisms. Food quality relates to the deterioration process of the food and its influence on consumer acceptability, e.g. sensory quality. Shelf life is determined by the extent of quality change that is apparent enough to influence the opinion of the consumer. Hence, shelf life is highly varying and dependent on consumer expectation.

#### 2.1.1 Date labelling

Date labelling is regulated by EU legislation to give the consumer information about how to use food products in a safe way (Nurttila *et al.* 2015). The date labels indicate how long a product can be stored under some specified conditions. In EU, the labels “best before” and “use by” are used on food products.

The best before date is the length of time that a food product can be stored and be expected to retain its original quality (Nurttila *et al.* 2015). Hence, the food product may be safe to eat after this date but the producer does not guarantee its quality. The use by date, however, is used for food products where food safety is a concern. It states the last day a food product can safely be consumed before it may pose a health risk. This kind of labelling is used for highly perishable food products that may pose a risk from a microbiological point of view. It is the last day the product is acceptable for consumption and allowed to be sold.

The manufacturer determines the durability of the food product and sets the best before date (Livsmedelsföretagen 2016). Usually, the date is determined based on experience from shelf life testing or by recommendations from a branch organization. According to EC 2073/2005, the company is responsible for ensuring that the food safety criteria are fulfilled during the entire shelf life of the product, throughout the supply chain until it reaches the consumer. For food products that requires storage at lower temperature, EC regulation 852/2004 applies, stating that the cold chain should not be disrupted. Further, information must be provided to the consumer if specific storage or usage conditions are necessary to maintain quality until best before or use by date.

### 2.1.2 Date labelling on the Swedish market

As a background to this thesis, a market survey was performed to investigate the date labelling of fermented milks of different market brands. Eleven brands of sour milk (3% fat) and twelve brands of mild yogurt (3% fat) were categorized based on the stated shelf life on their date labelling (Table 1). The information was collected on internet and by contacting the customer service of different dairy companies. The five largest Swedish dairy companies were included, which together have 98% of the Swedish milk market (Lingheimer *et al.* 2016). Also included in the survey were seven smaller local dairy companies as well as a Finnish brand present on the Swedish market.

Table 1. *Number of days after production labelled as best before date for eleven brands of sour milk and twelve brands of yogurt (3% fat) in the Swedish market*

Labelled shelf life	Number of sour milk brands	Number of yogurt brands
14 days	2	
16 days	2	
17 days	1	
18 days	3	
19 days	1	
21 days	2	3
25 days		1
28 days		6
32 days		1
35 days		1

Three of the five larger dairy companies were also asked questions about how they evaluate the shelf life of the product and determine the best before date. All three stated that the evaluation is based on both microbiological and sensory parameters.

They also reported that the sensory evaluation, taking place at best before date, is the most important parameter for products that already exist on the market. Some or all of the parameters visual appearance, consistency, aroma and taste are evaluated at best before date. For new products, a more extensive shelf life testing including both microbiological and sensory parameters is performed to determine the date labelling.

## 2.2 Fermented dairy products

### 2.2.1 Starter cultures

Starter cultures for fermented milks mainly consists of LAB, which are used in different combinations in different dairy foods (Puniya 2015). The most important LAB used in the dairy industry include several species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. Some are homofermentative, producing lactic acid as the major end product. Others are heterofermentative and produce a variety of end products, such as lactic acid, carbon dioxide, ethanol and acetic acid. The fermentation of the products provide dairy foods with flavor and texture characteristics as well as acidity that contributes to preservation of the food. The optimum product characteristics obtained with different strains within the LAB species have been frequently reported in the literature.

The type of strains included in the starter culture influence the technological properties of the fermented product, e.g. texture and flavor development and post-acidification (Xu *et al.* 2015). The strain specific proteolytic activity has been shown to have a major impact on the quality of fermented milks (Amani *et al.* 2016). LAB produce enzymes to break down milk proteins in order to get access to peptides and amino acids that is required as growth factors in their metabolism. The degree of proteolytic activity in the fermented milk influence the technological properties of the product.

### 2.2.2 Production of fermented milks

Production of fermented milks begins with pasteurization of the milk to kill pathogens as well as to denature whey proteins (Puniya 2015). The denaturation of the whey proteins gives a smooth and viscous texture to the product. The following steps in the production process includes standardization, homogenization and inoculation of starter culture. Yogurt can be produced as both set and stirred (Serra *et al.* 2009). A set yogurt is allowed to gel in the package, whereas in the case of stirred

yogurt, the gel is broken and stirred to get a smooth and viscous texture. In the case of stirred yogurt, the fermented milk may be packaged before or after the cooling step (Puniya 2015).

### 2.2.3 Sour milk

In the cool climate of the Nordic countries different fermented milks with mesophilic cultures have traditionally been consumed (Walstra 2006). These traditional fermented milks are usually characterized by high viscosity and, occasionally, ropiness due to the use of an exopolysaccharide (EPS) producing starter culture. Sour milk is characterized by a quite high viscosity and is manufactured with different fat contents, although the standard is 3%.

Mesophilic starter cultures are used in production of the traditional Swedish sour milk, which is allowed to ferment up to 24 hours at around 20°C (Walstra 2006). This culture usually contains the diacetyl and lactic acid producing *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* which provides the product with a characteristic aromatic flavor (Hui *et al.* 2004). Other common mesophilic cultures used in production of fermented milks are *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Leuconostoc* spp. (Ratray 2019). The main products of mesophilic starter culture metabolism are lactic acid, diacetyl and CO<sub>2</sub>.

### 2.2.4 Yogurt

In the range of fermented milk products different types of yogurts are by far the most consumed (Walstra 2006). The species *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* dominates the starter culture used in yogurt production. The main products of their metabolism are lactic acid and acetaldehyde (Ratray 2019). During fermentation, there is a continuous shift in domination between the two species. Their final ratio in the fermented milks is dependent on e.g. inoculation time and temperature. High temperature, prolonged fermentation time or insufficient cooling generally favors the growth of *L. bulgaricus*.

The species have a mutual beneficial effect on each other, a so called proto-cooperation, based on exchange of growth stimulating metabolites (Mchiouer *et al.* 2017). *L. bulgaricus* has a high proteolytic activity and provides *S. thermophilus* with amino acids and peptides necessary for its growth. *S. thermophilus* in turn produces metabolites, e.g. pyruvic acid, formic acid and lactic acid, which decrease the pH to a favorable level for growth of *L. bulgaricus*. The synergistic relationship results in a faster pH reduction as well as a lower final pH and a higher number of bacteria.

The manufacture process of stirred yogurt begins with pasteurization and inoculation of starter bacteria when the milk has cooled down to 30-32°C (Walstra 2006). The milk is incubated at around 45°C and during the fermentation process, the acidification solubilizes the calcium phosphate, which weakens the casein micelles. As the pH approaches the isoelectric point of casein (pH 4.6) the milk begins to form a gel (Muir & Banks 2000). The yogurt is then stirred and cooled either before or after packaging (Walstra 2006). During storage in refrigeration temperature the yogurt continues to acidify at a slower rate (Beal *et al.* 1999). If the fermentation process is not efficiently inhibited by cooling after the gel has formed, it may give rise to a higher acidity as well as an increased syneresis, i.e. separation of whey, during storage (Walstra 2006).

## 2.3 Physicochemical properties

### 2.3.1 pH

The change of pH in fermented dairy products is caused by the activity of the LAB starter culture (Walstra 2006). The bacteria produce lactic acid, as well as other metabolites, in their fermentation of the substrates present in the milk. Even after the fermentation process has been inhibited by cooling of the gel, the metabolic activity of the starter culture bacteria continues (Beal *et al.* 1999).

The continuous decrease in pH taking place in the packaged fermented milk during refrigerated storage is referred to as post-acidification (Beal *et al.* 1999). The post-acidification is dependent on the final pH of the fermentation process. A lower pH at the end of fermentation also gives rise to a lower pH during post-acidification. The strain type of starter culture bacteria, as well as the combination of strains, highly influence the acidification activity of the starter culture during storage (Amani *et al.* 2016).

### 2.3.2 Syneresis and water holding capacity

Syneresis is one of the major defects in fermented milks and occurs when the aqueous phase within the protein network is mobilized due to rearrangement and shrinking of the network (Walstra 2006). High incubation temperature and low casein content may increase the level of syneresis. As an example, in the manufacture of stirred yogurt, the incubation temperature has to be around 32°C if the milk has a low casein content to avoid syneresis. An increase in syneresis after stirring gives a more viscous and lumpier product.

Syneresis is known to increase during storage due to weakening of gel strength (Walstra 2006). In the literature, syneresis of stirred yogurt has been measured by both whey drainage and high-speed centrifugation. Whey drainage is a measure of the spontaneous expulsion of whey from the gel network. High-speed centrifugation, however, is rather a measure of the resistance of the gel network to external force (Lee & Lucey 2010). Hence, whey expulsion as a result of high-speed centrifugation is also mentioned in the literature as a measure of the water holding capacity (WHC). The WHC of the gel refers to its ability to maintain the aqueous phase within its structure (Bierzuńska *et al.* 2019).

## 2.4 Microbiological properties

Milk is a nutrient-rich medium for growth of many microorganisms, including spoilage microorganisms (Boor & Fromm 2006). Psychrotrophic bacteria are one of the main concerns for microbial spoilage of dairy products (Muir & Banks 2000). The hygienic quality of the raw milk is therefore of great importance as the initial microbial load is driving the deterioration process. Due to pasteurization, low pH and a high concentration of LAB, usually in a range of  $10^7$ - $10^9$  cfu/ml, the growth of spoilage bacteria is efficiently inhibited in fermented milks.

In fermented dairy products, e.g. yoghurt and sour milk, spoilage caused by fungi is a more common issue (Boor & Fromm 2006). Yeast can grow at low pH and temperature and is a major cause of spoilage in fermented milks. Spoilage yeast that have been found in commercial yogurt belong to e.g. the genera *Candida*, *Saccharomyces* and *Kluyveromyces*. The quality defects resulting from growth of these yeasts include off-flavor development and gas production.

## 2.5 Rheological properties

Rheology is the science of flow and deformation behavior of food materials (Lee & Lucey 2010). Although the fermented milks have a high moisture content they have the consistency of a gel and behave as solid-like materials (Tamime *et al.* 2007). The solidness of fermented milks is derived from the aggregation of casein micelles, which forms a three-dimensional network during the fermentation process. During heating, the casein micelles expand in size and form a matrix that immobilize the aqueous phase inside and form a continuous structure with solid-like properties. The texture of fermented milks may also be influenced by EPS, i.e. ropy filaments that contribute to the viscosity of the product. EPS are produced by some strains of bacteria, e.g. *S. thermophilus* in yogurt starter culture (Xu *et al.* 2015).

In rheological terms, stirred yogurt is defined as a viscoelastic fluid, meaning that the yogurt structure exhibits both elastic and viscous properties (Lee & Lucey 2010). In the stirring process it acquires rheological properties of a shear rate thinning non-Newtonian liquid (Tamime *et al.* 2007). The desired consistency in production of stirred yogurt is a smooth and quite viscous gel that can be achieved if the gel network is homogeneously formed in the production process.

The rheological behavior of a liquid system can be studied with a rheometer (Anton Paar 2019). An oscillation test measures the viscoelastic behavior of a material, and is conducted within the so called linear viscoelastic region (LVR). The LVR is the range of shear stress or strain which is low enough to prevent destruction of the sample structure and is determined by performing an amplitude sweep. The amplitude sweep is performed over a shear stress or strain range to determine at what point the applied stress will give rise to breakdown of the structure, i.e. the stability of the structure (Anton Paar 2019). Once information about the LVR of the material has been obtained, a frequency sweep is performed with a stress or strain within the LVR.

During a frequency sweep, the samples are continuously agitated by a sinusoidal stress or strain within the LVR region while their elastic modulus,  $G'$ , and viscous modulus,  $G''$ , is measured. The elastic, or storage, modulus and the viscous, or loss, modulus are measures of the storage and loss of energy per deformation cycle and describe the rheological properties of the material (Lee & Lucey 2010).

The relationship between the elastic modulus,  $G'$ , and the viscous modulus,  $G''$ , of a gel network is determined by the amount of interactions and their strength (Ahmed *et al.* 2016). In production of fermented milks, the  $G'$  will increase when the gel is formed as a result of casein micelle bonding, rearrangement of the protein network and addition of protein strands (Lee & Lucey 2010).

The firmness of yogurt generally increases during storage due to increasing bonds between proteins and the reinforcements of the gel microstructure. During storage, the microbial EPS production may also increase and further contribute to the firmness of the gel (Joyner 2019).

## 2.6 Sensory properties

### 2.6.1 Flavor characteristics of fermented milks

The flavor of fermented dairy products depend on many factors, e.g. the raw milk composition, type of starter culture and fermentation process and applied temperature (Routray & Mishra 2011). A wide range of volatile metabolites produced by the LAB is responsible for the sensory experience of the products.

The flavor characteristic of sour milk largely derives from the presence of diacetyl that is produced by *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* (Ratray 2019). As described by Routray & Mishra (2011), both acids and carbonyl compounds influence the yogurt aroma. As already mentioned, lactic acid and acetaldehyde are the main end products in the metabolism of yogurt starter culture (Ratray 2019). Besides this, almost a hundred different compounds have been identified as flavor contributors (Routray & Mishra 2011). During storage, the concentration of acetaldehyde in yogurt tend to decrease, which may give rise to a loss of flavour. In general, change in aroma compound concentrations during storage at high temperature have been shown to correlate with decrease of sensory quality.

### 2.6.2 Sensory defects

The most common sensory defects of yogurt are development of acid and bitter flavors, and usually, these defects determine shelf life (Routray & Mishra 2011). Acid flavor occurs because of post-acidification during storage and bitterness derives from proteolysis. Both defects are largely dependent on the starter culture. The continuous acidification has to be controlled by a very rapid cooling of the product after completion of the fermentation process. Even so, all of these processes continue in the product at refrigerated temperatures even though at a slower rate.

Other sensory defects mentioned by Routray & Mishra (2011) are derived from yeasts and moulds that may grow in the product. In sensory literature, these flavor defects are most often described using attributes such as yeasty, fruity, musty, cheesy or bitter. The off-flavors derived from fungi are usually detected at a threshold of  $10^4$  organisms per ml. Their growth is highly dependent on the availability of oxygen and will therefore increase in an opened package.

Yet another sensory defect of fermented milks is the lack of characteristic flavor<sup>1</sup>. This can occur if the production of acetaldehyde is too low, e.g. if *S. thermophilus* in yogurt culture is growing excessively at the expense of *L. bulgaricus* (Routray & Mishra 2011).

The mesophilic starter culture used in the production of sour milk is the reason for a more rapid development of an old and bland flavor during storage<sup>1</sup>. *Leuconostoc* spp. present in mesophilic cultures have a high influence on CO<sub>2</sub> production (Ratray 2019). Fresh sour milk is usually bubblier than stored sour milk due to a decreasing concentration of CO<sub>2</sub><sup>1</sup>. Other recurring sensory defects in stored sour milk are bitterness and aroma loss.

### 2.6.3 Pivot© Profile

The traditional descriptive profile methods frequently mentioned in the sensory literature have the drawback of being time consuming and costly, due to the demand for extensive panel training (Fonseca *et al.* 2016). In recent years, a range of different rapid profile methods have emerged that have become particularly popular in quality control. The most recently described methods include comparative approaches where the samples are evaluated in comparison to reference products. One of these methods is the Pivot© Profile (PP), first introduced by Thuillier *et al.* (2015). This method has the advantage of not being restricted to one simultaneous evaluation of the whole sample set. For that reason, the PP method was chosen for this experiment, considering that evaluation of the products took place at several occasions during storage time.

PP originates from the so called free description method (Thuillier *et al.* 2015). Free description allows the assessor to describe the perceived attributes in a completely non-restrictive manner. In addition, the PP method introduces an element of also relating the perceived attributes to a constant reference, the so called “pivot”. In a PP evaluation session, the assessor describes any attributes perceived as less intense or more intense than the pivot product, e.g. less acid or more thick. The panellist may use any sensory attribute they prefer but must relate the intensity of this attribute to the pivot. The comparative element of including a stable reference makes the subsequent data analysis easier to perform than for the free description method. It should be noted that PP should be viewed as an exploratory descriptive method which does not provide quantitative data.

The choice of pivot product highly influences the sensory evaluation and, therefore, should be chosen with consideration to the aim of the study. As suggested by Thuillier (2015) a benchmark within the company product range may be

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<sup>1</sup> Monica Björling, Falköpings mejeri, 2019-09-16

used as the pivot. In the case of this experiment, a fresh sour milk or yogurt as close to production date as possible was used as the pivot product at each session. Although it was far from ideal that the pivot product in each session originated from different batch, it was found to be the most suitable alternative. For this type of product, it was not possible to maintain a stable reference from the same batch, e.g. by freezing the pivot during storage time.

## 3 Experimental procedure

### 3.1 Products

The products chosen for the experiment were sour milk (3% fat, Coop) and mild yoghurt (3% fat, Coop Änglamark). The starter culture for the sour milk includes, according to the manufacturer, different strains of *Lactococcus lactis* and *Streptococcus thermophilus*. The yogurt starter culture consists of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Both products were unflavoured. The best before date of the yoghurt was set to 35 days after the production date. The sour milk had a shorter shelf life and the best before date was 19 days after production. Replicates of each product originated from the same batch, i.e. the same production day.

### 3.2 Sampling design and storage

The experimental design was based on a basic sampling design, which means that a single batch of product is stored under normal conditions and tested at different intervals (Giménez et al. 2012). Here, storage temperature was also added as an additional factor. The normal conditions represent storage at 8°C, which is the highest temperature consumers are recommended for storing the products. To challenge product quality and possibly simulate a worst case of how the consumer may handle the products, they were also stored at 15°C. This is also motivated by the fact that a package of yoghurt or sour milk may be opened and closed several times, including occasionally being placed at room temperature for some time, e.g. on the breakfast table.

The products were pre-ordered from a local Coop store (Stora Coop, Uppsala) and picked up less than 2 h after they had been delivered from the Coop central warehouse. The products were kept in the cold storage at Stora Coop until they were collected. The products were transported by car and placed in two separate cold storage rooms at SLU, Uppsala. The time passing from picking up the products from the cold storage in the store until placing them in the cold storage rooms was maximum 30 minutes. A total of 80 packages of each product type was picked up on different days. Half of the products of each product type was stored at 8°C ( $\pm 1^\circ\text{C}$ ) and the other half was stored at 15°C ( $\pm 1^\circ\text{C}$ ).

### 3.3 Experimental design

The yoghurt sensory analysis was performed on the best before date (d35), seven days past (d42), 14 days past (d49) and 21 days past best before date (d56). Sensory analysis of the sour milk was performed on the best before date (d19) as well as 7 days (d26) and 14 days (d33) beyond the best before date. The experimental time frame beyond the best before date was one week longer for the yoghurt, but motivated by equal ratios to the original shelf life of the products.

The physicochemical, rheological and microbiological analysis were performed on the subsequent day following the sensory analysis, except for one occasion when they were performed the day before due to unexpected circumstances.

Physicochemical, rheological and microbiological analysis were also performed on the same day as the products were collected from the store. For yogurt this was 7 days after the production date. However, the microbiological data presented is from 12 days after production, and the reason is that the analysis had to be repeated due to lack of growth of LAB in the selected dilutions. The sour milk samples were collected in the store 8 days after the production date and were analyzed the same day.

### 3.4 Physicochemical analysis

#### 3.4.1 Samples

On the testing day, two biological replicates, i.e. two packages of the product, were brought from 8°C and 15°C temperature storage, respectively. Each biological replicate was subject to the same experimental procedure. Before sampling, the individual packages were turned 180° by hand 30 times to homogenize the content. All physicochemical, rheological and microbiological samples were prepared

at the same time by distributing the replicates into Falcon tubes. Samples were stored at 4°C until analysis.

### 3.4.2 pH

The pH of the samples was measured using a digital pH-meter (Mettler Toledo, SevenCompact S210). All measurements were performed in triplicates.

### 3.4.3 Water holding capacity

The WHC of the gels was evaluated using a high-speed centrifugation method described by Uduwerella *et al.* (2017). It is used by Uduwerella *et al.* (2017) as a measure of syneresis, but since it is a high-speed centrifugation method, it is here considered a measure of WHC.

Approximately 10 ml of the product was weighed before centrifugation (Thermo Scientific, Sorvall LYNX 6000) at 1500 x *g* for 20 min at 8°C or 15°C, respectively. The expelled whey was collected and weighed. The WHC in percent was determined according to the formula described by (Bierzuńska *et al.* 2019). All measurements were made in triplicates.

## 3.5 Rheological analysis

A rheometer (Bohlin CVOR 150, Malvern Instruments) was used to analyse the viscoelastic properties of the yoghurt and sour milk gels. The temperature was set to 8°C and 15°C, respectively, representing each storage condition and the rheometer was operated with the bob and cap geometry. Approximately 15 mL product was used and the measurements were performed in triplicates for all samples. The loading of the samples into the cup geometry was conducted with care, not to disturb the gels more than necessary. An oscillatory amplitude sweep was performed for each product type before the start of the experiment in order to determine the linear viscoelastic region (LVR). The amplitude sweep was performed in a stress range between 0.1-100 Pa. During the experiment, frequency sweeps were performed with a shear stress of 0.2 Pa, which was a shear stress within the LVR found in the amplitude sweep for both products. The frequency range was 0.1-10 Hz.

## 3.6 Microbiological analysis

### 3.6.1 Bacteria counts

Serial dilutions with a dilution factor of 10 were made from two biological replicates from each storage temperature condition (four biological replicates in total on each testing day). Plate spreading was performed on five different agar media. Plate Count Agar (PCA) was used for total bacteria count and De Man, Rogosa and Sharpe (MRS) agar was for enumeration LAB. Specifically, MRS agar supports growth of *Lactobacillus*, however, *Leuconostoc*, *Lactococci* and *Streptococci* may also grow on this medium since it is not selective (Sigma-Aldrich 2013)

M17 agar with 10% lactose solution supports growth of *Streptococcus* in yogurt as well as *Lactococcus* present in the mesophilic starter culture of the sour milk, e.g. *Lactococcus lactis* spp. The medium is not optimized for *Lactobacillus* but they may also grow there to some extent (Merck 2018).

All plates were incubated for approximately 48 hours. Yogurt PCA and M17 plates were incubated aerobically in 30°C while the MRS plates were incubated anaerobically in 42°C to allow for growth of thermophilic LAB. Agar plates for evaluation of bacteria in the sour milk were incubated according to the same protocol apart from the MRS plates that were incubated at 30°C to allow for growth of mesophilic strains.

Finally, the samples were tested for presence of coliforms on Violet Red Bile (VRB) agar. The detection of coliforms is a common procedure to ensure hygienic quality of dairy products, since coliform bacteria are considered as indicator organisms of contamination (Martin *et al.* 2016). The VRB plates were incubated aerobically at 30°C for 24 h and growth was reported as detected or not detected.

### 3.6.2 Fungi detection

Presence of yeast and moulds was investigated by culturing on Potato Dextrose Agar (PDA). The PDA plates were aerobically incubated at 25°C for 6-7 days. The yeast and mould that were detected during the experiment were further isolated on PDA, collected in tubes and frozen. Due to time restraints, identification of the fungi could not be performed within the scope of this report.

## 3.7 Sensory evaluation

### 3.7.1 Samples and testing conditions

Each test day, two biological replicates from each storage temperature (in total four packages per product) were transported in a cool bag with ice packs to Coop headquarter (Solna, Stockholm). The transport time was approximately 2 h. Included in the transport was also the pivot product, which was a product bought from the store on the same day or the day before the sensory testing. The pivot yoghurt were 14 days old and the sour milk pivots were 12 days old. Since a basic sampling design was used, the pivots originated from different batches, but they were always produced an equal number of days before the sensory test.

The individual packages were turned 180° by hand 30 times to homogenize the content. An amount of 40 ml of each product was poured into plastic cups approximately 15-20 minutes before the beginning of the test. The temperature of the samples at serving was within a range of 10-15°C. It was considered appropriate to serve the samples at a temperature within this range since it enhances the perception of volatile flavors. Due to the transport and time restrictions it was not possible to control for a more exact serving temperature. Each assessor received a tray containing the pivot product (referred to as “Ref”) and five samples marked with random three-digit codes. The coded samples consisted of two biological replicates stored at 8°C and 15°C, respectively, as well as the freshly bought product that was served as a blind control besides being used as the pivot. Tap water and wheat wafers were used as palate cleansers between each sample. The sensory evaluation took place in a room designed according to ISO Standard 8589:2007 with some modifications.

### 3.7.2 Panel

The sensory analysis was performed with an in-house panel at Coop private label department (Solna, Stockholm). The panel consisted of nine individuals in total (age 22-55 years) and each individual participated in different number of evaluations. On each testing day, four individuals from the panel participated in the evaluation. The participation among the nine individuals was not balanced, i.e. they did not participate in an equal number of evaluations.

The in-house panel tests products on a weekly basis using a free description method. Thus, all assessors were familiar with the procedure of sensory evalua-

tions. They did not have any specific experience with sensory evaluation of fermented dairy products, i.e. they were not a trained panel. However, all of them reported to consume such products on a regular basis, i.e. monthly or weekly.

### 3.7.3 Test procedure

The test procedure was based on the Pivot Profile (PP) method, as described by (Thuillier *et al.* 2015). The experiment consisted of sensory testing on four occasions for the yoghurt (d35, d42, d49, d56) and three occasions for the sour milk (d19, d26, d33). The samples were tested in a monadic sequential design, i.e. one samples at a time, and compared to the pivot product according to the PP procedure. The pivot product could be tasted as much as preferred to decide on attributes that were less or more intense in the sample. The samples were served in a balanced serving order, i.e. each sample was served in each position only once, in an incomplete block design due to a lower number of assessors than samples (Lawless & Heymann 2010).

Prior to testing, provided written instructions of the procedure were explained by the panel leader. The assessors were asked to test the sample in the presented order and with their own words describe which attributes, if any, they perceived as less intense and more intense in the coded sample compared to the marked pivot. The attributes could be written in any of the categories appearance, aroma, consistency and flavor. According to the instructions, attributes should consist of descriptive words and exclude whole sentences as well as negative forms. If no differences in intensity were perceived between the sample and the pivot, the assessors were instructed to leave the fields blank.

### 3.7.4 Statistical analysis

All generated words were listed and grouped by semantic categories. For example, the words musty, cowshed, old, carton, paper, cheesy were considered to refer to the attribute off-flavor. A total of 12 semantic groups for sour milk and 10 semantic groups for yogurt were created. Subsequently, the negative frequencies, i.e. the number of times reported as less intense than the pivot, and the positive frequencies, i.e. the number of times reported as more intense than the pivot, of each attribute was compiled in the list of semantic categories. The negative frequencies were subtracted from the positive frequencies to give an estimate of the overall attribute intensity. According to the PP method, the absolute value of the minimum score was added to all scores to obtain only positive scores. Thereafter, these val-

ues were compiled in a contingency table and submitted to a simple Correspondence Analysis (CA) in Minitab® (version 18.1). The obtained results were descriptive product maps displaying two-dimensional graphs of the data sets.

## 4 Results

### 4.1 Physicochemical properties

#### 4.1.1 pH

The pH of sour milk ranged between 4.03 and 4.25 for the 8°C temperature treatment and between 3.98 and 4.20 for the 15°C temperature treatment (Figure 1a). Between the first sampling point at day 8 and the best before date (day 19), the pH of sour milk decreased to the lowest value observed during the experiment. At the best before date, sour milk stored at 8°C and 15°C had pH values of 4.03 and 3.98, respectively. One and two weeks after best before date, the 8°C sour milk had a pH similar to the pH of the first sampling point at day 8. The 15°C sour milk had more or less the same pH at day 8, day 27 and day 34. The observed difference between the temperature treatments, i.e. a lower pH in sour milk when stored at the higher temperature, was significant ( $p < 0.05$ ) at all testing days except day 8.

All pH measurements of yogurt ranged between 4.03 and 4.22 (Figure 1b). The pH decreased between day 7 and day 34 for both temperature treatments, which indicates metabolic activity of acid producing bacteria. During the subsequent three weeks, the pH increased slightly, although never to the level of day 7. Also for yogurt, the difference in pH between storage of the product at 8°C and 15°C, respectively, was significant ( $p < 0.05$ ) at day 34, 43 and 50.

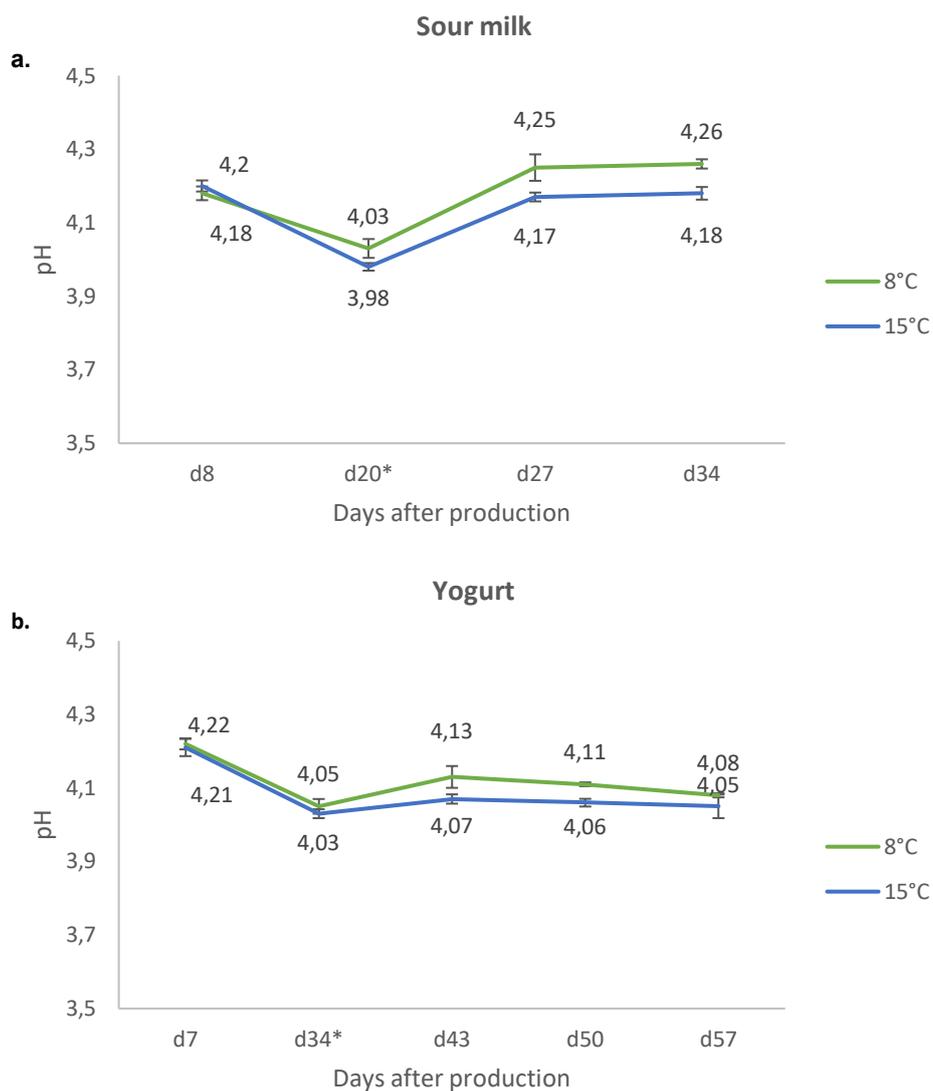


Figure 1. pH of sour milk (a) and yogurt (b) during storage measured days (d) after production. Best before date marked \*.

#### 4.1.2 Water holding capacity

The degree of whey expulsion, as a result of high-speed centrifugation, differed significantly between the two temperature treatments of sour milk on day 34 ( $p < 0.05$ ) (Figure 2a). The sour milk stored in 8°C had a significantly lower degree of whey expulsion on day 34, i.e. a higher WHC. There was no apparent change in whey expulsion over time when the sour milk was stored at 15°C.

The WHC of yogurt followed a similar pattern as the sour milk (Figure 2b) and the only significant difference between the storage temperatures was found on day 57 ( $p < 0.05$ ). In both sour milk and yogurt, a storage temperature of 8°C resulted in a lower degree of whey expulsion, i.e. a higher WHC, at the last sampling point.

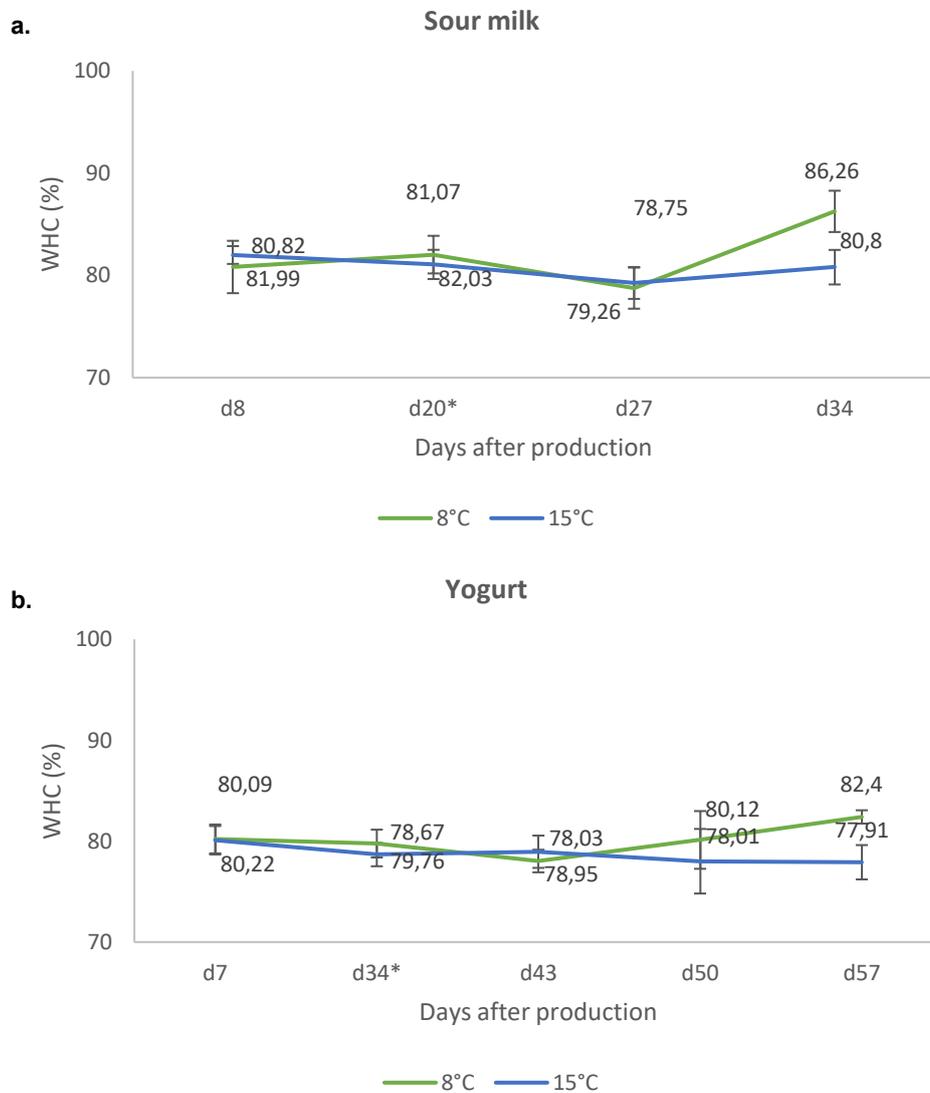


Figure 2. Water holding capacity (%) in sour milk (a) and yogurt (b) during storage measured days (d) after production. Best before date marked \*.

## 4.2 Rheological properties

### 4.2.1 Sour milk

The elastic modulus,  $G'$ , was higher than the viscous modulus,  $G''$ , at all sampling points (not displayed in Figure 3). It shows that elastic, i.e. solid-like, properties are dominating the rheological behavior of the gel rather than viscous liquid-like properties. The  $G''$  of sour milk was stable during the experiment time at both storage temperatures and, therefore, it is not included in Figure 3a-b.

During storage at 8°C (Figure 3a), the elastic modulus barely decreased during the experiment. During storage at 15°C (Figure 3b), however, a distinct reduction in elastic modulus was observed between day 8 and the following testing days at day 20, 27 and 34. The observation indicates that during storage at a higher temperature the solid-like properties of the sour milk decreased. This result is also reflected in the sensory evaluation, where the samples were perceived as thinner on day 33. However, the attribute thin was reported at both storage temperatures, while the decreased elastic modulus was only observed for the higher temperature.

### 4.2.2 Yogurt

The elastic modulus,  $G'$ , was higher than the viscous modulus,  $G''$ , for all measurements (not displayed in Figure 3). The  $G''$  of yogurt was stable during the experiment time at both storage temperatures and, therefore, it is not included in Figure 3c-d. When Figure 3a-b and 3c-d are compared, it is visible that values of  $G'$  were always higher in the yogurt than in the sour milk. Thus, the results indicate that yogurt exhibited a higher degree of solid-like properties than sour milk.

The viscoelastic behavior of yogurt was reversely influenced by storage time in comparison to sour milk. For both storage temperatures (Figure 3c-d), the elastic modulus increased between day 7 and the following measurements at day 34, 43, 50 and 57. The observation indicates that the solid-like properties of the gel increase during storage regardless if the temperature was 8°C or 15°C. However, the difference was larger in the higher temperature. The change in viscoelastic behavior took place between day 7 and day 34 and thereafter the material exhibited similar viscoelastic behavior.

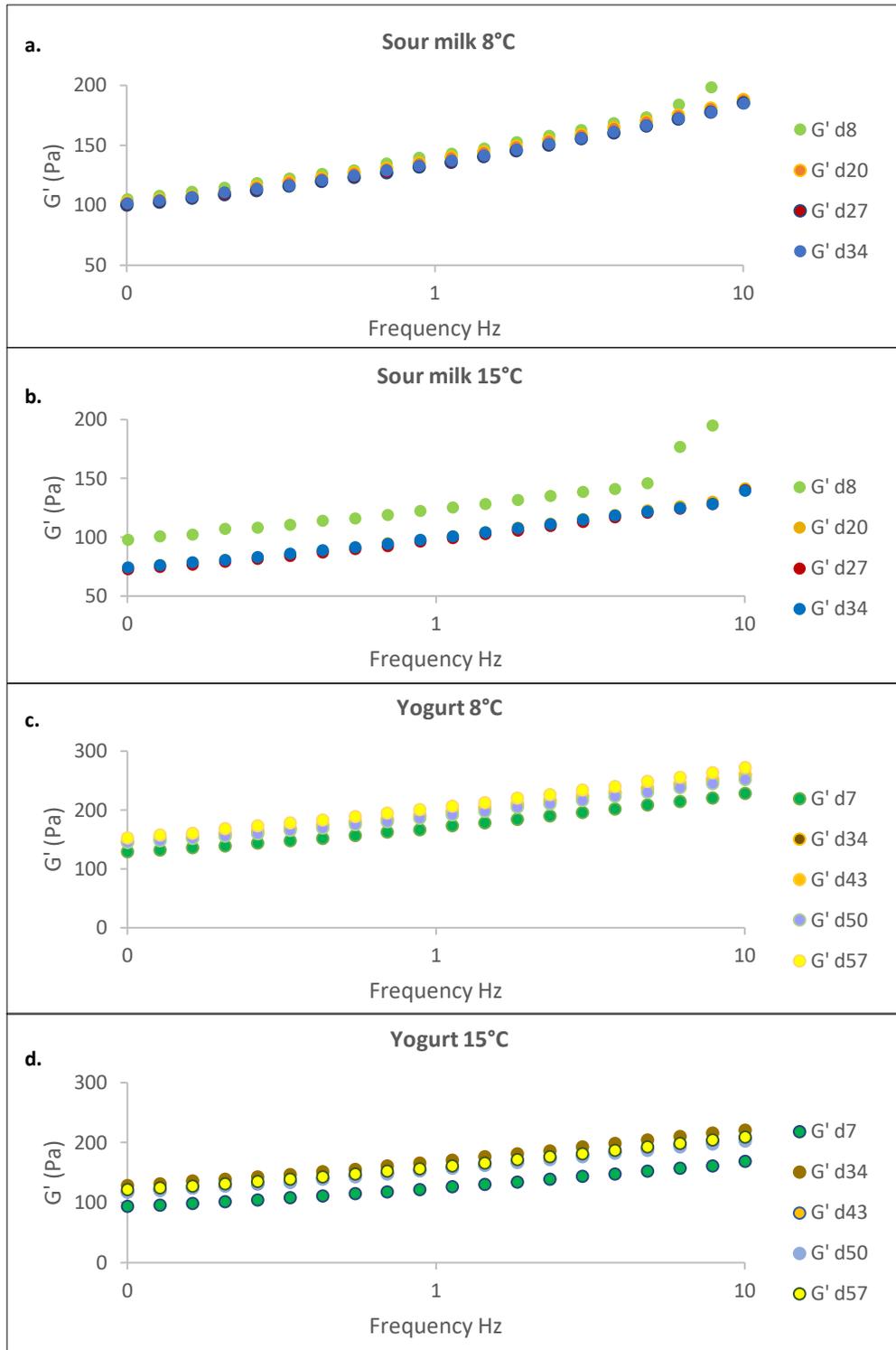


Figure 3. Elastic modulus ( $G'$ ) as a function of frequency (Hz) for sour milk (a-b) and yogurt (c-d) measured days (d) after production.

## 4.3 Microbiological counts

### 4.3.1 Sour milk

The bacteria counts in sour milk during storage is displayed in Figure 4a. Total bacteria count on PCA decreased from around  $10^7$  cfu/ml at day 8 to  $10^6$  cfu/ml at day 20 in the 8°C samples. In the 15°C samples, there was a slightly higher viability at day 20. During the remaining storage period, the viability was mainly unchanged.

The LAB enumerated on MRS agar decreased with less than one log reduction and the viability was rather stable around  $10^7$  cfu/ml during the entire storage time at both temperatures. The most noticeable difference during storage time appeared in the growth on M17 agar, which is most likely dominated by *Lactococcus* and *Streptococcus*. It decreased with a 2 log reduction between day 8 and 20 for the 8°C samples and a 3 log reduction for the 15°C samples. For the remaining sampling points at day 27 and 34 the population at 8°C had decreased to around  $10^3$  and no viable bacteria were observed in the 15°C samples. The result indicates that both storage time and storage temperature influenced viability of different LAB in sour milk.

### 4.3.2 Yogurt

The composition of bacteria in yogurt (Figure 4b) differed markedly from sour milk (Figure 4a), which is explained by the use of different starter cultures. At the first count on day 12, the total bacteria count was approximately  $2 \times 10^7$  cfu/ml. Between day 12 and day 57, it decreased with one log reduction at 8°C storage temperature and with 3 log reduction at 15°C storage.

A similar viability pattern was observed for bacteria enumerated on M17 agar, which most likely was dominated by *S. thermophilus*. The population remained around  $10^9$  until day 57 at 8°C storage, while in a higher storage temperature the numbers continuously decreased. Overall, a 2 log reduction was observed between day 12 and 57 at 15°C storage.

Growth on MRS agar, especially supporting growth of *Lactobacillus*, was only observed at day 12 when stored at 15°C. The viability at 8°C was slightly higher and growth was observed at day 12, 34 and 50, although the numbers continuously decreased. At day 43 and 57, data is missing because the colony forming units were too few to count even in the lowest dilutions. The result indicates that storage temperature influenced the viability of LAB since higher bacteria numbers were

observed on both M17 and MRS agar when yogurt was stored at 8°C. Also, storage time influenced growth on both types of agar.

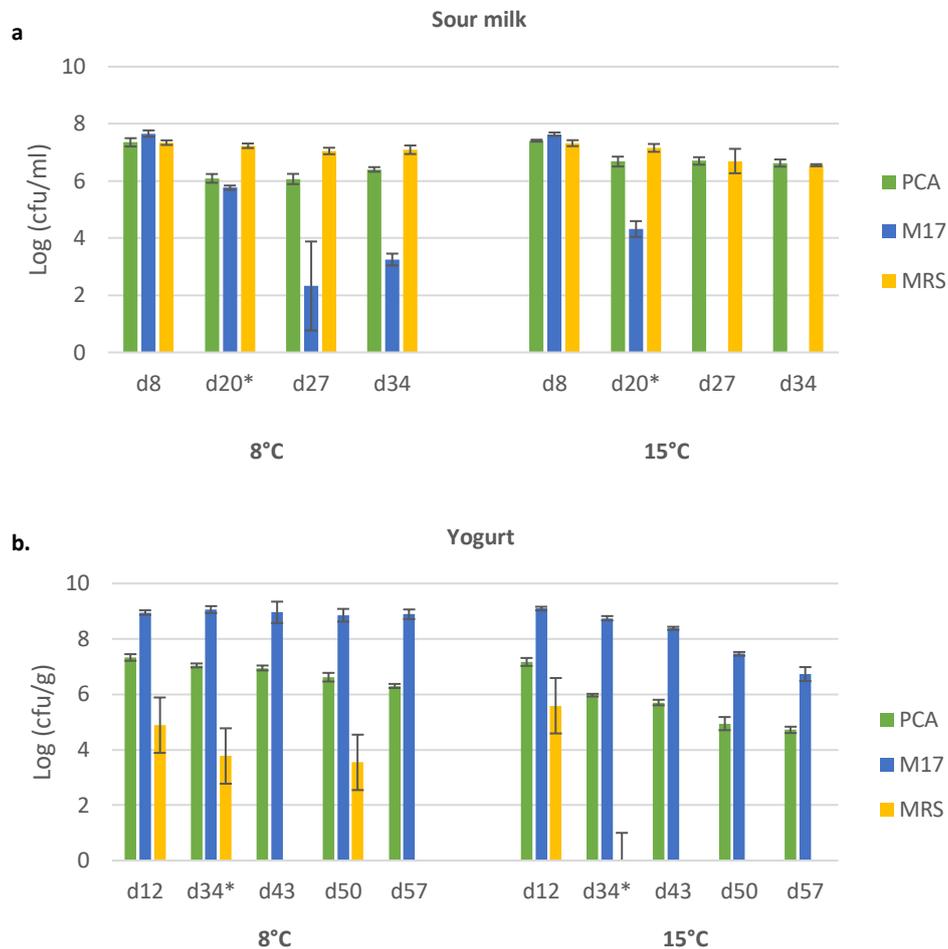


Figure 4. Log count (cfu/ml) of bacteria on PCA, MRS and M17 plates for sour milk (a) and yogurt (b) measured days (d) after production. Best before date marked \*.

#### 4.3.3 Fungi and coliform bacteria

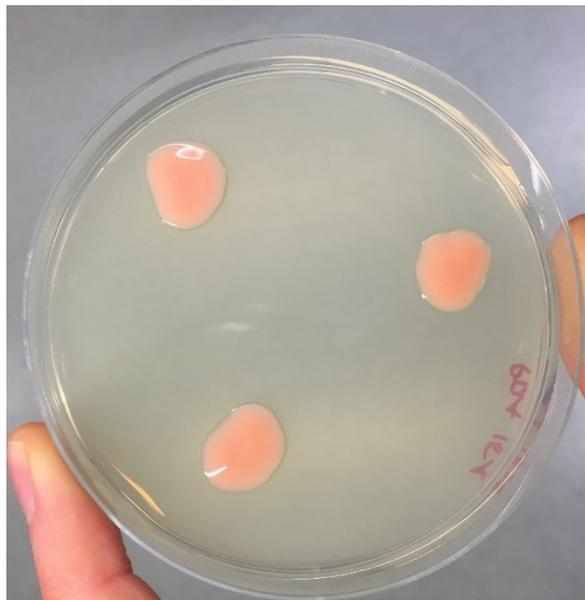
Culturing of potential coliform bacteria was performed to ensure hygienic quality of the products and detect possible processing contaminations. Although each batch is monitored at the dairy plant post production, it was also controlled during the

experiment to ensure safety of the products. Coliform colonies could not be observed in any of the products.

At each sampling occasion the presence of yeast or mould was tested by inoculation on PDA agar. Growth was reported as detected or not detected and observed after 6-7 days of incubation. For the sour milk, fungal growth was observed already at best before date. On day 20, fungal growth was observed on all four replicates stored at 15°C and in one of the replicates stored at 8°C. One week later, growth was observed on all plates except for one and at day 34, two weeks past best before date, growth was observed in all inoculated plates. All the detected colonies had the same visual appearance (Figure 5).

On one of the sour milk plates from day 34, some mould growth was observed as well. However, since only one colony was observed on one of the plates it was not possible to conclude if the mould originated from the sour milk package or if it was due to contamination during the plate inoculation.

No fungi organisms were detected during storage of yogurt except for one mould colony observed on one of the plates at day 57. The same difficulty to draw any conclusions applies here as above. Due to time restraints, it was not possible to perform the necessary investigations to identify the fungi within the scope of this thesis.



*Figure 5.* Representative example of fungal growth observed in sour milk 20, 27 and 34 days after production.

## 4.4 Sensory evaluation

### 4.4.1 Sour milk

The results of the correspondence analysis from the sensory evaluation of sour milk is displayed in Figure 6a as a symmetric biplot. The first two dimensions together explain 82% of the variance. Attributes (red circles) that are clustered together on the map have a similar profile and the same applies for samples (blue squares). Attributes that contribute most to the first two dimensions, i.e. located far from the middle, are most important in explaining the variability within the data set. Attributes that do not have a large influence on the first two dimensions, i.e. located close to the middle, are less important. Here, creaminess and fresh had the highest contribution to the positive pole of the first dimension. Thin, musty, astringent and acid contributed the most to the negative pole, although the influence was not as strong as for creaminess.

As seen in the plot, storage time seems to be of high importance for the sensory attributes since the samples from the same day are closely located to each other. Larger differences were observed between the samples with different storage time. On day 19 the sour milk was most similar to the blind control whereas on day 26 and day 33 the samples were less similar to the blind control. The attributes creaminess and fresh were in contrast to the attributes thin, musty, astringent and acid in the first dimension.

The relationships between samples and attributes displayed in Figure 6a were analyzed with help from the asymmetric plot from the correspondence analysis (Appendix 1). It appears that the day 19 samples related more to the attributes creaminess, fresh and sweet. The attributes creaminess, fresh and thin were most influential on the first component. Samples from day 26 were related to acid and fruity and day 33 samples were related to thin and musty.

### 4.4.2 Yogurt

The result of the sensory evaluation of yogurt is displayed in Figure 6b as a symmetric biplot from the correspondence analysis. The first two dimensions together explain 90% of the variance. Creaminess had the highest contribution to the positive pole of the first dimension, while thinness had the highest contribution to the negative pole.

Both samples from day 35 were more similar to the blind control compared to the other samples, however, the samples stored at 15°C showed most similarity to the blind control. Most different to the blind control were the 8°C samples from

day 49. Samples from day 56 appeared to be somewhat more similar to the pivot than samples from day 42 and day 49, respectively.

The creaminess attribute was most influential on the first dimension. Creaminess was associated with the blind control and samples from day 35. The attributes fresh, fullness and cream were also more related to the blind control and day 35 samples than the older samples. Samples from day 42, 49 and 56 were more related to the attributes thin, smooth, musty and astringent. Although, thin and smooth were the attributes that contributed most to the variation in the first dimension. The day 42 samples were somewhat more associated with the attribute smooth, while the day 49 samples were more associated with thin. The attributes musty and astringent had a very small contribution to the dimension, but the attribute musty was somewhat more related to samples from day 56.

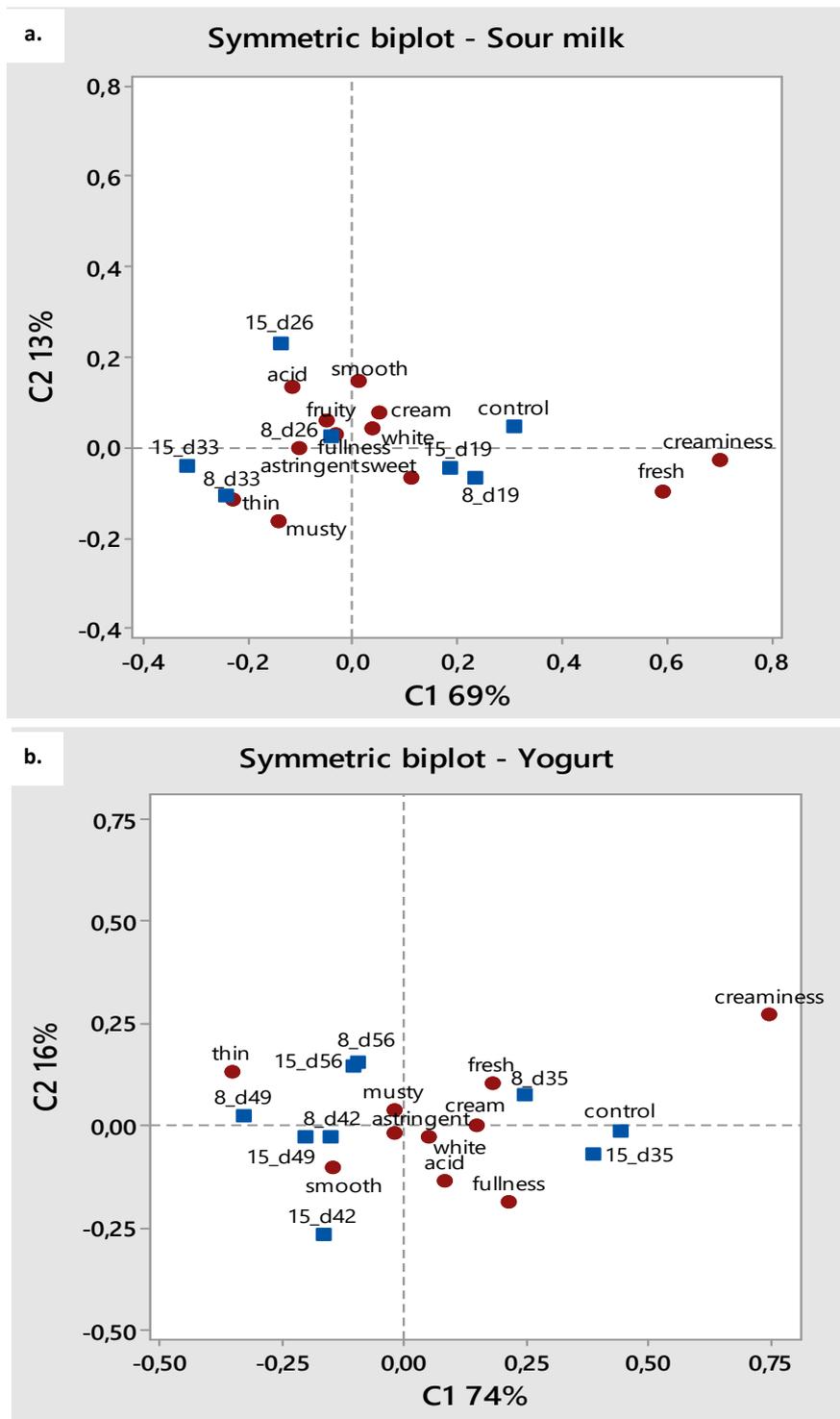


Figure 6. Symmetric product maps of sensory attributes (circles) and samples (squares) in the first two dimensions (C1, C2). Samples are marked with storage temperature (8 or 15) and days, d, after production.

## 5 Discussion

### 5.1 Physicochemical results

The low pH observed in both products at best before date (Figure 1) indicates that the metabolic activity of acid producing bacteria has been high. This relates to the fact that the highest concentration of viable bacteria was found at the first sampling point (Figure 4). The subsequent increase in pH is not easily explained, but may be related to the decreasing viability of LAB. A measure of titratable acidity could have been used in addition to pH measurements, to investigate the post-acidification more thoroughly.

At the last sampling point of both sour milk and yogurt (Figure 2), the WHC of the products stored at 8°C increased and were significantly different from the products stored at 15°C. An increasing WHC indicates an increased resistance to the forces applied in high-speed centrifugation. It was not unexpected that products stored at higher temperature would exhibit a lower WHC since higher temperature favors contraction of the gel network (Salvador & Fiszman 2004).

The results of this study agrees with those by Serra *et al.* (2009), who observed a tendency of the WHC to increase with storage time at 4°C. This was explained by stronger interactions within the gel network, giving rise to a more organized structure with time, which may be more resistant to high forces. However, hydrophobic interactions can not be part of the explanation, since they are known to decrease in strength in low temperature (Bhat *et al.* 2016). It is not clear what kind of interactions Serra *et al.* (2009) are referring to as a possible explanation to increase WHC during storage at low temperature. Possibly, EPS produced by some strains of the starter culture bacteria, e.g. *S. thermophilus* and *L. lactis* subsp. *cremoris*, may contribute to a stronger gel network and an increased WHC (Behare *et al.* 2009; Xu *et al.* 2015).

It should be noted that the variation in the WHC measurements were high (Figure 2), which may contribute to uncertainty in the results.

## 5.2 Rheological results

The shift in viscoelastic behavior of the sour milk seems to appear somewhere between day 8 and day 20 (Figure 3a-d). A possible reason for the decrease in elastic modulus is that rearrangements have taken place in the gel network together with fusion of particles. It may stress the strands of the gel network which in time will become thinner and in turn decrease the solidness of the gel (Serra *et al.* 2009).

It has been mentioned that stirred yogurt gels have the ability to recover their structure during cold storage after the destructive stirring treatment in the production process (Serra *et al.* 2009). However, it should be noted that the first tests of this experiment took place 7 days after production. Since the change of rheological behavior before the first sampling point is unknown, it is not possible to conclude if the gel had already recovered before the experiment began. The results indicate that the elastic modulus, i.e. the solid-like properties, of the yogurt increase after day 7.

A possible explanation for the increase in elastic modulus may be the rebuilding of the stirred gel network as proteins rearrange and their connections increase (Xu *et al.* 2015). Production of EPS contributes to increased viscosity and may also explain why the solidness of the gel increase. Both *L. bulgaricus* and *S. thermophilus* produce EPS, although *S. thermophilus* is the major EPS producer. The numbers of viable bacteria enumerated on M17 agar remained high during the entire storage time of yogurt, indicating continuous metabolic activity of *S. thermophilus*. In sour milk, the numbers of viable bacteria on M17 agar, corresponding to e.g. the EPS producing *S. thermophilus* and *L. lactis* subsp. *cremoris*, decreased during storage and were present in lower numbers at all sampling points compared to yogurt. This might explain why the elastic modulus of sour milk decreased and the opposite behavior was observed in the yogurt.

## 5.3 Microbiological results

Hamann & Marth (1984) observed that the production process and choice of starter culture have a high influence on LAB growth, as different patterns were observed for different manufacturers. It is therefore likely that the production process and starter culture strains used for these particular products have a large impact on LAB growth during storage. As conditions at the dairy plant are unknown, it is not possible to determine how these factors may have influenced the bacteria growth.

For both products, the highest number of bacteria counted on each plate type was found on the first testing day, i.e. 8 days after production in case of sour milk and 12 days in case of yogurt. The most apparent change in pH also occurred between the first day and the second testing day, i.e. the best before date (Figure 1), which indicates a high metabolic activity.

According to Hamann & Marth (1984) storage temperature is a highly influential factor for bacteria growth. The effect of storage temperature was evident in this experiment for bacteria grown on M17 agar in sour milk and on MRS agar in yogurt, as their numbers decreased to a higher extent when stored at 15°C. In addition, a decline in bacterial numbers on M17 agar of yogurt, most likely dominated by *S. thermophilus*, was only visible when stored at 15°C.

It has previously been shown that storage time influence the total number of bacteria in stirred yogurt. According to Beal et al. (1999), the major decrease in numbers appeared during post-acidification between day 7 and 21 and was more apparent for *S. thermophilus* than *L. bulgaricus*. In this case, the opposite was observed, as the growth on MRS agar was lower and decreased faster than growth on M17 agar. Hamann & Marth (1984) related the decreasing bacterial numbers to the pH reduction. It can also be related here, as the major pH reduction appeared between day 12 and 34 and growth on MRS agar had decreased at day 34. The fact that no growth on MRS plate was observed day 43 was due to technical errors.

*L. bulgaricus* is, according to Beal et al. (1999), expected to be more acid tolerant and exhibit a higher survival rate at low pH. Since the opposite was observed here, it may be explained by difference in acid tolerance among strains. The acid tolerance of LAB is also dependent on growth phase, e.g. they are considered to be more stress tolerant in the stationary phase (Zotta et al. 2008). According to Zotta et al. (2008) there is a large variability in stress response between different strains of *S. thermophilus* during its growth phases. If the same applies to all LAB, low pH on day 20 in the sour milk and day 34 in the yogurt may have given rise to different stress responses in the starter culture bacteria that influence their survival patterns.

## 5.4 Sensory results

First, it should be noted that the results of the sensory evaluation must be interpreted with caution. The PP method is in itself an exploratory method and does not provide an objective description of the products. Instead, it is a way of finding interesting associations that may be studied further in other sensory tests. Also, in the sensory evaluation conducted as part of this study, the number of assessors at each testing day was small (n=4). Sensory tests described in the literature rarely

consists of panels with such a low number of assessors, as it limits the generalizability of the results. With this in mind, the results of this experiment may be used as a starting point to find a new hypotheses to test concerning the sensory quality of the products after best before date.

The results of the experiment show that both products had a higher creaminess at the first sampling point and were thinner at the sampling points two and three weeks after best before date. Both attributes are related to the consistency of the product and it is reasonable that a creamy product is not perceived as thin and vice versa.

The thinness of the sour milk at day 33 (Figure 5a) can be related to the decrease in elastic modulus during the storage. It is likely that a gel with decreasing elastic properties would be perceived as thinner compared to the pivot. However, the same correlation was not observed in the sensory evaluation of yogurt. The elastic modulus of yogurt increased during storage but it was also perceived as thinner compared to the pivot at day 49.

Fungi growth is one possible explanation for the attributes musty and fruity that were observed in the microbiological analysis of sour milk from day 20 and onwards. At day 33, all replicates also showed growth of yeast like colonies in the microbiological analysis. It is a reasonable assumption that growth of fungi and the musty attribute to some extent might correlate.

The attribute acid, which was related to day 26 in the sensory evaluation of sour milk (Figure 5a), is likely to be explained by an increasing production of lactic acid by the starter culture. Most acid production appeared before day 20 where the lowest pH was observed (Figure 1a). Therefore, it is expected that the attribute acid would more frequently mentioned at the sampling points at the end of storage. Although, there is no explanation for why it was especially noticeable at day 26.

The smooth attribute, observed in yogurt on day 42, is most likely related to batch variability. The panel leader observed at this sampling point that the consistency of the pivot was noticeably different from the pivots that were used in the previous evaluations. The pivot at day 42 was grainier and more inhomogeneous in consistency and for that reason the stored samples may have been perceived as smoother.

## 5.5 General discussion

As mentioned in the background of this report, shelf life is a dynamic concept that is dependent on many parameters. This experiment was designed as an explorative pilot study and the aim was to gain insight about several of these parameters in relation to experimental objects. The comprehensive approach of an exploratory

study makes it possible to study several parameters in the same experiment. However, it also limits the possibility to gain a deeper understanding of each parameter.

The major limitation with this experiment was that only one batch of each product was tested. It is one batch out of many batches produced per year and the variability between batches is in this case unknown. During one of the sensory evaluations of yogurt the panel leader observed that the pivot product, originating from a different batch, had distinctly different appearance and consistency compared to the equally aged product from the test batch. The observation is an indicator of the batch-to-batch variability that may occur in the production.

Another important aspect of this experiment is that culturing was used as the method for microbial characterization of the samples. Since a culturing method is susceptible to human error it has an inherent uncertainty that must be taken into consideration. Especially in this case, when the results are based on two biological replicates from one single batch and the practice and the preparation time of the culturing method was limited, the results should be interpreted cautiously.

The report is lacking a characterization of the fungi growth that was observed in the microbiological analysis. Due to time restraints, it was not possible to perform an analysis to identify the fungi on a species level. It would have been of interest to identify the yeast-like colonies to confirm their species. Also, to identify the mould growth and investigate if it originated from the product or from contamination during the analysis. Besides identifying, it would have been interesting to enumerate the fungi, since it has been mentioned that off-flavors derived from fungi are detectable at levels of  $10^4$  organisms per ml. Having the numbers of fungi, it would have been interesting to relate these to the sensory analysis, e.g. the attribute musty that appeared on day 20.

## 5.6 Future research

Several other measurements may be used in future studies to evaluate the quality of fermented dairy products. The method used to measure WHC is not equivalent to the spontaneous syneresis that occur when the gel structure loses its strength during storage. A suggestion for future studies is to include a method for measuring the spontaneous syneresis during storage. Likewise, other rheological tests may be performed to measure the viscosity of the products. Texture analysis is frequently mentioned in relation to yogurt quality and may also be of interest to be further studied. An alternative for microbiological analysis is MALDI-TOF mass spectrometry to identify the bacteria in the products. In this experiment, the bacteria growing on different agars were assumed to be dominated by different species but the bacteria were not identified.

It may also be of interest to investigate the influence of the starter cultures on the quality parameters. Since different strains differ in their proteolytic activity and production of EPS, it may be of interest to correlate these properties to the physicochemical, rheological and sensory parameters that were investigated here. However, that requires knowledge about the specific strains of the starter culture.

In order to generalize results beyond a single batch, future experiment should include a larger number of batches to ensure that the findings are representative of the production in general. The experiment should include both microbiological and sensory parameters, since these are both crucial for shelf life. These parameters could be included in the evaluations made at the dairy plants.

As mentioned in the background of this report, shelf life is a highly dynamic concept that is dependent on consumer expectation. Since fermented milks, like sour milk and yogurt, generally are considered as safe food products, the sensory parameters become an important factor. It is not possible to say if the attributes reported in this experiment, e.g. the products became less creamy and more musty compared to the pivot during storage, are meaningful to the consumer. A consumer sensory testing, i.e. preference or acceptance test, should be performed with a larger number of persons, in order to find out if the perceived differences influence the consumer's acceptance.

## 6 Conclusion

In this study, physicochemical, rheological, microbiological and sensory parameters of yogurt and sour milk were investigated in relation to storage time and temperature. The purpose was to gain insight about possible improvements related to date labelling at manufacture and retail level.

The study indicates that most intense metabolic activity of LAB occurred before best before date, as it had the lowest pH. WHC was relatively unchanged during storage but showed some temperature dependence at the end of storage. The solid-like properties of sour milk decreased during storage when stored in higher temperature. In yogurt, the opposite pattern of solid-like rheological behavior was observed, as the elastic modulus increased. The microbiological counts showed that the highest number of LAB in yogurt appeared on M17 agar and their viability seemed to be influenced by storage temperature. Bacteria growing on MRS agar dominated the microbial population in sour milk and maintained viability during storage time at both temperatures. Fungal growth was observed in sour milk from best before date and onwards. The fungal species and their influence on sensory quality is of interest to further investigate. The sensory evaluation indicated influence of storage time. Sour milk was attributed as more creamy, fresh and sweet at best before date and more thin and musty two weeks later. Yogurt was attributed with higher creaminess at best before date and more thin after two and three weeks of storage.

The experiment was designed as an explorative pilot study and a single batch was included. This limits the possibilities for generalizations, and future research should include several batches. It is also necessary to investigate if the sensory changes in the products after best before date influence consumer acceptability. Date labelling is one factor influencing food waste at consumer level and, therefore, evaluation of optimum shelf life is a tool food industry can use to contribute to diminished food waste.

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## Appendix 1 – Popular Scientific Summary

### Best before – not always worse after

**Are you one of those people who carefully check the labelled date on your breakfast yogurt before you pour it in your plate? Then you are not alone. Studies show that in Sweden we discard about 6.5 kg of yogurt, sour milk and other fermented dairy products in the drain every year.**

Astonishingly, that is just a fraction of all the food we throw away in the bin. Every year each of us throw away almost 100 kg of food. Actually, a lot of this food is still edible and would contribute to a lot less damage on the environment if it were allowed to pass through our mouths instead of our bins.

To be honest, it is not all your fault though. The date labels have been invented for a good reason. The “use by” date is used on food products that may cause disease and should not be consumed after the date has passed. The label “best before”, however, is another matter. It is used so that you as a consumer can trust that the food product will retain its original quality during this period. This means that “best before” is more of a subjective kind of labelling, since the manufacturer decides what level of quality they want to guarantee the consumer. It is argued that even very small extensions of the labelled best before date could diminish food waste in consumer households considerably.

Fermented dairy products, such as yogurt, sour milk and crème fraiche, are good products to start with. That is why in this study I explored what happens in yogurt and sour milk after best before date had passed. I also wanted to see what happened if the products were treated a bit more harshly and were stored in a higher temperature than recommended. In reality, food products are not always treated in the most optimal conditions at consumer homes. Maybe you are enjoying a long Saturday breakfast and the package of sour milk happens to stay out on the table for quite a while. At least that happened to me more than once!

The results of this study indicated that yogurt maintained its quality three weeks after best before date. Sour milk was studied until two weeks after best before date, but for this product, I believe the microbiological quality needs to be further examined. Foods like yogurt and sour milk are generally very safe products be-

cause they are fermented. A fermented food is made by adding bacteria that produces lactic acid. Lactic acid makes the food sour and the bad bacteria, giving rise to disease, do not like that kind of environment. The major quality problems in fermented dairy products is rather the potential growth of yeasts and moulds that can spoil the product and influence the sensory quality. Sensory quality refers to the experience of the food connected to the physical senses, e.g. its flavor and consistency.

Both the yogurt and the sour milk I tested appeared to become thinner when it was stored for a long time. The individuals that tested the products also described the sour milk as mustier after the best before date passed and that it had lost some of its characteristic sour milk flavor. Both the yogurt and sour milk was also a lot creamier when it was fresh. All of these sensory changes may, or may not be, a deal-breaker for you as a consumer. That is why the sensory changes need to be related to consumer preference and acceptability. Would you buy the same yogurt again if it was less creamy and more musty at the end of its labelled shelf life, or would you be disappointed and choose another brand? That is the question the manufacturer wants to be sure of, because you as a customer is by far their most valued asset!

So what about the yeast and moulds? That is not something you would like to be surprised by when you pour up your plate. As for the yogurt I tested there is nothing to worry about. However, with the sour milk there is some question marks to be settled. At the day of best before some fungi growth appeared when the sour milk was stored in a higher temperature than recommended. Two weeks later, there was fungi growth regardless of which temperature it had been stored in. It might not be a problem, since fungi are not really something that makes you sick. However, when in high numbers they may give rise to some unpleasant off-flavors that make you not enjoy your breakfast as much. An interesting observation I made was that, as mentioned before, a musty flavor appeared in the sour milk after best before date has passed. It is possible that this flavor is related to the growth of fungi and that the spoilage of the product give rise to this sensory defect.

The take home message here is that best before date is not necessarily the worst after date. It is not possible to conclude anything about yogurt and sour milk in general terms, because the sample size of this study was far too small. However, the results show that the statement that you should “look, smell and taste” before you throw food away is not an accidental expression. Let us hope that the quality of these types of products will be investigated more in the future, so we can continue to challenge “best before” and diminish food waste.

## Appendix 2 – Additional plots sensory analysis

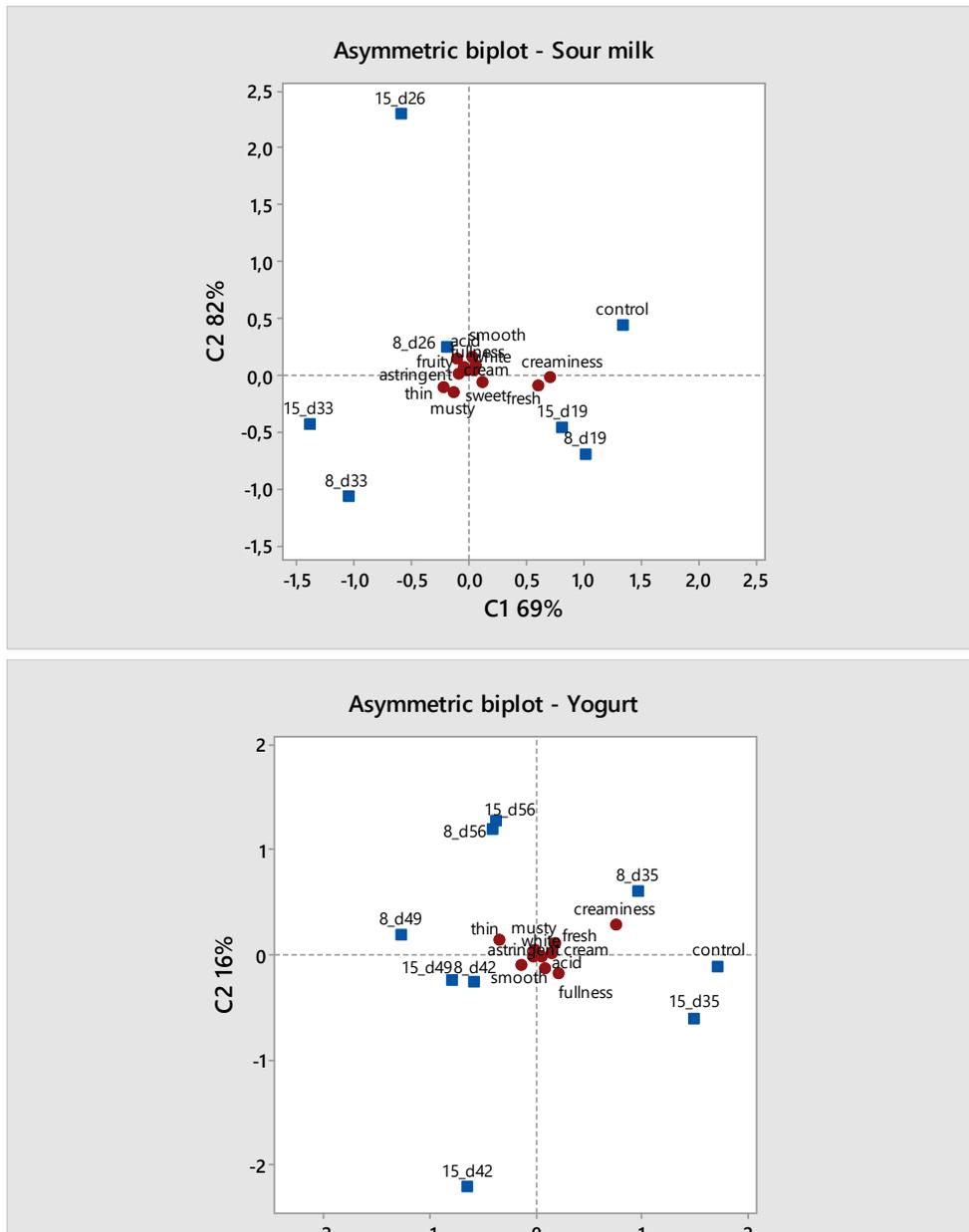


Figure 7. Asymmetric product maps displays sensory attributes (red circles) and samples (blue squares) in relation to the first two dimensions (C1 and C2). Samples are marked with storage temperature 8°C or 15°C and days, d, after production.