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Abstract

Plasmin (PL) is a native proteinase in milk, which incorporates a complex system of inhibitors and activators, whose activity is known to cause changes in ultra-high temperature (UHT) treated milk during storage. The objective of this study was to investigate if there were any seasonal variations in PL and plasminogen (PG) derived PL activity in Swedish silo and UHT milk, and if enzyme activity was affected by storage temperature and storage time. The reproducibility and detection limit also was calculated. Moreover, the relation between the somatic cell count (SCC) and PL and PG-derived PL activity was investigated. Milk collected monthly from November 2014 to November 2015 was ultra-centrifuged and analysed for PL and PG derived PL activities by a spectrometric assay using a chromogenic substrate. The reproducibility of the method was 10 % for the PL assay, and 9 % for the PG assay. The limit of detection was calculated to 0.59 U/ml. PL activity in silo milk was on average 3.35 U/ml ±15 % and PG-derived PL activity was on average 92.27 U/ml ±7 % over the year. Significant differences in PL and PG-derived PL activity between months indicated seasonal variations. The highest activity of PL activity was in milk collected during October, January and November 2014, and highest PG-derived PL activity in October, December and November 2015. Stage of lactation is believed to cause the seasonal variation of PL activity. No PL activity was detected in UHT milk hence it was inactivated by the UHT treatment. The UHT treatment decreased the PG-derived PL activity by 75 % to 21.16 U/ml ±17 %. Neither storage temperature nor storage time affected PG-derived PL activity, and variations observed during storage were most likely due to within batch variation. There was no significant correlation between SCC and total PL and PG-derived PL activity, PL activity, or PG-derived PL activity in silo milk. Although SCC has been reported to co-vary with PL and PG-derived activity, the relatively low cell counts in the Swedish milk seems to be a plausible explanation for not observing this correlation.

Keywords: plasmin, plasminogen, UHT, bovine milk, seasonal variation

Sammanfattning

Plasmin (PL) är ett endogent proteinas i mjölk, som ingår i ett komplext system av inhibitorer och aktivatorer, vars aktivitet är känd för att orsaka förändringar i ultrahög temperatur (UHT)-behandlad mjölk under lagring. Syftet med denna studie var att undersöka om det förekommer säsongsvariationer i PL-aktivitet och plasminogen (PG) härledd PL aktivitet i svensk silo- och UHT-mjölk, samt om enzymaktiviteten påverkades av lagringstemperatur och lagringstid. Metodens reproducerbarhet och detektionsgräns beräknades även. Dessutom undersöktes relationen mellan celltal och PL- och PG-aktivitet. Mjölk insamlad månadsvis från november 2014 till november 2015 ultracentrifugerades och analyserades med hjälp av en spektrometrisk metod med ett kromogent substrat. Reproducerbarheten för metoden var 10 % för PL-analysen och 9 % för PG-analysen. Detektionsgränsen beräknades till 0,59 U/ml. Medelvärdet under året för PL-aktiviteten i silomjölk var 3,35 U/ml ±15 % och den PG-härledda aktiviteten var 92,27 U/ml ± 7 %. Signifikanta skillnader i PL- och PG-härledda PL aktiviteten mellan månaderna indikerade en säsongsvariation. Högst PL aktivitet fanns i mjölk insamlad under oktober, januari och november 2014, och högst PG-härledda PL aktivitet i oktober, december och november 2015. Skillnader i laktationsstadium tros vara orsaken till PL-aktivitetens säsongsvariation. Ingen PL-aktivitet detekterades i UHT-mjölk vilket indikerar att enzymet inaktiverats under UHT behandlingen. UHT-behandlingen reducerade den PG-härledda PL aktiviteten med 75 % till 21,16 U/ml ±17 %. Varken temperaturer eller lagringstid påverkade den PG-härledda PL aktiviteten, och variationer som observerades under lagringen är mest sannolikt resultatet av variationer inom batcher. Det påfanns ingen signifikant korrelation mellan celltal och PL-aktivitet och/eller PG-härledd PL-aktivitet. Fastän celltal rapporterats samvariera med PL-aktivitet och PG-härledd PL-aktivitet så observerades detta ej i studien, detta troligtvis på grund av de relativt låga celltalen i svensk silomjölk.

Nyckelord: plasmin, plasminogen, UHT, mjölk, säsongsvariationer

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Abbreviations

ANOVA	Analysis of variance
EACA	ε-aminocaproic acid
PG	Plasminogen
PL	Plasmin
SCC	Somatic cell count
UHT	Ultra-high temperature treatment
UV	Ultra-violet radiation

1 Introduction

The Swedish milk association (Svensk Mjölk), today LRF mjölk and Växa Sverige, regularly investigates and compiles data on Swedish milk composition. In the latest report (Lindmark-Månsson 2012), the composition of milk showed a significant increase in total protein, casein, fat and ash content, together with a decrease in pH, lactose and urea. Variations in the unprocessed silo milk composition has also been noticed according to season, and may affect the quality and shelf-life of the final product, and is therefore of great importance for the dairy industry.

The advantages of ultra-high temperature (UHT) treated milk products are many. It is convenient to store milk at room temperature, especially for retailers and transport companies that save money without the need to maintain the cold chain. Some of the drawbacks involve sensory aspects that must be compensated for. Browning, fat separation, sedimentation, off flavours and gelation has been observed during long storage of UHT milk (Malmgren 2007). These issues are the result of chemical changes in milk and proteolytic activity of various active enzymes. The native proteinase plasmin (PL), due to its resistance to high temperatures, is said to be primarily responsible for proteolysis and is of great importance in the production of UHT milk (Rauh et al. 2014c). Pre-heating and altering of process conditions can adjust some of the quality concerns, including PL activity, although quality is greatly affected by the composition of milk (Rauh et al. 2014b).

This study was made as an independent project and carried out during one semester. The milk samples used were from a collaborative study by the Swedish University of Agricultural Sciences (SLU), Norrmejerier and Tetra Pak Processing Systems investigating seasonal variations in composition and properties of Swedish raw silo milk and impact on the quality of UHT.

1.1 Aim

The aim of this study was to investigate if there are any seasonal variations in PL and PG-derived PL activity in silo and UHT milk collected from November 2014

to November 2015. Moreover, the study aimed to investigate if PL and PG-derived PL activity in UHT milk was affected by storage time and storage temperature, as well as investigate the relation between somatic cell count (SCC) and PL and PG-derived PL activity. Additionally, the method's reproducibility and limit of detection will be calculated.

1.1.1 Research questions

- What is the limit of detection and reproducibility of the modified enzymatic method for determination of PL and PG-derived PL activity in milk?
- What are the levels of PL and PG-derived PL activity in silo and UHT milk, and how does the UHT process affect these levels?
- Is there a seasonal variation in PL and PG-derived PL activity in silo and UHT milk?
- Is PG and PG-derived PL activity in UHT milk affected by storage temperature and storage time?
- Is there a correlation between SCC and PL and PG-derived PL activity?

1.1.2 Limitations

Bacterial enzymes were not included in this study.

2 Literature review

Milk is a highly praised raw material with an enormous number of usages. Milk may be feed for calves, food for humans, and less commonly processed into fabrics or ingredients of cosmetic products and medical drugs.

Today, the most common bovine dairy breeds in Sweden are the Swedish Holstein and the Swedish red-and-white. These high-intensity milk producers yield up to 10,065 kg energy corrected milk a year. Sweden also has small herds with Jersey cows and the native breed Svensk fjällko (Växa Sverige 2015).

Milk from a typical dairy cow contain about 87.1 % water, 3.3 % protein, 4.6 % lactose, 4.0 % fat, 0.7 % minerals and vitamins, and 0.3 % of organic acids of which citrate is the most predominant (Walstra et al. 2006). The quantity of fat and protein in the milk, as well as the total number of somatic cells are the base of pricing. Spores and bacterial count, visually changed milk, smell and taste are other quality parameters which may reduce or enhance payment. (Arla, 2016). Milk composition varies among breeds due to the genetic diversity, and on-going breeding programs. The Jersey cow has for example a higher fat, protein and lactose content than the Holsteins (Walstra et al. 2006).

Two major groups of milk proteins are caseins and whey proteins, residual milk proteins are membrane proteins and enzymes (Walstra et al. 2006). The caseins constitute 80 % out of the 3.3 % total protein in milk. There are four different types of caseins; α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein. The chemical structure of casein micelles is not fully known but α_{s1}-casein, α_{s2}-casein and β-casein are by many researchers believed to compose the internal micelle by hydrophobic and ion binding, while hydrophilic κ-casein is situated on the micelle's surface, keeping the spherical structure stable. The micelle also includes inorganic matter, calcium phosphate and serine phosphate cross-linking with caseins in the internal design (De Kruif et al. 2012; Walstra et al. 2006).

The whey proteins consist of α-lactalbumin and β-lactoglobulin, as well as serum albumins and immunoglobulin, and are predominantly present dissolved in the serum fraction of milk (Walstra et al. 2006). These proteins are heat sensitive,

and denatured β-lactoglobulins are prone to form complexes with caseins, especially κ-caseins, after heat treatment (Kelly & Foley 1997).

2.1.1 Factors affecting milk composition

The synthesis of milk is affected by several parameters such as genetics, physiology and environment (Walstra et al. 2006).

Physiological conditions affecting milk composition are age, stage of lactation and udder health. Somatic cell count (SCC) is a marker of udder health and gives an indication of the severity level of an intramammary inflammation (mastitis). As milk is isotonic with blood, mastitic milk contains more low-molecular-mass blood components, due to leakage over membranes, at so called tight junctions. These components include e.g. somatic cells and enzymes. The SCC also increases with age, parity and vary with the stage of lactation (Walstra et al. 2006). Swedish cows calf all year round, although most calving occur from February to April (Seeman & Stenberg 2015). Consequently, many cows will be in the same stage of lactation and thus the milk composition will show a seasonal variation. International studies on seasonal variations report contradictory results as the calving pattern may differ between countries. In some countries cows calf all year round and will be in different lactation stages, thus there will be no seasonal variations (Walstra et al. 2006).

Milk composition has also been noticed to change in regards to environmental factors such as milking systems, feed and animal husbandry (Lindmark-Månsson 2012). Swedish legislation obliges cattle to graze during summer, and grazing can affect the levels of unsaturated fatty acids (Andrén 2010), although no significantly differences of unsaturated fatty acids were seen in the latest report by the Swedish milk association (Lindmark-Månsson 2012). The total amount of fat and protein vary with feed and lactation, and exhibit higher levels in colostrum and milk from later stages of lactation. Depending on milking frequency, fat content may vary on a day-to-day basis affected by the time elapsed between milking (Walstra et al. 2006).

Physiological and environmental factors are believed to be affected by the geographical location. Chen et al. (2014) believed that higher SCC in Holstein cows during winter could be due to different feed, animal husbandry and region. Walstra et al. (2006) and Lindmark-Månsson (2012) support the theory of variations in milk affected by geographically factors.

2.2 The dairy plant

At the dairy plant the incoming milk is subjected to several processes such as separation, standardisation, homogenisation, pasteurisation and packaging (Walstra et

al. 2006). Depending on the intended product, the extent of processing and process steps differ. For example, all drinking milk and cream produced in large-scale in Sweden must be pasteurized (LIVSFS 2005:20, 2005), and some traditional milk is not homogenised. A batch of milk intended to become an UHT product, will firstly be separated from the cream by centrifugal forces and secondly standardised with cream to desired fat content. The standardised milk is pre-pasteurized and depending on the dairy plant, homogenisation can occur before or after UHT treatment. The milk is cooled and packed aseptically with sterilized packaging (Bylund 2003).

2.2.1 Heat treatment

Heat treatment can be done in various ways and is a combination of time and temperature. As heating of milk cause some undesired changes affecting the sensory qualities, there is often a compensation between safety, useful properties and quality (Rauh 2014a).

Most milk in Sweden is sold as “fresh”, with a shelf-life of a one week. Fresh milk has undergone a mild heat treatment, so called low-pasteurization. Low-pasteurization improves the safety of milk as most pathogens are killed, although some heat-resistant bacteria, bacterial spores, and enzymes can remain (Walstra et al. 2006). The denaturation of enzymes by heat depends on several factors, and therefore varies between enzymes. Alkaline phosphatase is inactivated to a great extent and lipase activity only reduced during low-pasteurization, at e.g. 15 seconds at 72°C. PL requires a higher temperature to denature, and will retain its activity after low-pasteurization (Walstra et al. 2006). Fresh milk is usually consumed before PL causes changes, and PL is therefore not considered a problem in this product. However in UHT milk stored for several months at ambient temperature, PL activity can cause noticeable changes if not completely inactivated (Walstra et al. 2006).

In many parts of the world, consumption of UHT treated milk is common. During the UHT process the product is heated to a temperature exceeding 135°C for a few seconds. Such intense heat treatment kills all bacteria, destroys most spores and denatures most enzymes (Figure 1). UHT treatment can be done by indirect or direct heating. In indirect heating, a continuous flow of milk goes through a heat exchanger. In direct heating hot steam is either injected into a continuous flow of milk or milk is infused into hot steam (Bylund 2003). Before the milk is subjected to UHT treatment, it is commonly pre-heated to 80-90°C for a short time. At pre-heat treatment the indirect system takes a longer time to reach desired holding temperature and it has a longer holding time than the direct system. Consequently, the indirect system yields a larger heat load affecting chemical changes in milk

(Datta et al. 2002) and in turn more efficiently inactivate enzymes (Malmgren 2007).

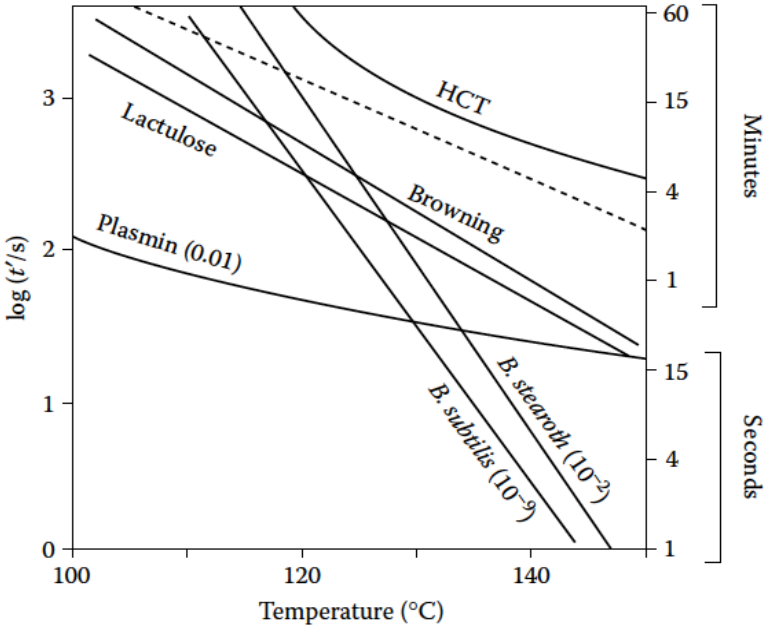

Figure 1. Heat treatment affecting enzymes, bacteria and browning in milk (Walstra et al. 2006), with permission to publish granted by Copyright Clearance Center.

2.3 Plasmin in milk

There are many native enzymes in milk; either originating from the cow or bacteria. Some enzymes play a physiological role in milk, while others are simply present as a consequence of contamination (Fox & Kelly 2006). PL is present in blood and ends up in the milk by leakage through the tissue of the mammary gland (Walstra et al. 2006). Its biological function in blood is to hydrolyse blood clots (Precetti et al. 1997). In milk it has been thought to play a physiological role in the involution of lactating mammals (Politis 1996). The enzyme exists in all mammals although there are differences in the amino acid sequence affecting the functional properties (Schaller et al. 1985).

2.3.1 The plasmin system

The PL system includes the active enzyme PL, the inactive form plasminogen (PG), PG activator inhibitors, PL inhibitors and PG activators (Figure 2). All com-

ponents in the PL system work together to regulate the proteolytic activity of PL (Precetti et al. 1997).

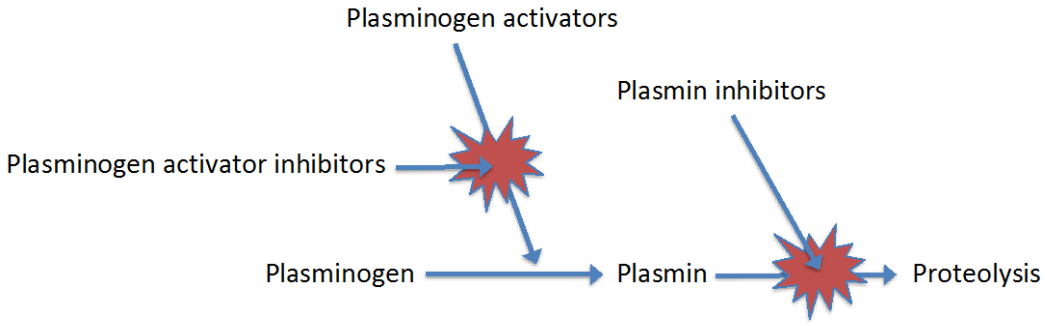

Figure 2. The PL system (modified from Ismail & Nielsen (2010)) with permission to publish granted by Copyright Clearance Center.

The interaction between the elements of the PL system is very complex, and the exact mechanisms are not fully understood. PL and PG in milk are associated with the casein micelle, but exactly where they adhere is yet unknown. The inhibitors of PL and PG are proteases associated to the milk serum, and other inhibitors of PL includes denatured serum proteins (Rauh 2014a).

The PL and PG-derived PL activities in milk vary due to multiple factors. Any expansion in the permeability of the mammary gland, as ageing of the cow, parity and stage of lactation, may affect the leakage of enzymes into the milk. Studies have shown that the PG-derived PL activity almost double from early stages of lactation to late (Korycha-Dahl et al. 1983; Nicholas et al. 2002). This implies that more PG enters the milk as the lactation advances, although the ratio PG:PL decreases as a continuous activation of PG into PL by PG activators occurs (Baldi et al. 1996). As the PG activators are associated to the somatic cells, PL activity increase with high cell count (Walstra et al. 2006; Kelly & Foley 1997). Activation of PG can occur within a wide range with an optimal temperature of 37°C and pH 7.4 (Fox & Kelly 2006).

Although many studies of PL activity in milk come to the same conclusion, contradictory results exist and are often explained by the variations within individual cows, and due to physical, environmental and geographical factors (Nicholas et al. 2002).

2.3.2 Thermal inactivation of the plasmin system

As parts of the PL system are heat sensitive, the PL activity in milk changes upon pasteurization. The heat stability of PL and PG is high and enzyme activities will

be reduced but persist through pasteurization (Lu & Nielsen 1993). PL concentration in silo milk has been reported to be 0.1-0.7 µg/ml, and PG 0.5-2.8 µg/ml (Ozen et al. 2003; Korycha-Dahl et al. 1983).
Korycha-Dahl et al. (1983) measured a 10 % reduction of PL or PG-derived PL activity in milk after low-pasteurization, followed by a 30 % increase of PL activity during the two forthcoming days whilst studying the effect of storage. The increase of PL activity after pasteurization is due to denaturation of heat labile inhibitors. In absence of inhibitors, thermally stable PG activators and residual PG are prone to initiate proteolytic activity during storage (Prado et al. 2007).

As mentioned, the two UHT processes, direct and indirect, are unequally efficient in inactivating enzymes, including PL and the even more thermally stable PG activators (Lu & Nielsen 1993). The indirect UHT system has shown to be more effective in inactivating PL and its activators due to the greater heat load than the direct UHT process, which has reported to exhibit a higher rate of proteolysis (Kelly & Foley 1997; Rauh et al. 2014b). Rauh et al. (2014b) measured a 70 % and 86 % reduction in PL and PG-derived PL activity respectively, using the direct UHT system, while Korycha-Dahl et al. (1983) measured an 100 % and 90 % reduction respectively, using a mixture of direct and indirect.

2.3.3 Proteolysis by plasmin

UHT milk is commonly stored at room temperature, a favorable temperature for PL proteolysis. PL hydrolyses α-caseins and β-caseins to proteose peptones and γ-caseins (Walstra et al. 2006). The κ-caseins are known not to be affected by PL hydrolysis, however they are thought to have a significant role in age gelation, a defect sometimes occurring in UHT milk stored for several months (Rauh et al. 2014b).

In UHT milk stored at refrigerated temperatures, proteolysis can occur, although at a slow rate, due to accessibility of β-caseins dissociated from the casein micelle (Walstra et al. 2006). The level of proteolytic activity in milk is affected by changes in milk that occur during milk processing (heat treatment, homogenisation, skimming) storage conditions (temperature, time) and milk composition (initial amount of PL and its elements) (Walstra et al. 2006).

The main issue with proteolytic activity during prolonged storage of milk is the formation of undesired bitter tasting peptides. Additionally, some of these peptides are thought to form complexes with other peptides causing gelation, also called age gelation, as it happens to occur after a long period of storage (Walstra et al. 2006; Rauh et al. 2014c). Enright et al. (1999) added PL in readily heat treated UHT milk, and observed an increased proteolysis, sedimentation and instability of the milk during storage. These findings led to several studies involving PL as a potential cause of age gelation, as proteolytic products react and form an unstable

and fragile gel network. Even though the mechanism behind age gelation is not fully understood, studies have shown that the level of gelation in milk is highly depended on storage time in combination with the UHT process (indirect or direct), pre-heat treatment (increase temperature to delay gelation) and storage temperature (Malmgren 2007).

Approximately 80 % of all potential PL activity arise from indigenous PG in milk, and the amount of PG is about 2 to 30 times higher than PL (Fox 1993; Benfeldt et al. 1995; Rauh 2014a). Rauh et al. (2014c), found that about 75 % of the proteolytic products in milk were solely due to PL activity indicating the important impact of PL compared to other enzymes.

3 Materials and method

3.1 Quantitative measurements

There are several ways to quantify PL and PG in milk including enzyme concentration, proteolytic products or enzyme activity. The advantage of measuring enzyme activity is to actually enumerate the active enzymes, and not the inactive, as well as the enzyme assays being fast and sensitive (Rauh 2014a). The disadvantages of the method are related to the overall usage of enzyme assays and material. As multiple methods are used in different studies, difficulties of comparing results apply. Since the assays measure activity and not the amount of enzyme present, the unit needs to be defined (Kelly et al. 2006). Other drawbacks with using multiple methods are factors affecting the results, such as the choice of substrate (level of affinity) and buffer, as well as sample composition (substrate interference by casein), and milk processing (de-fatting of high pressure homogenised milk remove some plasmin associated to caseins on the milk fat globules) (Rauh 2014a).

The method used in this study was based on a modified version of Korycha-Dahl et al. (1983). The enzyme assay measures PL and PG-derived PL activity by use of a chromogenic substrate that reacts with the proteolytic enzyme. Its hydrolysis cause a change in colour, which can be detected at a specific wavelength. To determine the PG-derived PL activity, one extra step, i.e. addition of the PG activator urokinase, is included in the assay. One unit of enzyme activity has been defined, according to Korycha-Dahl et al. (1983), as the amount of PL or PG-derived PL activity causing a change in absorbance at 405 nm of 0.001 in 1 minute at pH 7.4 at 37°C.

There are two known types of PG activators, urokinase PG activator and tissue-type activator. The method selected for the PG assay is based on only one of the activators, the urokinase PG activator, due to it being more effective and commonly used by previous authors (Prado et al. 2007).

3.2 Experimental overview

PL and PG-derived PL activity was analysed for each month, excluding August due to holiday season, in unprocessed silo milk and UHT milk. To study the effect of storage time and storage temperature, UHT milk from five months (November 2014, January, March, May, July) stored at different temperatures and for various time, was analysed for PL and PG-derived PL activity. All milk samples were equally prepared, treated, and analysed.

In total the study included approximately 150 samples.

3.3 Milk samples

Silo milk and UHT milk with 1.5 % fat were, on a monthly basis from November 2014 to November 2015 with an exception for August, received from Norrmejerier. The UHT milk was processed at Norrmejerier by indirect UHT treatment at 137°C for 4 seconds including pre-heating at 82°C. UHT milk produced in November 2014, January 2015, March 2015, and May 2015 were stored at 4, 20, 30, and 37°C and milk samples were taken out every fourth week. All collected milk samples were frozen and stored at -20°C until further analysis.

Low-pasteurized and homogenized milk with 3 % fat was bought from the local store and used to test the reproducibility of the assay.

3.4 Material preparation

A sample buffer was prepared by dissolving 20 mM ε-aminocaproic acid (EACA), 53 mM Trizma buffer and 1117 mM sodium chloride (Merck KGaA, Darmstadt, Germany) in de-ionized water. The pH was adjusted to 7.4 with 2.5 M sodium hydroxide using a pH meter (ProLab 3000, SI Analytics GmbH, Mainz, Germany) and pH electrode (IoLine, IL-pHT-A120MF, SI Analytics GmbH, Mainz, Germany) The buffer was stored at room temperature.

Chromogenic substrate CS 41(03) (HYPHEN BioMed, Neuville-sur-Oise, France) was diluted with 10 ml of de-ionized water to a concentration of 2.5 $mg \cdot ml^{-1}$. The substrate solution was aliquoted and stored at 8°C.

One vial of 10 000 IU of freeze-dried urokinase from human kidney cells was diluted with 0.6 ml of de-ionized water to a concentration of 16 666 IU urokinase$\cdot ml^{-1}$. The enzyme solution was aliquoted and stored at -20°C.

Chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Inc., Stockholm, Sweden) except where stated differently.

3.5 Sample preparation

Frozen milk samples were thawed at 4°C overnight and placed in a water batch at 45°C for 15 minutes.

Two millilitres of milk was de-fatted by centrifugation (Himac CT15RE, Hitachi Koki Co., LTD, Tokyo, Japan) at 1 500 *g* at 4°C for 10 minutes. A cotton stick was used to remove the fat layer from the surface.

3.6 Plasmin and plasminogen isolation

A volume of 320 µl of de-fatted milk was mixed with 4680 µl of buffer and incubated for two hours at room temperature. The samples were ultracentrifuged (Optima MAX-XP, Beckman Coulter, Inc., Bromma, Sweden) at 100 000 *g* at 4°C for one hour. The supernatant containing PL and PG was transferred into a clean Eppendorf tube and stored at -20°C.

3.7 Plasmin and plasminogen assay

A volume of 172 µl of the chromogenic substrate was mixed with 645 µl of PL and PG isolated from milk and 190 µl of the mix was added on a 96-wells plate. All samples were analysed in duplicates.

The same reaction mix of chromogenic substrate and isolated PG and PL was used for determination of the PG-derived PL activity. An addition of 4.5 µl of urokinase was pipetted to duplicate wells.

Buffer sample was used as blank for both assays.

PL, and PG-derived PL activity was measured spectrophotometrically (FLUOstar Omega, BMG LABTECH). Absorbance was measured at 405 nm, 41 cycles, 3 min/cycle, at 37°C. Data analysis of results was made with MARS Data Analysis Software (BMG LABTECH).

As the isolate contained both PL and PG, the assay measured both PL and PG-derived PL activity. PL activity was subtracted from the PG values to obtain PG-derived PL activity.

3.8 Reproducibility and limit of detection

The reproducibility of the PL and PG assay was measured using three samples of low-pasteurized and homogenized milk with 3 % fat, which was prepared, treated, and analysed equally to the silo and UHT milk samples in this study. The reproducibility was expressed as the relative standard deviation between measurements (n=3).

The limit of detection was calculated by calculating the mean of 10 blanks + 3 standard deviations.

3.9 SCC correlation

Unpublished data of somatic cell count (Karlsson, 2015) of silo milk from the different months were tested for correlation with PL and PG-derived PL activity by statistical measurements. Total PL and PG-derived PL activity, PL activity, and PG-derived PL activity were tested for correlation with SCC. In total three tests were performed.

3.10 Statistical analysis

Statistical analysis was carried out using the statistical software Minitab Express™ (Minitab Inc., 2014). Pearson's correlation was used to measure the strength of a linear correlation. A one-way Analysis of Variance (ANOVA) was used to test for differences between means. The level of significance was tested at 5 %, meaning a *p*-value of <0.05 was considered significant.

The ANOVA test gives information whether the means of sample data are equal or not, at a specific significance level. To determine exactly which of the sample data that are significantly different, further analysis by pairwise comparisons must be performed made (Williams & Abdi 2010). The Tukey's method was carried out to establish which sample datas that were significantly different, and grouped samples according to least significance similarities.

4 Results

4.1 Reproducibility and limit of detection

The reproducibility of the method was ±10 % for the PL assay, and ±9 % for the PG assay (Appendix 1). The deviations are not accounted for when presenting the data, but must be kept in mind whilst interpreting the results.

The limit of detection was calculated to 0.59 U/ml (Appendix 1).

4.2 Levels of plasmin and plasminogen-derived plasmin activity in silo milk

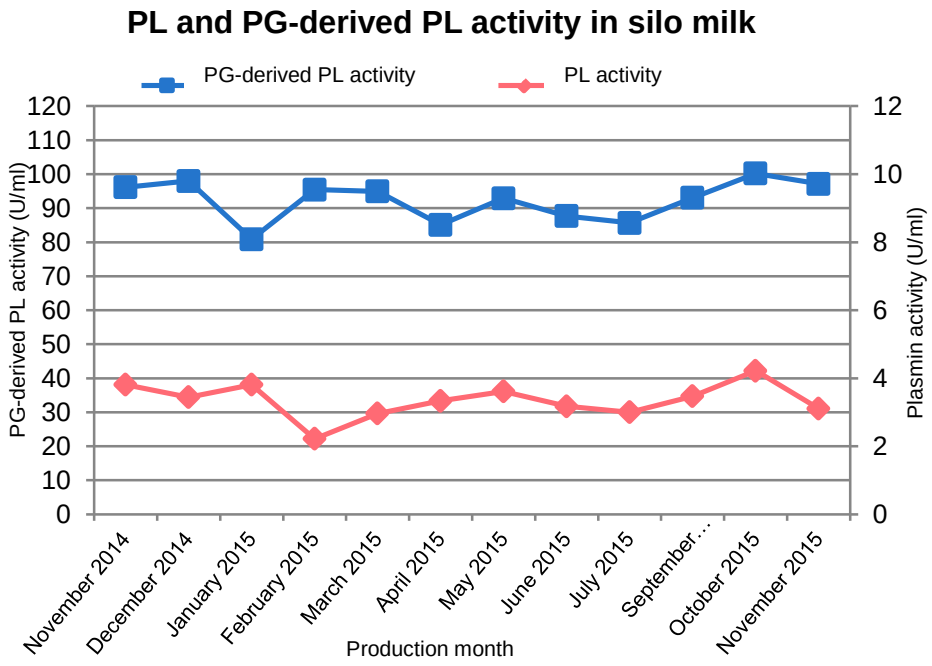

Figure 3. Variations in PL and PG-derived PL activity in silo milk, from November 2014 to November 2015. Data points present mean values of duplicates.

PL activity in silo milk was on average 3.35 U/ml ±15 % over the year (n=12). The highest PL activities were observed in October, January and November 2014; while the lowest was observed in February, March and July (Figure 3). The one-way ANOVA-test presented a *p*-value of <0.0001 in a 95 % confidence interval for PL activity in silo milk, meaning there is a significant difference between months. When conducting Tukey's method on PL activity of silo milk, five groups are formed (Table 1), with February being the only month differing significant from all the other months.

Table 1. *PL activity in silo milk and grouping of samples according to Tukey's method.*

Grouping Information Using the Tukey Method and 95% Confidence

Nov 2015	2	3.11	C	D

Means that do not share a letter are significantly different.

The PG-derived PL activity in silo milk was 92.27 U/ml ±7 %, which was approximately 30 times higher in comparison to PL activity (Figure 3). Highest PG-derived PL activities were observed in October, December and November 2015 and the lowest in January, April and July. The ANOVA-test presented a *p*-value of <0.0001 in a 95 % confidence interval for PG-derived PL activity in silo milk, meaning there is a significant difference between months. Five distinct groups are formed when conducting Tukey's method on PG-derived PL activity in silo milk (Table 2).

Table 2. *PG-derived PL activity in silo milk and grouping of samples according to Tukey's method*

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
		3.055		B	C
Oct	2	100.22	A		
Nov 2015	2	97.165	A	B	

Means that do not share a letter are significantly different.

There is no evident trend amongst monthly samples regarding PL and PG-derived PL activities, as February is low in PL but high in PG-derived PL activity and the reverse state seen for January. However, most winter months exhibited both high PL and PG-derived PL activity.

4.2.1 Somatic cell count

The SCC in silo milk was higher in November 2014, January and May and lower in March, April and July (Figure 4). Although similar pattern was visually seen between months of SCC (Figure 4) and enzyme activity (Figure 3 and 5), there was no significant correlation (Appendix 2, Table 11-13) between SCC and total PL and PG-derived PL activity (p = 0.8312.), PL activity (p = 0.3598), or PG-derived PL activity (p = 0.7520) in silo milk according to Pearson's correlation.

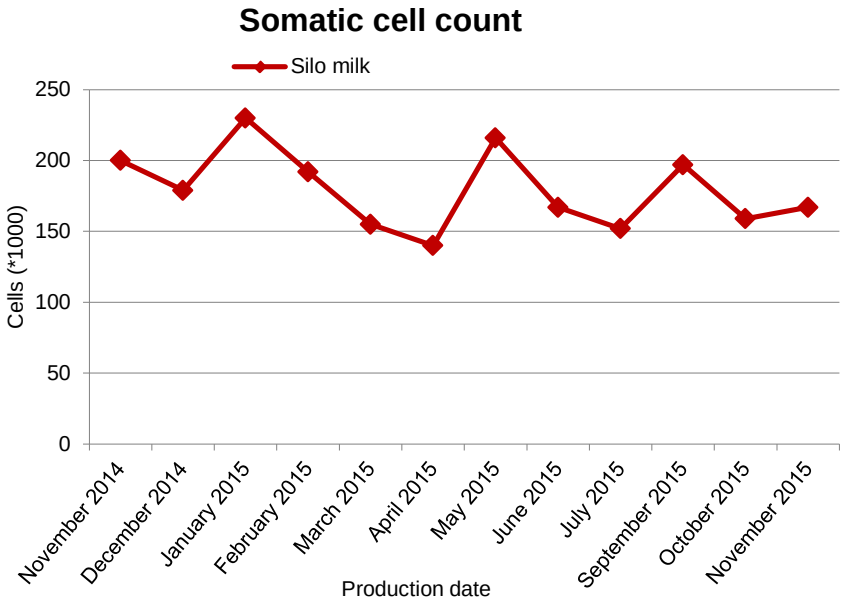

Figure 4. The SCC of silo and UHT milk (Karlsson, 2015, unpublished data).

4.3 Plasmin and plasminogen-derived plasmin activity in UHT milk

No PL activity was detected in UHT milk (Appendix 1). The PG-derived PL activity in UHT milk was 21.16 U/ml ±17 %. When comparing the PG-derived PL activity, autumn and winter months September to February had the highest activity, except December (Figure 5).

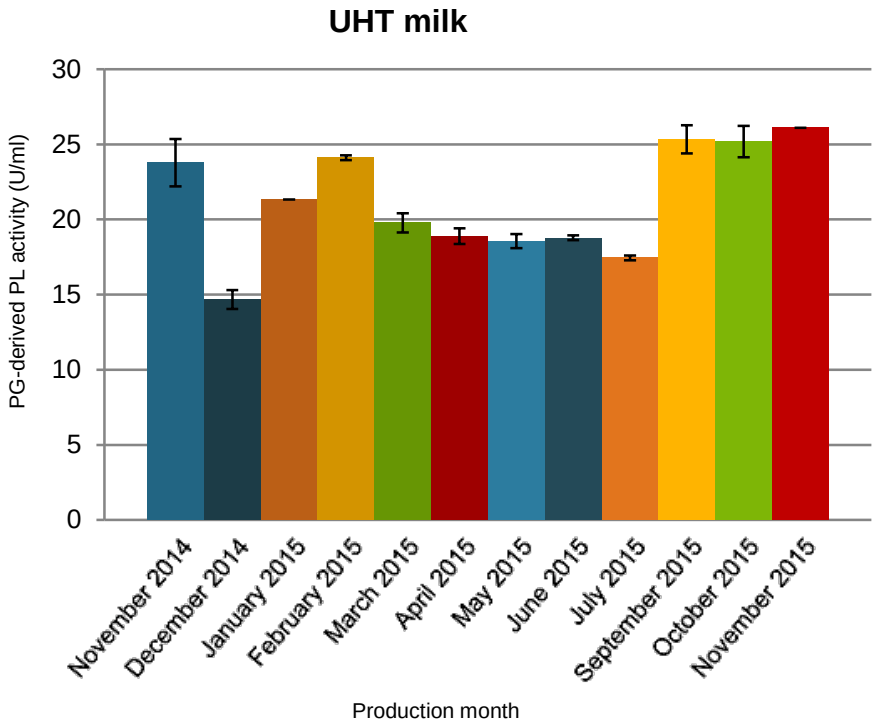

Figure 5. PG-derived PL activity in UHT milk (not stored) from different months. Every bar presents the mean value of duplicates, with an error bar indicating the standard deviation.

The one-way ANOVA test gave a *p*-value of <0.0001 in a 95 % confidence interval for PG-derived PL activity in UHT milk, indicating there is a significant difference between months. December was solely significantly different from other months, while remaining months formed four groups when conducting the Tukey's method (Table 3). Spring and summer months were grouped together, and did not differ significantly from each other.

Table 3. *PG-derived PL activity in UHT milk and grouping of samples according to Tukey's method*

Grouping Information Using the Tukey Method and 95% Confidence

		25.185	A
Nov 2015	2	26.11	A

Means that do not share a letter are significantly different.

Approximately 25 % of the PG-derived PL activity in silo milk remained after the UHT process (Figure 6). November 2014, September 2015 and November 2015 exhibited higher percentage of residual amounts in comparison to the other months. These months also had significantly higher PG-derived PL activity (Table 3).

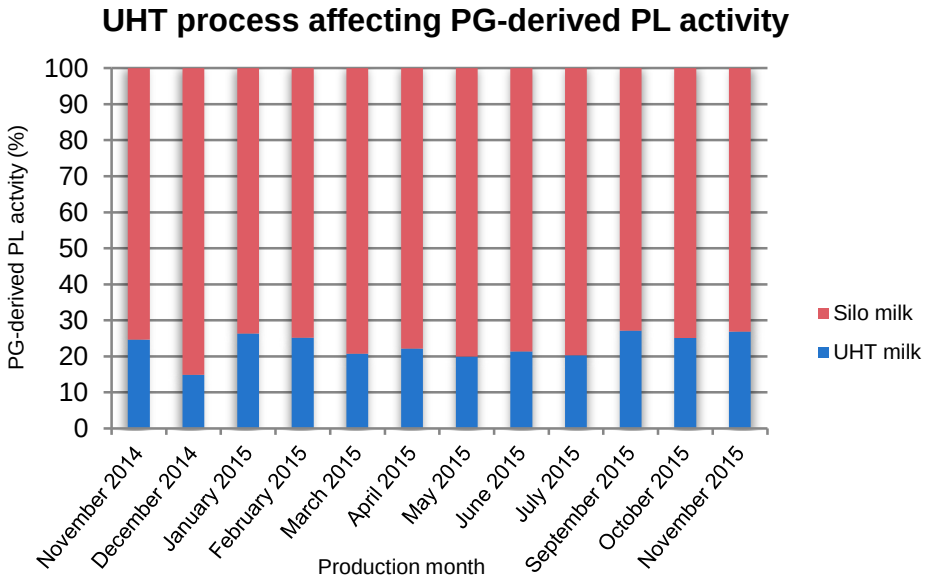

Figure 6. PG-derived PL activity in silo and UHT milk from November 2014 to November 2015.

4.4 Is plasminogen-derived plasmin activity affected by storage temperature and time?

No PL activity in UHT samples after storage was detected, thus the effect of storage time and storage temperature cannot be evaluated.

The PG-derived PL activity indicated, as expected, no sign of an increased or decreased deviating enzyme activity in any of the months as the variations overlap the initial and final activity values, although no statistical analysis was carried out due to lack of time.

The lines of the different temperatures could visually be seen to follow each other through time (Figure 7-9), except for a few data point where the enzyme activity varied noticeably more e.g., in January, week 24 (Figure 8), where the enzyme activity varied between 18.33 to 33.22 U/ml. The enzyme activity in November 2014 seemed to vary during the entire storage period. Most noticeably is the drop during the first eight weeks, and a similar drop occurring around week 36 (Figure 7) In relation to the two previous months, only minor variations in deviating enzyme activity were observed in March (Figure 9), May (data not shown) and July (data not shown). The visually observed variations are not due to a changing PG-derived PL activity in the product, as any decrease in PG-derived PL activity would yield an increase in PL activity, and no increase in PG-derived PL activity is possible.

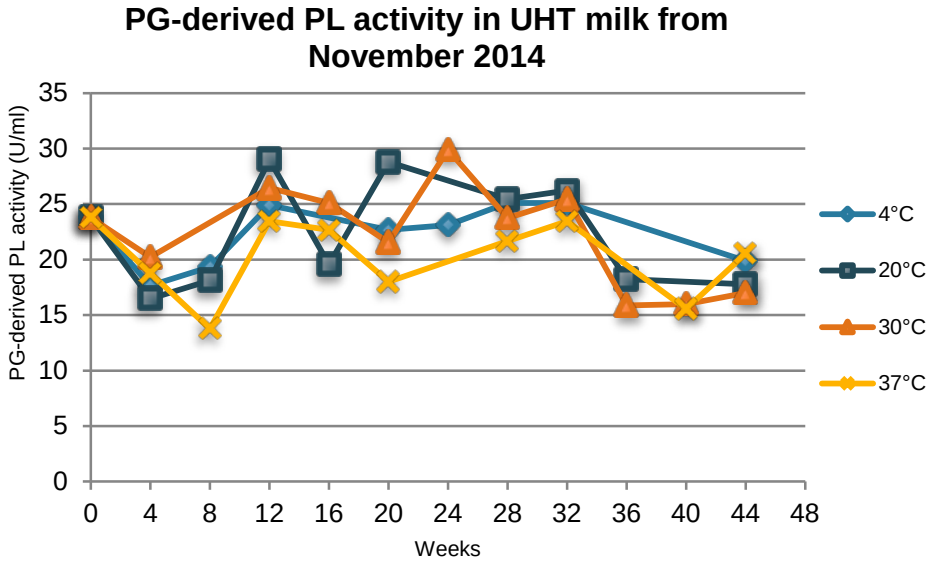

Figure 7. PG-derived PL activity in UHT milk from November 2014.

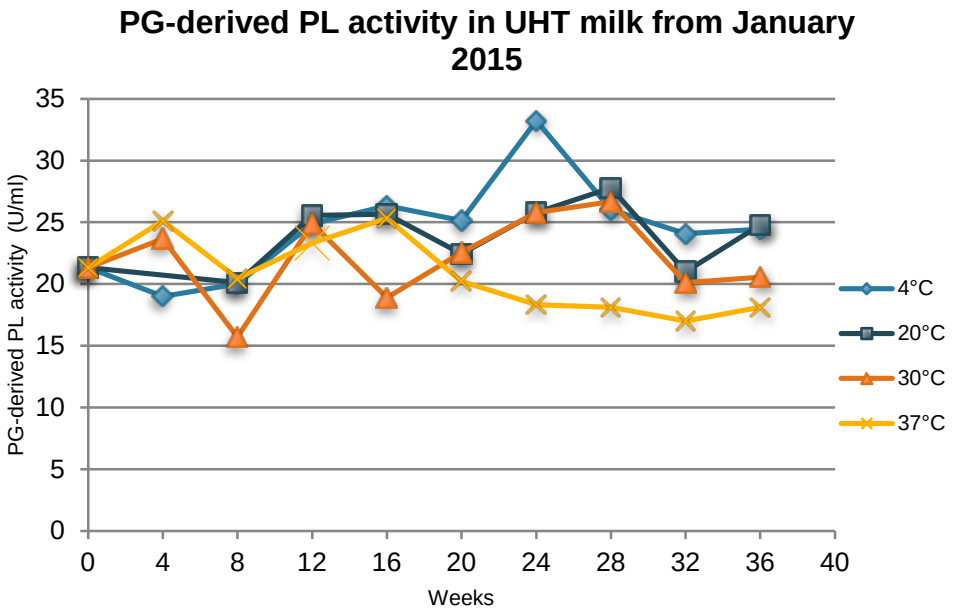

Figure 8. PG-derived PL activity in UHT milk from January 2015.

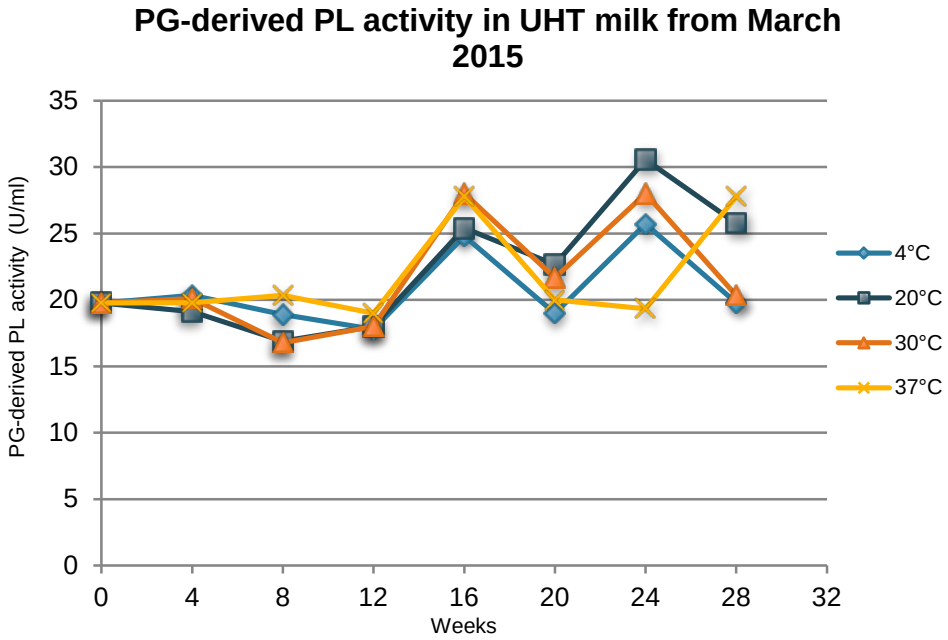

Figure 9. PL-derived PL activity in UHT from March 2015.

5 Discussion

There are many factors affecting the initial amount of enzymes in milk and various methods can be used to measure the total enzymatic activity or the activity of individual enzymes. It is thereby expected that results obtained by other authors differ somewhat from our results.

5.1 Reproducibility and limit of detection

The reproducibility in this study was 10 % for the PL assay, and 9 % for the PG-derived PL activity assay. These values are in agreement with Korycha-Dahl et al. (1983) which reported a variance coefficient of 10 %.

The limit of detection was measured to 0.59 U/ml for the instrument using blank samples. In UHT samples where no PL activity was detected, it is possible these samples contain up to 0.59 U/ml of PL activities. The study does not determine whether 0-0.59 U/ml of PL activity is sufficient enough to cause noticeable proteolysis during storage. For this, additional measurements of the proteolytic products are needed.

5.2 Plasmin and plasminogen-derived plasmin activity in silo milk

The PL activity in silo milk was in agreement with Korycha-Dahl et al. (1983) who reported 4.2-5.4 U/ml, in comparison with our mean value, 3.35 U/ml. Höök (2015) observed similar results in a study investigating differences in PL/PG-derived PL activity in milk from automatic (3.61 U/ml) and conventional milking systems (4.33 U/ml). The PG-derived PL activity with an average of 92.27 U/ml was in agreement with Höök (2015) who measured 88.73 U/ml in milk from automatic milking system and 94.93 U/ml in milk from conventional milking system. Our PG-derived PL activities are considerably higher compared to Korycha-Dahl et al. (1983) who measured 26.3-45.3 U/ml PG-derived PL activities in silo milk.

5.2.1 Modification of original method

This study used a modified method based on the previously published method by Korycha-Dahl et al. (1983). Factors affecting PL yield relates to the EACA concentration in the PL and PG assay. A higher concentration of EACAs lead to a higher degree of dissociated PL and PG from the casein micelle, although EACA can also have an inhibitory effect if not diluted properly prior to analysis (Rauh et al. 2014d). In contrast to Korycha-Dahl et al. (1983), the EACA concentration has in previous studies at SLU been optimized to gain maximal dissociation of PL from casein micelles without inhibition (Johansson, M., 2015, pers. comm., December) and could explain the higher PG-derived PL activity in our results. Other possible causes of this increase could be either the use of a different substrate, or a higher initial amount of PG in our silo milk.

5.2.2 Seasonal variations

The silo milk from Norrmejerier is a mixture of milk from different farms, with each farm using different milking systems, milking frequencies, and keeping cows of different ages and breeds. The diversity complicates the determination of factors influencing PL and PG-derived PL activity. Many studies report seasonal variations of PL and PG-derived PL activity, although results are conflicting as high and low levels are observed during different times of the year and some studies show no seasonal variation (Chen et al. 2014; Nicholas et al. 2002).

Variations in results are expected since studies are carried out under different conditions, and few physical and environmental factors are accounted for, however most studies do not include relevant information about individual cows or herds to make comparisons of physical or environmental factors possible.

5.2.3 Stage of lactation

One important physiological factor affecting milk composition and enzyme activity is the stage of lactation (Nicholas et al. 2002). Unfortunately, this factor cannot entirely be accounted for due to all milk being mixed together in bulk tanks at the farm. As many Swedish cows calf during spring, and PL and PG-derived PL activity is reported to increase at late stages of lactation, it is only reasonable that milk during autumn and winter has higher enzyme activity. In this study, lowest PL and PG-derived PL activities in silo milk are observed in spring and summer, with an increasing activity towards autumn and winter. This corresponds with results from other studies on PL and PG-derived PL activity and lactation (Korycha-Dahl et al. 1983; Nicholas et al. 2002), and indicates a seasonal variation of PL and PG-derived PL activity that co-varies with stage of lactation.

5.2.4 Somatic cell count

Somatic cell count has been reported to affect PL and PG-derived PL activity. Studies on SCC and PL activity often compare very low and very high cell count (Kelly & Foley 1997), thus no difference would be seen if only using low cell counts. In this study, there was no significant correlation between SCC and total PL and PG-derived PL activity, PL activity or PG-derived PL activity, and a plausible explanation could be the relatively low cell counts in the Swedish milk.

5.3 Plasmin and plasminogen-derived plasmin activity in UHT milk

PL activity in UHT milk was not detected as the UHT treatment completely inactivated the enzyme, and similar results have been obtained by Korycha-Dahl et al. (1983). This suggests that also PG activators are denatured to the extent that no activation of PG occurs during storage. The hypothesis was that proteolysis would increase with increasing storage temperature but results show that all PL is inactivated and no activation of PG into PL could be observed during storage of UHT milk.

Statistical analysis of PG-derived PL activity was not carried out for UHT milk as any activation of PG during storage would result in an increase of PL activity, and no PL activity was detected. The observed between week and sample variations are most probably due to variations within the batches, variations between packages, rather than an effect of storage.

Since neither PL activity nor an indication of PG activation cold be observed during storage, proteolytic activity by PL affecting the UHT milk's shelf-life must have occurred before the UHT treatment. Quality assurance should ideally work towards minimizing PL and PG levels in the unprocessed silo milk as a UHT product with low PL and PG-derived PL activity levels is expected to have a long shelf-life and cause minimum changes in milk during storage. There are, however additional heat-stable microbial enzymes in milk, which can cause proteolysis and changes in milk during storage. These, and cellular proteases, have not been not accounted for in this study.

PG-derived PL activity decreased when subjected to UHT treatment, and approximately 25 % of the PG-derived PL activity in silo milk was retained in the UHT milk. This figure will depend on the processing conditions and UHT system applied.

5.4 Future value

If there are seasonal variations in PL and PG-derived PL activity, and these show significant differences, this knowledge could be used to strategically produce high

quality UHT milk products by selecting the best-suited raw material. If simply milk with low PL and PG-derived PL activity was to be used for UHT milk, then age gelation and undesirable bitter peptides due to PL could be avoided or less of an issue. This is especially true for UHT milk produced by direct UHT treatment, as PL and PG activators are less efficiently inactivated compared to the indirect UHT method.

Although many factors affecting the initial PL and PG-derived PL activity in milk are identified, the interaction of the PL system and the mechanism of age gelation needs to be investigated further.

5.5 Method discussion

In this laboratory study, only one sample for each storage time and storage temperature was analysed. If using more samples, it would be possible to see if there were any variations between milk samples from the same batch, and if the method and sample preparations affected the result.

A critical detail in this assay is the importance of a pure and clear liquid after sample preparation, as the method is sensitive to inference by turbidity (Rauh et al. 2014d). As some of the samples, e.g., UHT milk from December, had lower activity than expected it might be possible that samples were contaminated with milk constituents due to sloppy pipetting after ultracentrifugation. Contamination could possibly interfere with hydrolysis of the substrate, and thereby yield a weaker colour shift.

Comparing results from different studies is difficult as there is no common method. As other authors has already expressed (Chavan et al. 2011), there is a need of a standardisation of a spectrophotometric assay.

6 Conclusions

- *What is the limit of detection and reproducibility of the modified enzymatic method for determination of PL and PG-derived PL activity in milk?*

The reproducibility of the method was ±10 % for the PL assay, and ±9 % for the PG assay.

The limit of detection was calculated to 0.59 U/ml, meaning that samples where no PL activity was detected may contain up to 0.59 U/ml of PL activities.

- *What are the levels of PL and PG-derived PL activity in silo and UHT milk, and how does the UHT process affect these levels?*

Silo milk from October had the highest PL activity (4.22 U/ml) and PG-derived PL activity (100.22 U/ml). Silo milk from February had the lowest PL activity (2.22 U/ml) and differed significantly compared to all other months. Silo milk from January had the lowest PG-derived PL activity (80.86 U/ml).

No PL activity was detected in the UHT milk. UHT milk from November 2015 had the highest PG-derived PL activity (26.11 U/ml), and December had the lowest (14.67 U/ml). The UHT treatment inactivated PG-derived PL activity by approximately 75 %.

- *Is there a seasonal variation in PL and PG-derived PL activity in silo and UHT milk?*

According to the statistical analyses of PL and PG-derived PL activity, significant differences appear between months, which indicates a seasonal variation. Highest activity was observed in silo milk gathered during winter, and lowest in summer. Variation in stage of lactation is the most probable cause for seasonal variation of plasmin activity, but other factors cannot be ruled out.

- *Is PL and PG-derived PL activity in UHT milk affected by storage temperature and storage time?*

No PL activity was detected in UHT milk. Conclusion can be drawn that either is the method used in this study not sensitive enough to detect low levels of active PL, or PL is entirely inactivated by the indirect UHT process applied and thus does not change during storage regardless of storage temperature.

It was not possible to study the effect of storage temperature or storage time of PL activity, as no PL activity was detected in UHT. PG was not activated during storage, and thereby not affected by storage time or temperature. The variation of PG-derived PL activity seen during storage is likely to originate from the within batch variation.

- *Is there a correlation between SCC and PL and PG-derived PL activity?*

There was no significant correlation between SCC and total PL and PG-derived PL activity, PL activity, or PG-derived PL activity in silo milk. Although SCC has been reported to co-vary with PL and PG-derived activity, the relatively low cell counts in the Swedish milk seems to be a plausible explanation for not observing this correlation.

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Appendix 1: Raw data

Table 4. *Overview of milk samples and enzyme activity*

Milk type	Production date	Storage time (weeks)	Storage temperature (°C)	Plasminogen (U/ml)	St Dev	CV (%)	Plasmin (U/ml)	St Dev	CV (%)
Silo	14-11-02	0	4	96,19	0,52	1	3,81	0,00	0
Silo	14-11-30	0	4	98,00	1,57	2	3,44	0,16	5
Silo	15-01-11	0	4	80,86	0,94	1	3,81	0,00	0
Silo	15-02-08	0	4	95,44	0,79	1	2,22	0,00	0
Silo	15-03-07	0	4	95,67	0,08	0	2,96	0,00	0
Silo	15-04-19	0	4	85,11	1,80	2	3,33	0,22	7
Silo	15-05-17	0	4	92,94	2,44	3	3,61	0,39	11
Silo	15-06-28	0	4	87,71	0,31	0	3,17	0,00	0
Silo	15-07-13	0	4	85,67	2,36	3	3,00	0,16	5
Silo	15-09-06	0	4	93,06	1,18	1	3,47	0,20	6
Silo	15-10-04	0	4	100,22	0,39	0	4,22	0,00	0
Silo	15-11-01	0	4	97,17	2,36	2	3,11	0,00	0
UHT	14-11-02	0	4	23,78	1,57	7	ND	ND	ND
UHT	14-11-02	4	4	17,67	0,16	1	ND	ND	ND
UHT	14-11-02	4	20	16,48	1,83	11	ND	ND	ND
UHT	14-11-02	4	30	20,22	0,94	5	ND	ND	ND
UHT	14-11-02	4	37	18,78	0,79	4	ND	ND	ND
UHT	14-11-02	8	4	19,33	0,00	0	ND	ND	ND
UHT	14-11-02	8	20	18,11	0,16	1	ND	ND	ND
UHT	14-11-02	8	30	22,59	1,05	5	ND	ND	ND
UHT	14-11-02	8	37	13,78	0,94	7	ND	ND	ND
UHT	14-11-02	12	4	24,89	0,31	1	ND	ND	ND
UHT	14-11-02	12	20	29,00	0,16	1	ND	ND	ND
UHT	14-11-02	12	30	26,44	0,63	2	ND	ND	ND
UHT	14-11-02	12	37	23,44	0,16	1	ND	ND	ND
UHT	14-11-02	16	4	21,81	1,37	6	ND	ND	ND
UHT	14-11-02	16	20	19,56	0,63	3	ND	ND	ND
UHT	14-11-02	16	30	25,11	0,00	0	ND	ND	ND
UHT	14-11-02	16	37	22,67	0,00	0	ND	ND	ND
UHT	14-11-02	20	4	22,67	0,00	0	ND	ND	ND
UHT	14-11-02	20	20	28,78	1,10	4	ND	ND	ND
UHT	14-11-02	20	30	21,56	0,31	1	ND	ND	ND
UHT	14-11-02	20	37	18,00	1,26	7	ND	ND	ND
UHT	14-11-02	24	4	23,11	0,31	1	ND	ND	ND
UHT	14-11-02	24	20	20,28	0,79	4	ND	ND	ND
UHT	14-11-02	24	30	29,90	1,71	6	ND	ND	ND
UHT	14-11-02	24	37	27,22	1,73	6	ND	ND	ND
UHT	14-11-02	28	4	25,11	0,63	3	ND	ND	ND
UHT	14-11-02	28	20	25,44	0,47	2	ND	ND	ND
UHT	14-11-02	28	30	23,75	0,98	4	ND	ND	ND
UHT	14-11-02	28	37	21,67	0,47	2	ND	ND	ND
UHT	14-11-02	32	4	25,11	0,63	3	ND	ND	ND
UHT	14-11-02	32	20	26,22	1,26	5	ND	ND	ND
UHT	14-11-02	32	30	25,44	0,79	3	ND	ND	ND
UHT	14-11-02	32	37	23,44	1,10	5	ND	ND	ND
UHT	14-11-02	36	4	27,96	1,83	7	ND	ND	ND
UHT	14-11-02	36	20	18,22	1,26	7	ND	ND	ND
UHT	14-11-02	36	30	15,87	1,80	11	ND	ND	ND
UHT	14-11-02	36	37	18,33	2,04	11	ND	ND	ND
UHT	14-11-02	40	4	29,88	0,70	2	ND	ND	ND
UHT	14-11-02	40	20	24,11	1,41	6	ND	ND	ND
UHT	14-11-02	40	30	16,00	0,63	4	ND	ND	ND
UHT	14-11-02	40	37	15,56	0,39	3	ND	ND	ND
UHT	14-11-02	44	4	19,89	0,79	4	ND	ND	ND
UHT	14-11-02	44	20	17,78	1,57	9	ND	ND	ND
UHT	14-11-02	44	30	17,00	0,47	3	ND	ND	ND
UHT	14-11-02	44	37	20,56	0,16	1	ND	ND	ND
UHT	14-11-30	0	4	14,67	0,63	4	ND	ND	ND
UHT	15-01-11	0	4	21,33	0,00	0	ND	ND	ND

Milk type	Production date	Storage time (weeks)	Storage temperature (°C)	Plasminogen (U/ml)	St Dev	CV (%)	Plasmin (U/ml)	St Dev	CV (%)
UHT	15-01-11	4	4	19,00	0,16	1	ND	ND	ND
UHT	15-01-11	4	20	29,00	2,36	8	ND	ND	ND
UHT	15-01-11	4	30	23,67	0,16	1	ND	ND	ND
UHT	15-01-11	4	37	25,11	1,89	8	ND	ND	ND
UHT	15-01-11	8	4	20,00	1,26	6	ND	ND	ND
UHT	15-01-11	8	20	20,11	0,47	2	ND	ND	ND
UHT	15-01-11	8	30	15,71	0,67	4	ND	ND	ND
UHT	15-01-11	8	37	20,44	0,00	0	ND	ND	ND
UHT	15-01-11	12	4	24,89	1,89	8	ND	ND	ND
UHT	15-01-11	12	20	25,56	0,00	0	ND	ND	ND
UHT	15-01-11	12	30	24,89	1,57	6	ND	ND	ND
UHT	15-01-11	12	37	23,33	1,57	7	ND	ND	ND
UHT	15-01-11	16	4	26,33	1,41	5	ND	ND	ND
UHT	15-01-11	16	20	25,67	0,16	1	ND	ND	ND
UHT	15-01-11	16	30	18,89	0,31	2	ND	ND	ND
UHT	15-01-11	16	37	25,33	0,63	2	ND	ND	ND
UHT	15-01-11	20	4	25,15	1,57	6	ND	ND	ND
UHT	15-01-11	20	20	22,44	0,31	1	ND	ND	ND
UHT	15-01-11	20	30	22,56	1,41	6	ND	ND	ND
UHT	15-01-11	20	37	20,22	0,94	5	ND	ND	ND
UHT	15-01-11	24	4	33,22	1,73	5	ND	ND	ND
UHT	15-01-11	24	20	25,78	0,94	4	ND	ND	ND
UHT	15-01-11	24	30	22,67	0,00	0	ND	ND	ND
UHT	15-01-11	24	37	18,33	0,16	1	ND	ND	ND
UHT	15-01-11	28	4	26,03	0,45	2	ND	ND	ND
UHT	15-01-11	28	20	27,78	0,31	1	ND	ND	ND
UHT	15-01-11	28	30	26,67	0,31	1	ND	ND	ND
UHT	15-01-11	28	37	18,11	0,16	1	ND	ND	ND
UHT	15-01-11	32	4	24,07	0,52	2	ND	ND	ND
UHT	15-01-11	32	20	21,00	0,16	1	ND	ND	ND
UHT	15-01-11	32	30	20,11	1,10	5	ND	ND	ND
UHT	15-01-11	32	37	17,00	0,47	3	ND	ND	ND
UHT	15-01-11	36	4	24,44	0,31	1	ND	ND	ND
UHT	15-01-11	36	20	24,78	0,16	1	ND	ND	ND
UHT	15-01-11	36	30	20,56	0,16	1	ND	ND	ND
UHT	15-01-11	36	37	18,11	0,47	3	ND	ND	ND
UHT	15-02-08	0	4	24,11	0,16	1	ND	ND	ND
UHT	15-03-07	0	4	19,77	0,64	3	ND	ND	ND
UHT	15-03-07	4	4	20,33	1,41	7	ND	ND	ND
UHT	15-03-07	4	20	19,11	0,63	3	ND	ND	ND
UHT	15-03-07	4	30	20,11	0,47	2	ND	ND	ND
UHT	15-03-07	4	37	19,78	0,63	3	ND	ND	ND
UHT	15-03-07	8	4	18,89	0,63	3	ND	ND	ND
UHT	15-03-07	8	20	16,89	0,31	2	ND	ND	ND
UHT	15-03-07	8	30	16,78	0,79	5	ND	ND	ND
UHT	15-03-07	8	37	20,33	1,10	5	ND	ND	ND
UHT	15-03-07	12	4	17,78	0,39	2	ND	ND	ND
UHT	15-03-07	12	20	18,00	0,94	5	ND	ND	ND
UHT	15-03-07	12	30	18,00	0,00	0	ND	ND	ND
UHT	15-03-07	12	37	19,00	1,10	6	ND	ND	ND
UHT	15-03-07	16	4	24,81	0,52	2	ND	ND	ND
UHT	15-03-07	16	20	25,40	0,90	4	ND	ND	ND
UHT	15-03-07	16	30	28,00	0,00	0	ND	ND	ND
UHT	15-03-07	16	37	27,78	1,89	7	ND	ND	ND
UHT	15-03-07	20	4	19,00	1,41	7	ND	ND	ND
UHT	15-03-07	20	20	22,67	0,94	4	ND	ND	ND
UHT	15-03-07	20	30	21,67	1,41	7	ND	ND	ND
UHT	15-03-07	20	37	20,00	0,00	0	ND	ND	ND
UHT	15-03-07	24	4	25,71	1,80	7	ND	ND	ND
UHT	15-03-07	24	20	30,56	0,16	1	ND	ND	ND
UHT	15-03-07	24	30	28,00	0,00	0	ND	ND	ND
UHT	15-03-07	24	37	19,33	0,00	0	ND	ND	ND
UHT	15-03-07	28	4	19,78	0,63	3	ND	ND	ND
UHT	15-03-07	28	20	25,78	1,26	5	ND	ND	ND
UHT	15-03-07	28	30	20,33	0,47	2	ND	ND	ND
UHT	15-03-07	28	37	27,78	0,00	0	ND	ND	ND
UHT	15-04-19	0	4	18,89	0,52	3	ND	ND	ND
UHT	15-05-17	0	4	18,56	0,47	3	ND	ND	ND
UHT	15-05-17	4	4	18,57	0,67	4	ND	ND	ND
UHT	15-05-17	4	20	19,11	0,63	3	ND	ND	ND
UHT	15-05-17	4	30	19,78	0,94	5	ND	ND	ND
UHT	15-05-17	4	37	16,56	0,79	5	ND	ND	ND

Sample name	Milk type	Production date	Storage time (weeks)	Storage temperature (°C)	Plasminogen (U/ml)	St Dev	CV (%)	Plasmin (U/ml)	St Dev	CV (%)
5/8/4	UHT	15-05-17	8	4	19,22	0,16	1	ND	ND	ND
5/8/20	UHT	15-05-17	8	20	24,92	1,57	6	ND	ND	ND
5/8/30	UHT	15-05-17	8	30	18,89	1,57	8	ND	ND	ND
5/8/37	UHT	15-05-17	8	37	19,44	0,47	2	ND	ND	ND
5/12/4	UHT	15-05-17	12	4	18,89	0,00	0	ND	ND	ND
5/12/20	UHT	15-05-17	12	20	15,67	0,79	5	ND	ND	ND
5/12/30	UHT	15-05-17	12	30	16,67	0,63	4	ND	ND	ND
5/12/37	UHT	15-05-17	12	37	17,67	0,47	3	ND	ND	ND
5/16/4	UHT	15-05-17	16	4	21,11	0,63	3	ND	ND	ND
5/16/20	UHT	15-05-17	16	20	20,33	0,47	2	ND	ND	ND
5/16/30	UHT	15-05-17	16	30	19,11	0,00	0	ND	ND	ND
5/16/37	UHT	15-05-17	16	37	15,00	0,79	5	ND	ND	ND
6/0	UHT	15-06-28	0	4	18,78	0,16	1	ND	ND	ND
7/0	UHT	15-07-12	0	4	17,44	0,16	1	ND	ND	ND
7/4/4	UHT	15-07-12	4	4	22,00	0,63	3	ND	ND	ND
7/4/20	UHT	15-07-12	4	20	22,00	0,31	1	ND	ND	ND
7/4/30	UHT	15-07-12	4	30	20,56	0,16	1	ND	ND	ND
7/4/37	UHT	15-07-12	4	37	20,19	1,83	9	ND	ND	ND
7/8/4	UHT	15-07-12	8	4	20,33	0,16	1	ND	ND	ND
7/8/20	UHT	15-07-12	8	20	19,89	0,16	1	ND	ND	ND
7/8/30	UHT	15-07-12	8	30	19,44	0,16	1	ND	ND	ND
7/8/37	UHT	15-07-12	8	37	18,11	0,16	1	ND	ND	ND
9/0	UHT	15-09-06	0	4	25,33	0,94	4	ND	ND	ND
10/0	UHT	15-10-04	0	4	25,19	1,05	4	ND	ND	ND
11/0 (15)	UHT	15-11-01	0	4	26,11	0,00	0	ND	ND	ND
Control milk 1	Low-pasteurized	ND	1	8	55,89	1,10	2	6,89	0,31	5
Control milk 2	Low-pasteurized	ND	1	8	67,89	2,04	3	6,33	0,47	7
Control milk 3	Low-pasteurized	ND	1	8	66,05	1,54	2	7,78	0,14	2
Blank 1	Blank 1	15-09-30	ND	ND	ND	ND	ND	0,11	0,47	424
Blank 2	Blank 2	15-09-30	ND	ND	ND	ND	ND	-0,33	0,16	-47
Blank 3	Blank 3	15-09-30	ND	ND	ND	ND	ND	-0,11	0,47	-424
Blank 4	Blank 4	15-10-01	ND	ND	ND	ND	ND	0,00	0,00	0
Blank 5	Blank 5	15-10-01	ND	ND	ND	ND	ND	0,11	0,16	141
Blank 6	Blank 6	15-10-01	ND	ND	ND	ND	ND	-0,11	0,16	-141
Blank 7	Blank 7	15-10-02	ND	ND	ND	ND	ND	0,11	0,16	141
Blank 8	Blank 8	15-10-02	ND	ND	ND	ND	ND	0,11	0,16	141
Blank 9	Blank 9	15-10-02	ND	ND	ND	ND	ND	0,00	0,00	0
Blank 10	Blank 10	15-10-05	ND	ND	ND	ND	ND	0,33	0,16	47

Appendix 2: Statistical data

Table 5. *ANOVA test on PG-derived PL activity in silo milk.*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	11	799,012	72,6375	33,04	<0,0001
Error	12	26,381	2,1985		
Total	23	825,393			

Table 6. *Tukey's test on PG-derived PL activity in silo milk.*

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	95% CI	T-Value	Adjusted P-Value
Dec-Nov 14	1,81	(-4,082; 7,702)	1,22	0,976
Jan-Nov 14	-15,335	(-21,227; -9,443)	-10,34	<0,0001
Feb-Nov 14	-0,745	(-6,637; 5,147)	-0,5	1
Mar-Nov 14	-1,265	(-7,157; 4,627)	-0,85	0,9985
Apr-Nov 14	-11,08	(-16,972; -5,188)	-7,47	0,0003
May-Nov 14	-3,245	(-9,137; 2,647)	-2,19	0,5824
June-Nov 14	-8,475	(-14,367; -2,583)	-5,72	0,0033
July-Nov 14	-10,525	(-16,417; -4,633)	-7,1	0,0005
Sept-Nov 14	-3,135	(-9,027; 2,757)	-2,11	0,6239
Oct-Nov 14	4,03	(-1,862; 9,922)	2,72	0,3187
Nov 15-Nov 14	0,975	(-4,917; 6,867)	0,66	0,9999
Jan-Dec	-17,145	(-23,037; -11,253)	-11,56	<0,0001
Feb-Dec	-2,555	(-8,447; 3,337)	-1,72	0,8287
Mar-Dec	-3,075	(-8,967; 2,817)	-2,07	0,6466
Apr-Dec	-12,89	(-18,782; -6,998)	-8,69	<0,0001
May-Dec	-5,055	(-10,947; 0,837)	-3,41	0,1194
June-Dec	-10,285	(-16,177; -4,393)	-6,94	0,0006
July-Dec	-12,335	(-18,227; -6,443)	-8,32	0,0001
Sept-Dec	-4,945	(-10,837; 0,947)	-3,34	0,1335
Oct-Dec	2,22	(-3,672; 8,112)	1,5	0,9155
Nov 15-Dec	-0,835	(-6,727; 5,057)	-0,56	1
Feb-Jan	14,59	(8,698; 20,482)	9,84	<0,0001
Mar-Jan	14,07	(8,178; 19,962)	9,49	<0,0001
Apr-Jan	4,255	(-1,637; 10,147)	2,87	0,2606
May-Jan	12,09	(6,198; 17,982)	8,15	0,0001
June-Jan	6,86	(0,968; 12,752)	4,63	0,0177
July-Jan	4,81	(-1,082; 10,702)	3,24	0,1529
Sept-Jan	12,2	(6,308; 18,092)	8,23	0,0001
Oct-Jan	19,365	(13,473; 25,257)	13,06	<0,0001
Nov 15-Jan	16,31	(10,418; 22,202)	11	<0,0001
Mar-Feb	-0,52	(-6,412; 5,372)	-0,35	1
Apr-Feb	-10,335	(-16,227; -4,443)	-6,97	0,0006
May-Feb	-2,5	(-8,392; 3,392)	-1,69	0,8451
June-Feb	-7,73	(-13,622; -1,838)	-5,21	0,0071
July-Feb	-9,78	(-15,672; -3,888)	-6,6	0,001
Sept-Feb	-2,39	(-8,282; 3,502)	-1,61	0,8755

Oct-Feb	4,775	(-1,117; 10,667)	3,22	0,1583
Nov 15-Feb	1,72	(-4,172; 7,612)	1,16	0,9831
Apr-Mar	-9,815	(-15,707; -3,923)	-6,62	0,0009
May-Mar	-1,98	(-7,872; 3,912)	-1,34	0,9569
June-Mar	-7,21	(-13,102; -1,318)	-4,86	0,0123
July-Mar	-9,26	(-15,152; -3,368)	-6,25	0,0016
Sept-Mar	-1,87	(-7,762; 4,022)	-1,26	0,9701
Oct-Mar	5,295	(-0,597; 11,187)	3,57	0,0933
Nov 15-Mar	2,24	(-3,652; 8,132)	1,51	0,9113
May-Apr	7,835	(1,943; 13,727)	5,28	0,0064
June-Apr	2,605	(-3,287; 8,497)	1,76	0,8132
July-Apr	0,555	(-5,337; 6,447)	0,37	1
Sept-Apr	7,945	(2,053; 13,837)	5,36	0,0057
Oct-Apr	15,11	(9,218; 21,002)	10,19	<0,0001
Nov 15-Apr	12,055	(6,163; 17,947)	8,13	0,0002
June-May	-5,23	(-11,122; 0,662)	-3,53	0,0998
July-May	-7,28	(-13,172; -1,388)	-4,91	0,0114
Sept-May	0,11	(-5,782; 6,002)	0,07	1
Oct-May	7,275	(1,383; 13,167)	4,91	0,0114
Nov 15-May	4,22	(-1,672; 10,112)	2,85	0,269
July-June	-2,05	(-7,942; 3,842)	-1,38	0,9466
Sept-June	5,34	(-0,552; 11,232)	3,6	0,089
Oct-June	12,505	(6,613; 18,397)	8,43	0,0001
Nov 15-June	9,45	(3,558; 15,342)	6,37	0,0013
Sept-July	7,39	(1,498; 13,282)	4,98	0,0102
Oct-July	14,555	(8,663; 20,447)	9,82	<0,0001
Nov 15-July	11,5	(5,608; 17,392)	7,76	0,0002
Oct-Sept	7,165	(1,273; 13,057)	4,83	0,0129
Nov 15-Sept	4,11	(-1,782; 10,002)	2,77	0,297
Nov 15-Oct	-3,055	(-8,947; 2,837)	-2,06	0,6541

Table 7. *ANOVA test on PL activity in silo milk.*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	11	5,82571	0,52961	21,34	<0,0001
Error	12	0,29785	0,024821		
Total	23	6,12356			

Table 8. *Tukey's test on PL activity in silo milk.*

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	95% CI	T-Value	Adjusted P-Value
Dec-Nov 2014	-0,365	(-0,9911; 0,2611)	-2,32	0,5119
Jan-Nov 2014	0	(-0,6261; 0,6261)	0	1
Feb-Nov 2014	-1,59	(-2,2161; -0,9639)	-10,09	<0,0001
Mar-Nov 2014	-0,85	(-1,4761; -0,2239)	-5,4	0,0054
Apr-Nov 2014	-0,48	(-1,1061; 0,1461)	-3,05	0,2037
May-Nov 2014	-0,2	(-0,8261; 0,4261)	-1,27	0,9688
June-Nov 2014	-0,64	(-1,2661; -0,0139)	-4,06	0,0433
July-Nov 2014	-0,81	(-1,4361; -0,1839)	-5,14	0,008
Sept-Nov 2014	-0,34	(-0,9661; 0,2861)	-2,16	0,5994
Oct-Nov 2014	0,41	(-0,2161; 1,0361)	2,6	0,3688
Nov 2015-Nov 2014	-0,7	(-1,3261; -0,0739)	-4,44	0,0237

Jan-Dec	0,365	(-0,2611; 0,9911)	2,32	0,5119
Feb-Dec	-1,225	(-1,8511; -0,5989)	-7,78	0,0002
Mar-Dec	-0,485	(-1,1111; 0,1411)	-3,08	0,1946
Apr-Dec	-0,115	(-0,7411; 0,5111)	-0,73	0,9996
May-Dec	0,165	(-0,4611; 0,7911)	1,05	0,992
June-Dec	-0,275	(-0,9011; 0,3511)	-1,75	0,8185
July-Dec	-0,445	(-1,0711; 0,1811)	-2,82	0,2769
Sept-Dec	0,025	(-0,6011; 0,6511)	0,16	1
Oct-Dec	0,775	(0,1489; 1,4011)	4,92	0,0112
Nov 2015-Dec	-0,335	(-0,9611; 0,2911)	-2,13	0,6172
Feb-Jan	-1,59	(-2,2161; -0,9639)	-10,09	<0,0001
Mar-Jan	-0,85	(-1,4761; -0,2239)	-5,4	0,0054
Apr-Jan	-0,48	(-1,1061; 0,1461)	-3,05	0,2037
May-Jan	-0,2	(-0,8261; 0,4261)	-1,27	0,9688
June-Jan	-0,64	(-1,2661; -0,0139)	-4,06	0,0433
July-Jan	-0,81	(-1,4361; -0,1839)	-5,14	0,008
Sept-Jan	-0,34	(-0,9661; 0,2861)	-2,16	0,5994
Oct-Jan	0,41	(-0,2161; 1,0361)	2,6	0,3688
Nov 2015-Jan	-0,7	(-1,3261; -0,0739)	-4,44	0,0237
Mar-Feb	0,74	(0,1139; 1,3661)	4,7	0,0159
Apr-Feb	1,11	(0,4839; 1,7361)	7,05	0,0005
May-Feb	1,39	(0,7639; 2,0161)	8,82	<0,0001
June-Feb	0,95	(0,3239; 1,5761)	6,03	0,0021
July-Feb	0,78	(0,1539; 1,4061)	4,95	0,0107
Sept-Feb	1,25	(0,6239; 1,8761)	7,93	0,0002
Oct-Feb	2	(1,3739; 2,6261)	12,69	<0,0001
Nov 2015-Feb	0,89	(0,2639; 1,5161)	5,65	0,0037
Apr-Mar	0,37	(-0,2561; 0,9961)	2,35	0,4948
May-Mar	0,65	(0,0239; 1,2761)	4,13	0,0391
June-Mar	0,21	(-0,4161; 0,8361)	1,33	0,9574
July-Mar	0,04	(-0,5861; 0,6661)	0,25	1
Sept-Mar	0,51	(-0,1161; 1,1361)	3,24	0,1545
Oct-Mar	1,26	(0,6339; 1,8861)	8	0,0002
Nov 2015-Mar	0,15	(-0,4761; 0,7761)	0,95	0,9962
May-Apr	0,28	(-0,3461; 0,9061)	1,78	0,8036
June-Apr	-0,16	(-0,7861; 0,4661)	-1,02	0,9937
July-Apr	-0,33	(-0,9561; 0,2961)	-2,09	0,635
Sept-Apr	0,14	(-0,4861; 0,7661)	0,89	0,9979
Oct-Apr	0,89	(0,2639; 1,5161)	5,65	0,0037
Nov 2015-Apr	-0,22	(-0,8461; 0,4061)	-1,4	0,9434
June-May	-0,44	(-1,0661; 0,1861)	-2,79	0,2889
July-May	-0,61	(-1,2361; 0,0161)	-3,87	0,0584
Sept-May	-0,14	(-0,7661; 0,4861)	-0,89	0,9979
Oct-May	0,61	(-0,0161; 1,2361)	3,87	0,0584
Nov 2015-May	-0,5	(-1,1261; 0,1261)	-3,17	0,1696
July-June	-0,17	(-0,7961; 0,4561)	-1,08	0,99
Sept-June	0,3	(-0,3261; 0,9261)	1,9	0,7394
Oct-June	1,05	(0,4239; 1,6761)	6,66	0,0009
Nov 2015-June	-0,06	(-0,6861; 0,5661)	-0,38	1
Sept-July	0,47	(-0,1561; 1,0961)	2,98	0,2228
Oct-July	1,22	(0,5939; 1,8461)	7,74	0,0002
Nov 2015-July	0,11	(-0,5161; 0,7361)	0,7	0,9997

Oct-Sept	0,75	(0,1239; 1,3761)	4,76	0,0144
Nov 2015-Sept	-0,36	(-0,9861; 0,2661)	-2,29	0,5291
Nov 2015-Oct	*-1,11*	*(-1,7361; -0,4839)*	*-7,05*	*0,0005*

Table 9. *ANOVA test on PG-derived PL activity in UHT milk*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	11	298,502	27,1366	55,83	<0,0001
Error	12	5,833	0,4861		
Total	23	304,335			

Table 10. Tukey's test on PG-derived PL activity in UHT milk

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	95% CI	T-Value	Adjusted P-Value
Dec-Nov 2014	-9,115	(-11,8856; -6,3444)	-13,07	<0,0001
Jan-Nov 2014	-2,45	(-5,2206; 0,3206)	-3,51	0,1018
Feb-Nov 2014	0,33	(-2,4406; 3,1006)	0,47	1
Mar-Nov 2014	-4,01	(-6,7806; -1,2394)	-5,75	0,0032
Apr-Nov 2014	-4,89	(-7,6606; -2,1194)	-7,01	0,0006
May-Nov 2014	-5,225	(-7,9956; -2,4544)	-7,49	0,0003
June-Nov 2014	-5	(-7,7706; -2,2294)	-7,17	0,0005
July-Nov 2014	-6,335	(-9,1056; -3,5644)	-9,09	<0,0001
Sept-Nov 2014	1,555	(-1,2156; 4,3256)	2,23	0,5591
Oct-Nov 2014	1,405	(-1,3656; 4,1756)	2,02	0,6792
Nov 2015-Nov 2014	2,33	(-0,4406; 5,1006)	3,34	0,1322
Jan-Dec	6,665	(3,8944; 9,4356)	9,56	<0,0001
Feb-Dec	9,445	(6,6744; 12,2156)	13,55	<0,0001
Mar-Dec	5,105	(2,3344; 7,8756)	7,32	0,0004
Apr-Dec	4,225	(1,4544; 6,9956)	6,06	0,002
May-Dec	3,89	(1,1194; 6,6606)	5,58	0,0041
June-Dec	4,115	(1,3444; 6,8856)	5,9	0,0025
July-Dec	2,78	(0,0094; 5,5506)	3,99	0,0487
Sept-Dec	10,67	(7,8994; 13,4406)	15,3	<0,0001
Oct-Dec	10,52	(7,7494; 13,2906)	15,09	<0,0001
Nov 2015-Dec	11,445	(8,6744; 14,2156)	16,42	<0,0001
Feb-Jan	2,78	(0,0094; 5,5506)	3,99	0,0487
Mar-Jan	-1,56	(-4,3306; 1,2106)	-2,24	0,5552
Apr-Jan	-2,44	(-5,2106; 0,3306)	-3,5	0,104
May-Jan	-2,775	(-5,5456; -0,0044)	-3,98	0,0492
June-Jan	-2,55	(-5,3206; 0,2206)	-3,66	0,0816
July-Jan	-3,885	(-6,6556; -1,1144)	-5,57	0,0041
Sept-Jan	4,005	(1,2344; 6,7756)	5,74	0,0032
Oct-Jan	3,855	(1,0844; 6,6256)	5,53	0,0044
Nov 2015-Jan	4,78	(2,0094; 7,5506)	6,86	0,0007
Mar-Feb	-4,34	(-7,1106; -1,5694)	-6,23	0,0016
Apr-Feb	-5,22	(-7,9906; -2,4494)	-7,49	0,0003
May-Feb	-5,555	(-8,3256; -2,7844)	-7,97	0,0002
June-Feb	-5,33	(-8,1006; -2,5594)	-7,64	0,0003
July-Feb	-6,665	(-9,4356; -3,8944)	-9,56	<0,0001
Sept-Feb	1,225	(-1,5456; 3,9956)	1,76	0,8131
Oct-Feb	1,075	(-1,6956; 3,8456)	1,54	0,901
Nov 2015-Feb	2	(-0,7706; 4,7706)	2,87	0,2609

Apr-Mar	-0,88	(-3,6506; 1,8906)	-1,26	0,97
May-Mar	-1,215	(-3,9856; 1,5556)	-1,74	0,8198
June-Mar	-0,99	(-3,7606; 1,7806)	-1,42	0,9375
July-Mar	-2,325	(-5,0956; 0,4456)	-3,33	0,1336
Sept-Mar	5,565	(2,7944; 8,3356)	7,98	0,0002
Oct-Mar	5,415	(2,6444; 8,1856)	7,77	0,0002
Nov 2015-Mar	6,34	(3,5694; 9,1106)	9,09	<0,0001
May-Apr	-0,335	(-3,1056; 2,4356)	-0,48	1
June-Apr	-0,11	(-2,8806; 2,6606)	-0,16	1
July-Apr	-1,445	(-4,2156; 1,3256)	-2,07	0,6473
Sept-Apr	6,445	(3,6744; 9,2156)	9,24	<0,0001
Oct-Apr	6,295	(3,5244; 9,0656)	9,03	<0,0001
Nov 2015-Apr	7,22	(4,4494; 9,9906)	10,36	<0,0001
June-May	0,225	(-2,5456; 2,9956)	0,32	1
July-May	-1,11	(-3,8806; 1,6606)	-1,59	0,883
Sept-May	6,78	(4,0094; 9,5506)	9,72	<0,0001
Oct-May	6,63	(3,8594; 9,4006)	9,51	<0,0001
Nov 2015-May	7,555	(4,7844; 10,3256)	10,84	<0,0001
July-June	-1,335	(-4,1056; 1,4356)	-1,91	0,7337
Sept-June	6,555	(3,7844; 9,3256)	9,4	<0,0001
Oct-June	6,405	(3,6344; 9,1756)	9,19	<0,0001
Nov 2015-June	7,33	(4,5594; 10,1006)	10,51	<0,0001
Sept-July	7,89	(5,1194; 10,6606)	11,32	<0,0001
Oct-July	7,74	(4,9694; 10,5106)	11,1	<0,0001
Nov 2015-July	8,665	(5,8944; 11,4356)	12,43	<0,0001
Oct-Sept	-0,15	(-2,9206; 2,6206)	-0,22	1
Nov 2015-Sept	0,775	(-1,9956; 3,5456)	1,11	0,9876
Nov 2015-Oct	*0,925*	*(-1,8456; 3,6956)*	*1,33*	*0,9586*

Table 11. Pearson's correlation with SCC and total PL and PG-derived PL activity

Correlation
Pearson correlation of SCC and PL+PG = -0,069023
P-value = 0,8312

Table 12. Pearson's correlation with SCC and PG-derived PL activity

Correlation
Pearson correlation of SCC and PG = -0,102186
P-Value = 0,7520

Table 13. Pearson's correlation with SCC and PL activity

Correlation
Pearson correlation of SCC and PL = 0,290417
P-Value = 0,3598

Appendix: Popular summary in Swedish

Mjölk som värmebehandlats vid ultrahög temperatur (UHT-mjölk) och kan förvaras i rumstemperatur är praktiskt, men bara om kvalitén håller. Känt sedan länge är enzymet plasmins oönskade aktivitet under lagring. Vi utreder hur, när, och varför plasmin är aktiv i mjölk. Kanske finns det rum för förbättringar?

Svensk mjölk, numera LRF mjölk och Växa Sverige, sammanställer regelbundet uppgifter om mjölkens sammansättning och visar i sin senaste rapport hur den svenska mjölken har förändrats under de senaste åren. Då förändringar i mjölksammansättningen kan påverka kvalitén och hållbarheten på slutprodukten, är denna information av stor betydelse. UHT-mjölk kan med fördel förvaras i rumstemperatur flera månader eftersom värmebehandlingen vid ultrahöga temperaturer (135-150°C) under några få sekunder gör den i det närmaste steril. Det är praktiskt för både transportföretag och privatpersoner att kunna förvara mjölk i rumstemperatur då det inte längre krävs en obruten kylkedja.

Vid otillräcklig upphettning förblir värmestabila enzymer aktiva i mjölken, och enzymet plasmin är ett av dem. Plasmin ingår i ett komplicerat system som inkluderar den inaktiva formen plasminogen, samt aktivatorer och inhibitorer. Plasmin bryter ner mjölkproteiner till mindre komponenter (peptider) som när de ökar i antal ger bitter smak. Dessa peptider kan efter en tids lagring bilda klumpar i mjölken. Genom att förändra förhållanden under förvärmning, UHT-behandling och lagring kan en del av dessa problem bemästras.

På uppdrag av Norrmejerier och i samarbete med Tetra Pak Processing Systems har Sveriges lantbruksuniversitet undersökt plasminaktiviteten och dess ev. säsongsvariation i färsk silo- och UHT-mjölk. Mjölkprover uttagna månadsvis under ett år, samt UHT-behandlad mjölk lagrad olika lång tid i olika temperaturer, analyserades. Vi kom fram till att UHT-behandlingen förstör all plasminaktivitet, aktiviteten som härrör från plasminogen förstörs till ca 75 %. Aktivatorer reduceras till den grad där inget plasminogen omvandlas till aktivt plasmin. Nivåerna av plasminaktivitet i obehandlad mjölk och aktivitet som härrör från plasminogen i UHT-behandlad mjölk, var högre under höst och vinterhalvåret, och lägst under vår och sommar. Säsongsvariationerna beror troligtvis på att de flesta korna kalvar tidigt på våren, och enzymaktiviteten förändrades i takt med kornas laktationsstadium. Tidigare publicerade studier har sett liknande samband. Med vetskap om hur plasminaktiviteten varierar över året skulle vi kunna producera UHT-mjölk när

plasminaktiviteten är som lägst i mjölken, enligt vår studie under vår- och sommarhalvåret, för att få en välsmakade och hållbar produkt.