Final report

Increase the shelf-life of milk and thereby increase profitability

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Part 1: Detailed summary

Mjölkens kvalitet bestäms, utöver den mikrobiologiska kvaliteten, till stor del av enzymer som finns i obehandlad mjölk, enzymer som överlever värmebehandlingar samt enzymer som produceras av produktförstörande bakterier. Dessa enzymer försämrar hållbarheten och ger upphov till smak, lukt och produktdefekter, vilket i sin tur ger ett ökat svinn. Det är därför viktigt att mjölk som levereras till de svenska mejerierna är av hög kvalitet, vilket ställer krav både på gårds- och mejerinivå. Syftet med projektet var att undersöka enzymer som finns i obehandlad mjölk och hur dessa påverkar kvaliteten på mjölk och mejeriprodukter. I denna första del av projektet var syftet därför att undersöka lipas- och proteasaktiviteten i svensk obehandlad mjölk. Enzymaktiviteten i obehandlad mjölk utvärderades med avseende på effekter av säsong och geografiskt ursprung samt för skillnader mellan gårds- och mejerinivå. Denna kunskap är värdefull på lång sikt för att identifiera faktorer som kan användas för kvalitetssäkring av obehandlad mjölk och därmed förlänga hållbarheten på mjölk och mejeriprodukter.

Projekt har fått finansiering genom:
Stiftelsen Lantbruksforskning

Obehandlad mjölk från tre olika geografiska regioner i Sverige undersöktes; södra, mellersta och norra Sverige. Från varje region provtogs 20 gårdar och 10 mejerier, fördelat på två säsonger (betessäsong och stallperiod). Proverna har analyserats för lipasaktivitet med fluorometrisk teknik samt gaskromatografi, medan proteas- och plasminaktivitet har analyserats med spektrofotometri och HPLC-MS. Proverna analyserade även för kemisk och mikrobiologisk sammansättning genom användning av olika standardmetoder. Data har utvärderats med statistiska modeller.

Lipas- och proteasaktiviteten i obehandlad mjölk utvärderades med avseende på effekter av säsong och geografiskt ursprung såväl som för skillnader mellan gårds- och mejerinivå. Lipasaktiviteten i obehandlad mjölk på gårdsnivå påverkades både av regionala och säsongsmässiga variationer, medan på mejerinivå hade endast säsongen effekt. Resultaten indikerar emellertid att enbart region och säsong inte helt kan förklarar variationen i lipasaktivitet, vilket visar att andra faktorer kan vara av betydelse. Ingen skillnad i lipasaktivitet observerades mellan gårds- och mejerinivå, vilket tyder på att det är på gårdsnivå som den största kontamineringen inträffar. För proteasaktivitet kunde ingen signifikant effekt av varken region eller säsong ses i obehandlad mjölk på gårdsnivå. Till skillnad från lipasaktivitet kunde emellertid en intressant trend med högre proteasaktivitet under stallperioden noteras. Nedbrytningsprodukter som härrör från lipolys, såsom totala och fria fettsyror, och från proteolys, såsom peptider, individuella proteiner och plasminnedbrytningsprodukter, har visat sig variera något mellan säsong och geografiskt ursprung för obehandlad mölk på både gårds- och mejerinivå. Lipas- och proteasaktiviteten visade sig korrelera med flera av nedbrytningsprodukterna på både gårds- och mejerinivå, men det fanns en skitfning i vissa korrelationer mellan gårds- och mejerinivå. Resultaten av detta projekt indikerar därför att både lipas- och proteassystem är viktiga för kvaliteten på obehandlad mjölk, särskilt på gårdsnivå där den första kontamineringen inträffar.

En ökad förståelse för hur lipas- och proteasaktiviteten och resulterande nedbrytningsprodukter i obehandlad mjölk påverkas av säsongs- och regionala skillnader, såväl som skillnader mellan gårds- och mejerinivå, har uppnåtts. Resultaten av detta projekt kan vidare användas för att studera processeffekter på enzymaktivitet och hur kvaliteten på slutprodukter påverkas av enzymer som finns i mjölk. Detta skulle ge värdefull kunskap på såväl gårds- som mejerinivå för att identifiera faktorer som kan användas för kvalitetssäkring av obehandlad mjölk och därmed förlänga hållbarheten på mjölk och mejeriprodukter. Detta projekt är ett första steg mot ett praktiskt genomförande för att kontrollera mjölk och produktion av mejeriprodukter och dess kvalitetsparametrar för förbättrad hållbarhet och minskat svinn. Ytterligare studier behövs dock för att nå detta slutmål.

Part 2: The report (max 10 pages)

Introduction

The world demand for milk as raw material will grow by 2.5 percent per year, while at the same time the demand for dairy products such as consumer milk, long shelf-life milk and milk products (ESL and UTH), cheese, fermented products, butter and milk powder is even higher. Milk quality is, in addition to the microbiological quality, controlled to a large extent by enzymes found in raw milk and enzymes that survive heat treatments as well as enzymes produced by spoilage bacteria. These enzymes impair the shelf-life and gives rise to taste, odor and product defects, which in turn gives higher waste. It is therefore essential that the milk delivered to the Swedish dairies are of high quality, which put demands both at farm and dairy industry level.

The hypothesis is that by controlling the spoilage enzymes and their activities, raw milk can be directed towards an extended shelf-life. The aim of the project was to investigate enzymes present in raw milk and how these affect the quality of milk and dairy products. In this first part of the project, the aim was therefore to investigate the lipase and protease activity in Swedish raw milk. The enzyme activities in raw milk were evaluated for effects of season and geographical origin as well as for differences between farm and dairy level. This knowledge is valuable in the long term to identify factors that can be used for quality assurance of raw milk and thus prolong the shelf-life of milk and dairy products.

This final report summarizes the findings of the first part of a PhD-project. The project was terminated before the planned project end, approved by SLF in October 2018.

Materials and methods

Milk samples

Untreated milk samples from three different geographical regions of Sweden were investigated; south Sweden (Malmö), mid Sweden (Linköping) and north Sweden (Umeå). From each region, milk from 20 farms and 10 dairies were sampled. Farm milk samples were collected from the milk tanks on the farms where the milk was stored at 4ºC. The farms that were chosen had a population of 40-100 cows, were non organic with a non-robotic milking system and where the milk collection occurred every second day. The dairy milk samples were collected from the reception tanks at the dairy and were also non organic milk. Half of the milk samples were collected during indoor season (October 2017-March 2018) and the other half during the outdoor grazing season (May 2017-September 2017), from each geographical regions. After collection, an aliquot of the samples were transported directly to a certified dairy analysis laboratory (Eurofins Steins Laboratory, Jönköping, Sweden) for microbiological analyses and the rest of the samples were transported to Lund University where they were stored at 4ºC overnight. The following day, free fatty acids (FFA) and pH was measured and pH 4.6 filtrates for peptide profile analyses were prepared. The remaining analyses was performed on frozen milk stored at -20ºC until the day of analysis.

Chemical composition and microbiological assessment

Fat, protein, lactose, total solids (TS) and solids-non-fat (SNF) contents as well as freezing point depression have been analyzed using Fourier Transform Infrared (FTIR) technique and ionic calcium using an ion selective electrode. Protein composition was analyzed using high-performance liquid chromatography - mass spectrometry (HPLC-MS). Somatic cell count (SCC) and total bacterial count (TBC) were measured using flow cytometry, whereas psychrotrophic bacterial count (PBC) was analyzed using a plate count method. All microbiological analyses were performed by a certified dairy analysis laboratory (Eurofins Steins Laboratory, Sweden).

Protease methods
Plasmin and plasminogen activity were analyzed with a p-nitroanilide assay using a spectrophotometric technique, where the enzyme directly cleaves the detectable chromogenic substrate. Total proteolytic activity was measured with an azocasein assay using a spectrophotometric technique, where the enzyme directly cleaves the detectable chromogenic substrate. Peptides from bacterial proteolysis were determined in a TCA-filtrate using HPLC-MS, whereas peptides from total proteolysis were measured in pH 4.6 filtrate using HPLC-MS.

Lipase methods
Total lipase activity was analyzed using a fluorometric technique, where the enzyme directly cleaves detectable fluorogenic substrate. Total lipolysis, in the form of free fatty acids (FFA), and individual fatty acids were determined indirectly using solid phase extraction (SPE) and gas chromatography flame ionization detector (GC-FID).

Statistical analysis
To estimate the effects of region and season on the enzyme activities and the chemical and microbiological composition of the raw milk, the following univariate general linear fixed effects model was used to analyze the data:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (1)$$

where y_{ij} = response variable; μ = overall mean; τ_i= ith fixed treatment effect (i = season or region); ε_{ij} = random error (j = jth observation taken under treatment i). Main and interaction effects were evaluated, however, interaction effects were always excluded from the model if found insignificant. Variables failing the normality assumption of the general linear model, the effects of season and region were separately analyzed using the non-parametric Kruskall-Wallis on ranked data. The analysis of difference between farm and dairy level samples for all parameters, except protease activity, was performed with a one-way Anova. Upon failing the normality assumption, the Mann-Whitney U test on ranked data was used instead.

Spearman's rank correlation coefficient was used to determine the monotonic relationship between lipase and protease activity and the chemical and microbiological composition in milk from both farm and dairy level**.**

Statistical analyses were carried out using IBM SPSS Statistics Version 24 and Microsoft Office Excel 2016. All statistical tests were performed at a significance level of $P < 0.05$ if not stated otherwise.

Results and discussion
Data presented in this final report is under reviewer revision for an international peer-reviewed scientific journal.

Lipase and protease activity
In Figure 1, the lipase activity in raw milk at farm and dairy level with effects of season and geographical origin are presented. On farm level, both region and season had a significant effect ($P < 0.05$) on the lipase activity, however, on dairy level only season seems to have an effect. From the data (not presented) it could be seen that dairy samples have larger standard errors (SE $\approx$ 10 pkat/mL) compared to farm samples (SE $\approx$ 5 pkat/mL) which might explain why no significant effect of region could be seen for this sample group. It should be noticed that the statistical models have a low adjusted R^2, $R^2 = 0.29$ and $R^2 = 0.23$ for farm and dairy samples

respectively, which indicates that region and season alone do not explain the variation in lipase activity. In farm samples the regional differences is located between south and north Sweden (P = 0.017).

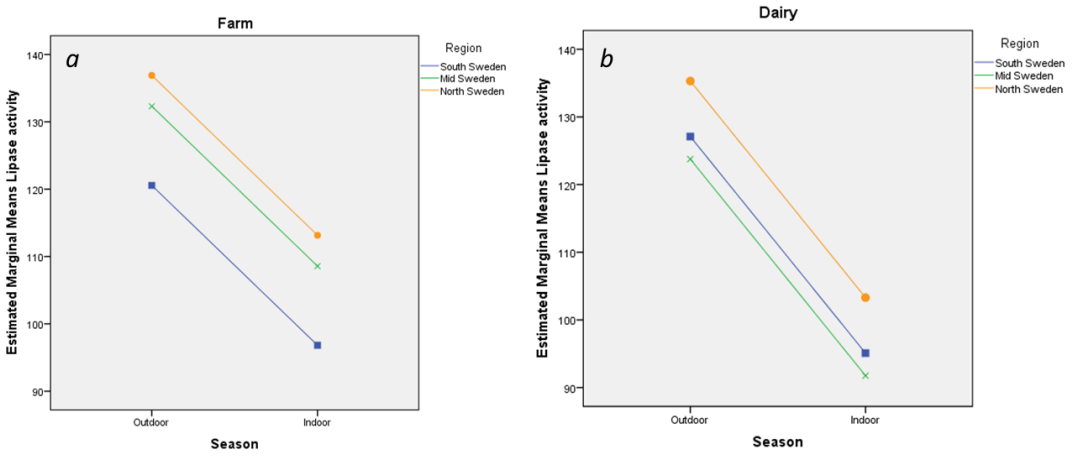

Figure 1. Estimated marginal means for lipase activity in raw milk at farm (a) and dairy (b) level from South Sweden (■), Mid Sweden (×) and North Sweden (●) during outdoor and indoor season.

Comparing farm and dairy level samples without any correction for season and region, no significant difference can be seen. This means that from farm to dairy no additional lipases have entered the milk, indicating that it is on farm level the major contamination occurs.

In Figure 2, the protease activity in raw milk at farm level with effects of season and geographical origin is presented. No significant effect of either region or season could be seen. However, an interesting trend of higher protease activity during indoor season could be indicated from Figure 2. This trend is opposite the trend for lipase activity, where indoor season had a significantly lower lipase activity compared to outdoor season.

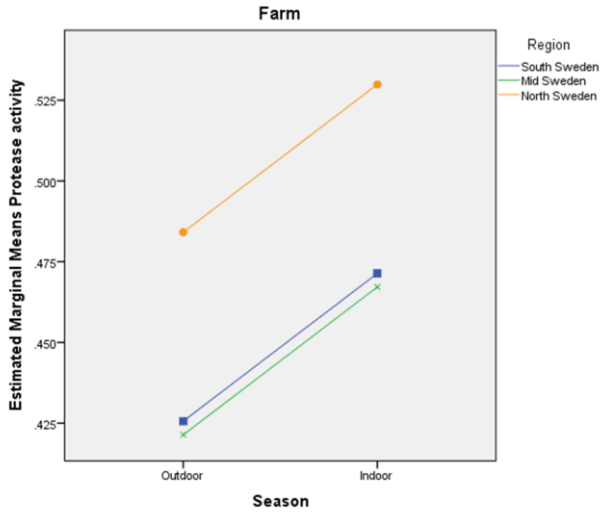

Figure 2. Estimated marginal means for protease activity in raw milk at farm level from South Sweden (■), Mid Sweden (×) and North Sweden (●) during outdoor and indoor season.

For dairy samples, the non-parametric Kruskall-Wallis test was used due to the non-normal distribution of protease activity. For both region and season no effect on protease activity could be seen.

Chemical and microbiological composition of milk at farm level

In Table 1, the chemical and microbiological composition of farm level milk is presented and in Table 2 effects of region and season are displayed.

Table 1. Chemical and microbiological composition (estimated marginal means ± standard error) in raw milk at farm level during outdoor (O) and Indoor (I) season in south, mid and north Sweden.

Variable[1]	South Sweden		Mid Sweden		North Sweden	
	O	I	O	I	O	I
pH	6.76 ± 0.01	6.74 ± 0.01	6.74 ± 0.01	6.72 ± 0.01	6.69 ± 0.01	6.74 ± 0.01
Fat (%)	3.91 ± 0.07	3.99 ± 0.07	4.08 ± 0.07	4.17 ± 0.07	4.15 ± 0.07	4.24 ± 0.07
Protein (%)	3.31 ± 0.02	3.35 ± 0.02	3.38 ± 0.02	3.43 ± 0.02	3.43 ± 0.02	3.48 ± 0.02
Lactose (%)	4.61 ± 0.02	4.55 ± 0.02	4.52 ± 0.02	4.52 ± 0.02	4.56 ± 0.02	4.61 ± 0.02
SNF (%)	8.74 ± 0.03	8.71 ± 0.03	8.72 ± 0.03	8.80 ± 0.03	8.81 ± 0.03	8.97 ± 0.03
TS (%)	12.73 ± 0.09	12.56 ± 0.09	12.75 ± 0.09	13.01 ± 0.09	12.94 ± 0.09	13.23 ± 0.09
FP (°C)	-0.505 ± 0.002	-0.503 ± 0.002	-0.501 ± 0.002	-0.502 ± 0.002	-0.505 ± 0.002	-0.514 ± 0.002
Ionic calcium (mM)[2]	2.8 ± 0.1	2.2 ± 0.0	2.5 ± 0.0	2.4 ± 0.0	3.6 ± 0.3	2.4 ± 0.0
FFA total (mg/L)	131.5 ± 7.4	110.7 ± 7.4	149.4 ± 7.4	128.6 ± 7.4	112.2 ± 7.4	91.4 ± 7.4
C4:0 (mg/L)	3.1 ± 0.3	3.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	3.0 ± 0.3	3.0 ± 0.3
C6:0 (mg/L)	2.8 ± 0.3	3.0 ± 0.3	3.4 ± 0.3	3.6 ± 0.3	2.6 ± 0.3	2.8 ± 0.3
C8:0 (mg/L)	1.9 ± 0.2	2.1 ± 0.2	2.4 ± 0.2	2.6 ± 0.2	1.9 ± 0.2	2.0 ± 0.2
C10:0 (mg/L)	3.3 ± 0.3	3.7 ± 0.3	4.2 ± 0.3	4.6 ± 0.3	3.2 ± 0.3	3.7 ± 0.3
C12:0 (mg/L)	4.2 ± 0.4	4.0 ± 0.4	5.3 ± 0.4	5.2 ± 0.4	4.2 ± 0.4	4.0 ± 0.4
C14:0 (mg/L)	8.3 ± 0.7	8.7 ± 0.7	9.1 ± 0.7	9.5 ± 0.7	7.3 ± 0.7	7.7 ± 0.7
C16:0 (mg/L)	47.6 ± 2.4	37.5 ± 2.4	51.1 ± 2.4	41.0 ± 2.4	38.3 ± 2.4	28.2 ± 2.4
C18:0 (mg/L)[2]	30.7 ± 1.4	17.5 ± 1.0	29.1 ± 2.4	19.8 ± 0.7	19.7 ± 0.7	16.6 ± 0.8
C18:1 (mg/L)	28.9 ± 2.5	25.7 ± 2.5	36.5 ± 2.5	33.2 ± 2.5	27.0 ± 2.5	23.8 ± 2.5
C18:2 (mg/L)	2.2 ± 0.3	2.2 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	2.2 ± 0.3	2.1 ± 0.3
C18:3 (mg/L)	1.8 ± 0.2	0.9 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.2 ± 0.2	0.9 ± 0.1
PBC (log cfu/g)[2]	2.8 ± 0.3	8.1 ± 2.5	2.4 ± 0.2	2.6 ± 0.3	1.9 ± 0.2	1.9 ± 0.3
SCC (x 1000/mL)	167 ± 19	179 ± 19	177 ± 19	189 ± 19	184 ± 19	195 ± 19
TBC (log cfu/mL)	4.0 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
Peptides (4-14) (Area-%)	3.91 ± 0.14	3.26 ± 0.14	3.83 ± 0.14	3.18 ± 0.14	3.86 ± 0.14	3.22 ± 0.14
GL- κ-CN (Area-%)	4.80 ± 0.06	4.40 ± 0.06	4.79 ± 0.06	4.40 ± 0.06	4.86 ± 0.06	4.46 ± 0.06
κ-CN A/E (Area-%)	4.30 ± 0.11	4.12 ± 0.11	4.61 ± 0.11	4.43 ± 0.11	4.66 ± 0.11	4.48 ± 0.11
κ-CN B (Area-%)	2.59 ± 0.11	2.40 ± 0.11	2.43 ± 0.11	2.23 ± 0.11	2.16 ± 0.11	1.97 ± 0.11
α_{s2}-CN (Area-%)	7.65 ± 0.13	8.81 ± 0.13	8.44 ± 0.13	8.63 ± 0.13	8.57 ± 0.13	9.07 ± 0.13
Peptides (19-22) (Area-%)	3.31 ± 0.28	4.00 ± 0.28	3.49 ± 0.28	2.81 ± 0.28	3.13 ± 0.28	3.66 ± 0.28
α_{s1}-CN (Area-%)	26.63 ± 0.33	25.44 ± 0.33	26.13 ± 0.33	26.61 ± 0.33	26.23 ± 0.33	25.71 ± 0.33
β-CN (Area-%)	34.48 ± 0.28	33.90 ± 0.28	35.08 ± 0.28	34.50 ± 0.28	34.39 ± 0.28	33.81 ± 0.28
Plasmin DG (Area-%)	0.95 ± 0.11	1.61 ± 0.11	1.07 ± 0.11	1.14 ± 0.11	0.89 ± 0.11	1.48 ± 0.11
α-LA (Area-%)[2]	3.20 ± 0.11	2.99 ± 0.03	2.86 ± 0.08	2.96 ± 0.10	2.82 ± 0.03	3.12 ± 0.06
β-LG B (Area-%)	2.50 ± 0.11	2.81 ± 0.11	2.93 ± 0.11	3.24 ± 0.11	3.07 ± 0.11	3.39 ± 0.11
β-LG A (Area-%)	5.82 ± 0.18	6.46 ± 0.18	4.85 ± 0.18	5.49 ± 0.18	5.18 ± 0.18	5.82 ± 0.18

[1] SNF = solids non fat, TS = total solids, FP = freezing point depression, PBC = psychtrophic bacterial count, SCC = somatic cell count, TBC = total bacterial count, GL = glycosilated, CN = casein, DG = degradation products, LA = lactalbumin, LG = lactoglobulin

[2] Mean values without correction for season and region

The fat content varied throughout the country where the northern and mid parts of Sweden had significantly higher content compared to the southern part (highest content 4.24% in the north and lowest 3.91% in the south), however, no seasonal variations could be seen. Protein content also varied between regions where south had significantly lower content compared to mid and north Sweden (highest 3.48% in the north and 3.31% in the south). For all regions a higher protein content could be seen for indoor season. Regional differences could also be seen in lactose content. The differences shifted between season where south Sweden had the highest record during outdoor season (4.61%) and north Sweden during indoor season (4.61%). Seasonal

variation within regions could not be seen. For ionic calcium regional differences were not recorded but seasonal variations could be seen where the content is significantly higher during outdoor season. The total FFAs was significantly higher in south and mid Sweden and higher during outdoor season. For all individual FFA differences between regions could be seen where mid Sweden either was significantly different from the southern or the northern parts. Seasonal differences were only seen for C16:0 and C18:0, where the content during outdoor season was higher than during indoor season. C18:3 showed the same seasonal difference but only in the southern regions. Variations in microbiology did not seem to be an effect of season or region except for southern Sweden which had significantly higher total bacterial count compared to mid and north Sweden. Several proteins had significantly different levels in different regions. Smaller peptides (peptides 4-14), glycosylated κ-CN, β-CN and α-LA did not show any differences between regions. Seasonal variations were different for different proteins. Smaller peptides (peptides 4-14), glycosylated κ-CN, β-CN and αS1-CN were significantly higher for outdoor season than indoor season. The opposite relationship, where indoor season had significantly higher levels than outdoor season, was found for αS2-CN, plasmin degradation products (plasmin DG), β-LG A and B.

Table 2. Comparisons between season and region for milk sampled at farm level. Seasonal differences between outdoor (O) and indoor (I) are displayed as either significantly ($P < 0.05$) higher (↑), significantly lower (↓) or not significant (-). Regional differences between south (S), mid (M) and north (N) Sweden are displayed as significantly different ($P < 0.05$) with different letter or as not significantly different (-). Failing the assumptions of Kruskal Wallis is denoted as not recorded (n.r.).

	South Sweden		Mid Sweden		North Sweden		Outdoor			Indoor		
	O	I	O	I	O	I	S	M	N	S	M	N
pH	↑	↓	-	-	↓	↑	a	b	c	a	b	a
Fat	-	-	-	-	-	-	a	b	b	a	b	b
Protein	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
Lactose	-	-	-	-	-	-	a	b	ab	ac	ab	c
SNF	-	-	-	-	↓	↑	ac	ab	c	a	b	c
TS	-	-	↓	↑	↓	↑	-	-	-	a	b	b
FP	-	-	-	-	↑	↓	-	-	-	a	a	b
Ionic calcium	↑	↓	↑	↓	↑	↓	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
FFA total	↑	↓	↑	↓	↑	↓	a	a	b	a	a	b
C4:0	-	-	-	-	-	-	a	b	a	a	b	a
C6:0	-	-	-	-	-	-	ab	a	b	ab	a	b
C8:0	-	-	-	-	-	-	ab	a	b	ab	a	b
C10:0	-	-	-	-	-	-	a	b	a	a	b	a
C12:0	-	-	-	-	-	-	a	b	a	a	b	a
C14:0	-	-	-	-	-	-	ab	a	b	ab	a	b
C16:0	↑	↓	↑	↓	↑	↓	a	a	b	a	a	b
C18:0	↑	↓	↑	↓	↑	↓	a	a	b	a	a	b
C18:1	-	-	-	-	-	-	a	b	a	a	b	a
C18:2	-	-	-	-	-	-	a	b	a	a	b	a
C18:3	↑	↓	-	-	-	-	a	ab	b	a	b	a
PBC	-	-	-	-	-	-	-	-	-	-	-	-
SCC	-	-	-	-	-	-	-	-	-	-	-	-
TBC	-	-	-	-	-	-	a	b	b	a	b	b
Peptides (4-14)	↑	↓	↑	↓	↑	↓	-	-	-	-	-	-
Glu-κ-CN	↑	↓	↑	↓	↑	↓	-	-	-	-	-	-
κ-CN A/E	-	-	-	-	-	-	a	b	b	a	b	b
κ-CN B	-	-	-	-	-	-	a	ab	b	a	ab	b
α_{s2}-CN	↓	↑	-	-	↓	↑	a	b	b	ab	a	b
Peptides (19-22)	-	-	-	-	-	-	-	-	-	a	b	a
α_{s1}-CN	↑	↓	-	-	-	-	-	-	-	a	b	ab
β-CN	↑	↓	↑	↓	↑	↓	-	-	-	-	-	-
Plasmin DG	↓	↑	-	-	↓	↑	-	-	-	a	b	a
α-LA	-	-	-	-	-	-	-	-	-	-	-	-
β-LG B	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
β-LG A	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b

[1] SNF = solids non fat, TS = total solids, FP = freezing point depression, PBC = psychtrophic bacterial count, SCC = somatic cell count, TBC = total bacterial count, GL = glycosilated, CN = casein, DG = degradation products, LA = lactalbumin, LG = lactoglobulin

Chemical and microbiological composition of milk at dairy level

In Table 3, the chemical and microbiological composition of farm level milk is presented and in Table 4 effects of region and season are displayed.

Table 3. Chemical and microbiological composition (estimated marginal means ± standard error) in raw milk at dairy level during outdoor (O) and indoor (I) season in south, mid and north Sweden.

Variable[1]	South Sweden		Mid Sweden		North Sweden	
	O	I	O	I	O	I
pH	6.72 ± 0.01	6.72 ± 0.01	6.68 ± 0.01	6.74 ± 0.01	6.70 ± 0.01	6.77 ± 0.01
Fat (%)	3.87 ± 0.04	4.12 ± 0.04	4.03 ± 0.04	4.28 ± 0.04	4.14 ± 0.04	4.39 ± 0.04
Protein (%)	3.22 ± 0.02	3.37 ± 0.02	3.40 ± 0.02	3.41 ± 0.02	3.34 ± 0.02	3.40 ± 0.02
Lactose (%)	4.57 ± 0.01	4.64 ± 0.01	4.51 ± 0.01	4.57 ± 0.01	4.52 ± 0.01	4.58 ± 0.01
SNF (%)	8.62 ± 0.02	8.79 ± 0.02	8.71 ± 0.02	8.88 ± 0.02	8.69 ± 0.02	8.86 ± 0.02
TS (%)	12.47 ± 0.07	12.84 ± 0.07	12.70 ± 0.07	13.07 ± 0.07	12.84 ± 0.07	13.21 ± 0.07
FP (°C)	-0.499 ± 0.001	-0.507 ± 0.001	-0.500 ± 0.001	-0.508 ± 0.001	-0.500 ± 0.001	-0.508 ± 0.001
Ionic calcium (mM)[2]	2.4 ± 0.0	2.5 ± 0.0	2.5 ± 0.0	2.2 ± 0.0	3.9 ± 0.4	2.3 ± 0.1
FFA total (mg/L)	135.4 ± 4.6	119.4 ± 4.6	134.8 ± 4.6	118.8 ± 4.6	146.5 ± 4.6	130.4 ± 4.6
C4:0 (mg/L)	3.8 ± 0.2	3.6 ± 0.2	4.6 ± 0.2	4.4 ± 0.2	4.9 ± 0.2	4.7 ± 0.2
C6:0 (mg/L)	3.3 ± 0.2	3.4 ± 0.2	3.9 ± 0.2	4.1 ± 0.2	4.1 ± 0.2	4.2 ± 0.2
C8:0 (mg/L)	1.9 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	2.9 ± 0.2	2.9 ± 0.2
C10:0 (mg/L)	3.5 ± 0.3	4.5 ± 0.3	5.2 ± 0.3	4.8 ± 0.3	4.9 ± 0.3	5.0 ± 0.3
C12:0 (mg/L)	3.8 ± 0.2	4.6 ± 0.2	6.0 ± 0.2	6.1 ± 0.2	5.8 ± 0.2	5.2 ± 0.2
C14:0 (mg/L)	8.3 ± 0.4	8.5 ± 0.4	9.5 ± 0.4	9.7 ± 0.4	10.3 ± 0.4	10.5 ± 0.4
C16:0 (mg/L)	53.3 ± 1.4	35.0 ± 1.4	39.6 ± 1.4	36.7 ± 1.4	45.2 ± 1.4	40.0 ± 1.4
C18:0 (mg/L)	31.4 ± 0.5	18.0 ± 0.5	18.8 ± 0.5	17.6 ± 0.5	21.8 ± 0.5	19.5 ± 0.5
C18:1 (mg/L)	29.6 ± 1.8	27.1 ± 1.8	35.5 ± 1.8	33.0 ± 1.8	39.8 ± 1.8	37.3 ± 1.8
C18:2 (mg/L)	2.6 ± 0.2	2.9 ± 0.2	3.4 ± 0.2	2.6 ± 0.2	2.8 ± 0.2	2.9 ± 0.2
C18:3 (mg/L)	1.3 ± 0.2	1.7 ± 0.2	1.1 ± 0.2	1.6 ± 0.2	0.7 ± 0.2	1.2 ± 0.2
PBC (log cfu/g)	3.9 ± 0.2	3.5 ± 0.2	4.0 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.2 ± 0.2
SCC (x 1000/mL)	215 ± 8	208 ± 8	216 ± 8	208 ± 8	187 ± 8	180 ± 8
TBC (log cfu/mL)[2]	4.4 ± 0.0	4.9 ± 0.4	4.6 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	4.4 ± 0.2
Peptides (4-14) (Area-%)	3.99 ± 0.30	3.74 ± 0.30	4.41 ± 0.30	4.16 ± 0.30	3.46 ± 0.30	3.21 ± 0.30
GL- κ-CN (Area-%)	5.13 ± 0.08	4.66 ± 0.08	4.82 ± 0.08	4.35 ± 0.08	4.71 ± 0.08	4.23 ± 0.08
κ-CN A/E (Area-%)	4.58 ± 0.09	4.43 ± 0.09	5.01 ± 0.09	4.85 ± 0.09	4.47 ± 0.09	4.31 ± 0.09
κ-CN B (Area-%)	2.68 ± 0.09	2.44 ± 0.09	2.24 ± 0.09	2.00 ± 0.09	2.32 ± 0.09	2.08 ± 0.09
α_{s2}-CN (Area-%)	8.33 ± 0.13	8.79 ± 0.13	8.85 ± 0.13	9.31 ± 0.13	8.45 ± 0.13	8.90 ± 0.13
Peptides (19-22) (Area-%)	3.41 ± 0.32	3.43 ± 0.32	4.46 ± 0.32	4.49 ± 0.32	3.08 ± 0.32	3.10 ± 0.32
α_{s1}-CN (Area-%)	25.76 ± 0.39	26.08 ± 0.39	25.25 ± 0.39	27.17 ± 0.39	26.36 ± 0.39	26.16 ± 0.39
β-CN (Area-%)	34.88 ± 0.48	34.15 ± 0.48	33.35 ± 0.49	32.61 ± 0.55	34.87 ± 0.48	34.13 ± 0.48
Plasmin DG (Area-%)	0.89 ± 0.04	0.87 ± 0.04	0.96 ± 0.04	1.95 ± 0.04	0.88 ± 0.05	1.56 ± 0.04
α-LA (Area-%)	2.82 ± 0.06	2.78 ± 0.06	2.64 ± 0.06	3.00 ± 0.06	2.82 ± 0.06	3.14 ± 0.06
β-LG B (Area-%)	2.31 ± 0.06	2.77 ± 0.06	2.89 ± 0.06	3.35 ± 0.06	2.77 ± 0.06	3.24 ± 0.06
β-LG A (Area-%)	5.31 ± 0.12	5.80 ± 0.12	5.25 ± 0.12	5.73 ± 0.12	5.64 ± 0.12	6.13 ± 0.12

[1] SNF = solids non fat, TS = total solids, FP = freezing point depression, PBC = psychtrophic bacterial count, SCC = somatic cell count, TBC = total bacterial count, GL = glycosilated, CN = casein, DG = degradation products, LA = lactalbumin, LG = lactoglobulin

[2] Mean values without correction for season and region

At dairy level, the fat content varied throughout the country where the northern and mid parts of Sweden had significantly higher content compared to the southern part (highest content 4.39% in the north and lowest 3.87% in the south). For fat content, also seasonal variations could be seen, with the highest content during the indoor season for all regions. The same is valid for lactose content regarding both seasonal and regional variations, with the highest lactose content in the south (4.64%) and the lowest lactose content in the mid (5.51%). For protein content, a regional difference could be seen for the outdoor season, whereas a seasonal difference only was observed in the south of Sweden. No regional difference could be seen for ionic calcium, however, a higher ionic calcium content was found during the outdoor season. Total FFAs showed both a regional and seasonal difference. The FFA content was higher in the north compared to mid Sweden, whereas the total FFA content was highest for outdoor season. The individual free FFAs differed somewhat for both region and season (see Table 4). For C18:3, a significantly regional difference

Table 4. Comparisons between season and region for milk sampled at dairy level. Seasonal differences between outdoor (O) and indoor (I) are displayed as either significantly ($P < 0.05$) higher (↑), significantly lower (↓) or not significant (-). Regional differences between south (S), mid (M) and north (N) Sweden are displayed as significantly different ($P < 0.05$) with different letter or as not significantly different (-). Failing the assumptions of Kruskal Wallis is denoted as not recorded (n.r.).

	South Sweden		Mid Sweden		North Sweden		Outdoor			Indoor		
	O	I	O	I	O	I	S	M	N	S	M	N
pH	-	-	↓	↑	↓	↑	a	b	ab	a	a	b
Fat	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
Protein	↓	↑	-	-	-	-	a	b	b	-	-	-
Lactose	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
SNF	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
TS	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
FP	↑	↓	↑	↓	↑	↓	-	-	-	-	-	-
Ionic calcium	↑	↓	↑	↓	↑	↓	-	-	-	-	-	-
FFA total	↑	↓	↑	↓	↑	↓	ab	a	b	ab	a	b
C4:0	-	-	-	-	-	-	a	b	b	a	b	b
C6:0	-	-	-	-	-	-	a	b	b	a	b	b
C8:0	↓	↑	-	-	-	-	a	b	b	-	-	-
C10:0	↓	↑	-	-	-	-	a	b	b	-	-	-
C12:0	↓	↑	-	-	-	-	a	b	b	a	b	c
C14:0	-	-	-	-	-	-	a	b	b	a	b	b
C16:0	↑	↓	-	-	↑	↓	a	b	c	a	ab	b
C18:0	↑	↓	-	-	↑	↓	a	b	c	a	a	b
C18:1	-	-	-	-	-	-	a	b	b	a	b	b
C18:2	-	-	↑	↓	-	-	a	b	ab	-	-	-
C18:3	↓	↑	↓	↑	↓	↑	a	ab	b	a	ab	b
PBC	-	-	-	-	-	-	-	-	-	-	-	-
SCC	-	-	-	-	-	-	a	a	b	a	a	b
TBC	-	-	-	-	-	-	-	-	-	-	-	-
Peptides (4-14)	-	-	-	-	-	-	ab	a	b	ab	a	b
Glu-κ-CN	↑	↓	↑	↓	↑	↓	a	b	b	a	b	b
κ-CN A/E	-	-	-	-	-	-	a	b	a	a	b	a
κ-CN B	↑	↓	↑	↓	↑	↓	a	b	b	a	b	b
α_{s2}-CN	↓	↑	↓	↑	↓	↑	a	b	a	a	b	a
Peptides (19-22)	-	-	-	-	-	-	a	b	a	a	b	a
α_{s1}-CN	-	-	↓	↑	-	-	-	-	-	-	-	-
β-CN	-	-	-	-	-	-	a	b	a	a	b	a
Plasmin DG	-	-	↓	↑	↓	↑	-	-	-	a	b	c
α-LA	-	-	↓	↑	↓	↑	a	b	a	a	b	b
β-LG B	↓	↑	↓	↑	↓	↑	a	b	b	a	b	b
β-LG A	↓	↑	↓	↑	↓	↑	a	a	b	a	a	b

[1] SNF = solids non fat, TS = total solids, FP = freezing point depression, PBC = psychtrophic bacterial count, SCC = somatic cell count, TBC = total bacterial count, GL = glycosilated, CN = casein, DG = degradation products, LA = lactalbumin, LG = lactoglobulin

was observed between south and north Sweden and a higher content was found during the indoor season. For glycosylated κ-CN and κ-CN B, regional differences were found, with the highest contents in the south of Sweden. Seasonal variations were also observed for these proteins, with the highest contents during the outdoor season. However, for αS2-CN, the seasonal variation was the opposite, with the highest content during the indoor season. No seasonal differences could be seen for smaller peptides (peptides 4-14) or longer peptides (peptides 19-22), whereas a seasonal difference could be seen for plasmin degradation products (plasmin DG) in the mid and north Sweden, with higher contents during indoor season. A seasonal variation was also observed for β-LG A and B, with higher contents during indoor season.

The difference between farm and dairy level regarding chemical and microbiological composition can be seen in Table 5. There were only significant differences between farm and dairy level for most FFA, the microbiological variables and the two proteins κ-CN A/E and α-LA. In common for all, except α-LA, was that dairy level was higher than farm level.

Table 5. Comparison of chemical and microbiological composition between farm and dairy level. Differences are displayed as either significantly ($P < 0.05$) higher (↑), significantly lower (↓) or not significant (-).

Variable[1]	Farm	Dairy	Variable[1]	Farm	Dairy
pH	-	-	PBC	↓	↑
Fat	-	-	SCC	↓	↑
Protein	-	-	TBC	↓	↑
Lactose	-	-	Peptides (4-14)	-	-
SNF	-	-	Glu-κ-CN	-	-
TS	-	-	κ-CN A/E	↓	↑
FP	-	-	κ-CN B	-	-
Ionic calcium	-	-	α_{s2}-CN	-	-
FFA total	-	-	Peptides (19-22)	-	-
C4:0	↓	↑	α_{s1}-CN	-	-
C6:0	↓	↑	β-CN	-	-
C8:0	↓	↑	Plasmin DG	-	-
C10:0	↓	↑	α-LA	↑	↓
C12:0	↓	↑	β-LG B	-	-
C14:0	↓	↑	β-LG A	-	-
C16:0	-	-			
C18:0	-	-			
C18:1	↓	↑			
C18:2	↓	↑			
C18:3	-	-			

[1] SNF = solids non fat, TS = total solids, FP = freezing point depression, PBC = psychtrophic bacterial count, SCC = somatic cell count, TBC = total bacterial count, GL = glycosilated, CN = casein, DG = degradation products, LA = lactalbumin, LG = lactoglobulin

Correlations between enzyme activity and chemical and microbiological status of raw milk

In farm samples, a positive correlation was found between protease activity and total protein, SNF, αS2-CN, larger peptides (peptides (19-22) and plasmin degradation products. A negative correlation was found with C16:0 and αS2-CN. For lipase activity, a positive correlation was found with ionic calcium and glycosylated κ-CN and a negative correlation with C18:3, total bacterial count and β-LG A. An indicator of proteolysis is plasmin DG which was found to have a negative correlation with ionic calcium and total FFA, where the contributing FFAs were C12:0, C16:0, C18:0 and C18:1. Similar lipolysis is indicated by the amount of FFA in the milk and C4:0 is a volatile FFA that is known to cause off-flavors. For total FFA there was a negative correlation with SNF, αS2-CN, plasmin DG and β-LG A. For C4:0, negative correlations with lactose and β-LG A were found. An important quality parameter of milk is the microbiological status. It was found that psychrotrophic bacterial count and total bacterial count had a negative correlation with total protein and SNF. Psychrotrophic bacteria also had a negative correlation with β-LG B and total bacterial count had negative correlations with fat content and total solids. SCC only had correlations with larger peptides (positive) and αS1-CN (negative).

From farm to dairy level a shift in certain correlations occur. Protease activity now have a positive correlation with SCC and only β-LG B and plasmin DG of the proteins. Lipase activity have a negative correlation with pH and lactose and a positive correlation with ionic calcium, C18:1 and total bacterial count (note the opposite sign of the correlation on farm level). On dairy level, total FFAs correlates negatively to lactose but not at all to any of the proteins (as it did on farm level). This is also true for C4:0 which no longer have any correlation to the protein β-LG A. Proteolysis (plasmin DG) has lost its correlation to total FFA and C18:1 but instead gained positive correlations with pH, fat, protein, SNF and TS. The correlation with C12:0 has changed from negative to positive from farm to dairy level. For the psychrotrophic bacteria additional correlations were found at the dairy level. pH, fat, TS and α-LA all had negative correlations with psychrotrophic bacteria, freezing point depression had a positive correlation and the correlation with β-LG B was lost. SCC was found to have two additional positive correlations (GL-κ-CN and

C18:3) and two negative correlations (α-LA and β-LG A). For total bacteria count only the correlation with lipase activity remained intact from farm to dairy level, however, as previously mentioned the sign of the correlation changed from negative to positive.

Heat treatment and storage conditions

The first part of the project was aimed to be followed by a study on the influence of different heat treatments and storage conditions on enzyme activity and content. Milk samples were planned to be collected before and after heat treatment processes at dairy level and stored for different times in varying temperatures. This second part of the project was fully planned with the industry partners, however, no experimental parts and analyses were conducted before the project ended.

Conclusions

The lipase activity in raw milk at farm level was affected both by regional and seasonal variations, whereas at dairy level only season had an effect. However, the results indicate that region and season alone do not fully explain the variation in lipase activity, thus other factors may be of importance. No difference in lipase activity was observed between farm and dairy level, thus indicating that it is on farm level the major contamination occurs. For protease activity, no significant effect of either region or season could be seen in raw milk at farm level. However, opposite from lipase activity, an interesting trend of higher protease activity during indoor season could be noticed. Degradation products resulting from lipolysis, such as total and free FFAs, and from proteolysis, such as peptides, individual proteins and plasmin degradation products, have shown to vary somewhat differently between season and geographical origin of the raw milk at both farm and dairy level. The lipase and protease activity were found to correlate to several of the degradation products at both farm and dairy level, however, there was a shift in some correlations between the farm and dairy level. Thus, the results of this project indicate that both lipase and protease systems are important for raw milk quality, especially at farm level where the first contamination occurs.

Benefits for stakeholders and recommendations

An increased understanding of how the lipase and protease activity and resulting degradation products in raw milk are affected by seasonal and regional differences, as well as which differences that occur between farm and dairy level, has been achieved. The outcome of this project could be used to further study processing effects on enzyme activity and how the quality of end products are affected by enzymes present in milk. This would give valuable knowledge at both farm and dairy level to identify factors that can be used for quality assurance of raw milk, and thus prolong the shelf-life of milk and dairy products. This project is a first step towards a practical implementation in order to control milk and production of dairy products and its quality parameters towards improved sustainability and reduced waste. However, further studies are needed to reach this end goal.

Part 3: Dissemination of results

Peer-reviewed scientific publications	**Submitted manuscript:**
	Glantz M., Lindmark-Månsson H., Rosenlöw M., Hartmann J., Rauh V., Waak, E., Xiao Y., Hallin Saedén H., Höjer A., Löfgren R., Svensson C., Svensson B., Lindau J., Paulsson M. 2019. Protease and lipase activities and the impact on raw milk quality in Swedish milk. *Submitted and under revision*.
Other publications (Popular science publications etc)	Paulsson M., Glantz M., Hedlund M., Nilsson K., Andersson I-M. and Edén J., ”Mejeriteknologi vid Lunds Universitet.” *Mjölk Special*, January 13, 2017.
Oral communication	Glantz M. and Paulsson M. ”Forskning inom mejeriteknologi vid Lunds Universitet. ”Aktuell forskning och utbildning vid Lunds Universitet”. *Mejeritekniskt Forum, Lund, Sweden*; February 4, 2016.
	Hedlund M. ”Milk quality and increased shelf-life of milk”. *Research meeting within Nordic-Baltic Dairy Network, web-based,* October 28, 2016.
	Hedlund M. ”Enzymatic activity in milk”. *Research meeting within Nordic-Baltic Dairy Network, web-based,* October 10, 2017.
	Glantz M. and Paulsson M. ”Senaste nytt inom mejeriforskning och utbildning vid Lunds Universitet”. *Annual meeting within Mejeritekniskt Forum, Malmö, Sweden*, March 16, 2017.
	Hedlund M. “Enzymatic quality evaluation as an approach to predicting shelf-life in milk.” *Workshop within Nordic-Baltic Dairy Network, Larkollen, Norway,* November 20-22, 2017.
	Hedlund M. ”Enzyme activity in milk”. *Annual meeting within Mejeritekniskt Forum, Halmstad, Sweden*, March 14, 2018.
	Hedlund M. ”Enzymatic quality evaluation as an approach to predicting shelf-life in milk”. *Research meeting within Nordic-Baltic Dairy Network, web-based,* May 16, 2018.
	Glantz M. and Paulsson M. ”Forskning inom mejeriteknologi vid Lunds Universitet.” *Aktuell forskning och utbildning vid Lunds Universitet, Mejeritekniskt Forum, Lund, Sweden*; October 24, 2019.
	Paulsson M. and Glantz M., “Dairy Technology at Lund University.” *Steering Committee meeting for Education and Research in Dairy Technology at Lund University, Lund, Sweden,* December 1, 2015; June 2, 2016; December 1, 2016; June 1, 2017; December 1, 2017; June 1, 2018; November 30, 2018; May 23, 2019; November 21, 2019.
Student theses	Hartmann J. “Lipase activity in Swedish raw milk”, MSc. degree project, Lund University, Sweden, 2018.