Quality goat milk for rennet coagulated cheese

Kvalitetsmelk for hvit geitost

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I   POSTER, Ystingskvalitet av norsk geitmelk. Geitedagene i Tromsø, 2011.

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FORORD

Un dessert sans fromage est une belle à qui il manque un œil.
En dessert uten ost er en skjønnhet som mangler et øye.
J. A. Brillat-Savarin

Her har dere svart på hvitt beviset på at skjære sin egen sti gjennom naturvitenskapens jungel fungerer! Det startet ut med miljøfilosofi, svingte innom Niels Bohr for å bli lærd i fysikk og matematikk, endte nesten opp med å redde verden med økotoxologi og naturbasert rensing av avløpsvann. Reiste landevegs med cirkus, startet min egen restaurant, dro til seters for å gjøre meg fet, nektet å ta generelle fag og å leve et generelt liv. Fordypet meg i meieriets verden og surfet solbrun inn i geitemelksprosjektet.

Er det en mening med det? Jeg har i hvert fall trua på at jeg en dag skulle møte Ludvig Funder. Etter mitt syn er det mest spennende av alt som har kommet ut av mitt første forskningsår utviklingen av blåmuggosten. Startet som et matauk. Fikk overbærende positive tilbakemeldinger fra bønder, forskere, professorer, teknikere, produsenter, ledere, forbrukere, familie, venner og mannen i gata. Men var ikke helt fornøyd selv, osten måtte utvikles. Fredag kveld, fest? Nei, hvem vil på fest når man kan yste blåmuggost i Piloten og høre på p2? Matauk ble før jeg visste ordet av det til produktutvikling.

Et godt stykke utpå høsten 2011, geitene var tørrlagte og masteren skulle skrives, ble jeg oppmerksom på en ansatt fra drift og service, bærende på bunker av gamle bøker ut fra sjakta i meieribygget. Hva skjer! Samme ettermiddag finner jeg tilfeldigvis boka Norsk Ost av Anders Oterholm på Biblioteket i Ås. Slår opp midt i boka; Capra – Norsk blåmuggost av geitmelk! Mitt første møte med L. Funder var unnagjort. De neste dagene ble støvfullt tilbrakt i sjakta som en detektiv for å redde gamle publikasjoner, beretninger og notater. Forsøksleder L. Funder (1875-1949) ved Statens Meieriforsøk startet forsøk med ysting av roquefortost av geitmelk i 1931. Resultatene fra de tre første forsøkssårene var så vellykkede at Funder fortsatte å yste Capra, på Sunnylven Meieri, hver sommer fram til andre verdenskrig. Flere norske meierifagfolk så enda lysere enn Funder på den nye osten og hevdet at en nå hadde utviklet en ost som var praktisk talt likeverdig med ekte Roquefort. Denne osten ville bety et veldig oppsving både i norsk geithold og i norsk osteproduksjon og det ble antydet at den

kunne til og med bli verdenskjent. Men Capra kom aldri på markedet. Var det andre verdenskrig som la de storveis planene på is? Funder døde, capra ble glemt. Og etter hva detektiv Brunborg har funnet ut har det ikke vært forsket på blåmuggost av geitmelk siden. Etter 70 tørrlagte år har jeg altså tatt opp forskningen til Funder, uten å ha en anelse om det. Er det på tide at Capra finner sin veg ut til mengdene?

ABBREVIATIONS

A_{30} - firmness after 30 min

ANOVA – analysis of variance

CN - casein

DM - dry matter

FA – fatty acids

FAA - free amino acids

FFA - free fatty acids

FID – flame ionizing detector

GC - gas chromatography

HPLC - high-performance liquid chromatography

IS - internal standard

K_{20} - time before reaching 20 mm between branches on formagraph

LAB – lactic acid bacteria

LCFA - long chain fatty acids

LPL - lipoprotein lipase

MCFA - medium chain fatty acids

MFGM – milk fat globule membrane

PCA - principal component analysis

PUFA – poly unsaturated fatty acids

rct - Rennet clotting time

RH – relative humidity

SCFA - short chain fatty acids

SNF - solids-not-fat

SPE - solid phase extraction

PTFE – Polytetra Fluor ethylene

LIST OF ORIGINAL PAPERS

The thesis is based on the following original papers and they are referred to in the text by their Roman numerals.

I   Jakobsen, Lise Brunborg, 2012. The influence of the α_{s1}-casein genotype of goats on the quality of cheese milk and cheese quality. Unpublished manuscript.

II   Jakobsen, Lise Brunborg, 2012. Effect of supplementation of saturated and unsaturated fat in the goat diet on the quality of cheese milk and cheese quality. Unpublished manuscript.

III   Jakobsen, Lise Brunborg, 2012. Development of a semi-hard blue veined cheese of goat milk. Unpublished manuscript.

1. GENERAL INTRODUCTION

The production of goat milk has a long tradition in Norway. The milk was essentially used for production of whey cheese but the demand for rennet coagulated goat milk cheese in Norway is increasing. The manufacture of rennet coagulated cheese depend more on a suitable and stabile milk quality than the whey cheese. The cheese produced in Norway has been of varying quality and unstable coagulation properties alongside a rancid and tart taste of the milk has been a major problem for the dairy industry. If the rennet coagulated goat milk cheese produced in Norway should be able to compete with the imported goat milk cheese the condition must be an improved milk quality. The quality of the goat milk varies trough the lactation, between individual goats, livestock's and farming practise. Part of the problem is due to the genetic variations of the Norwegian goat breed and until recently the lack of directional breeding. But the diet will also influence the quality of the milk, particularly the protein and fat composition and the sensory properties, which is of importance to the cheese making properties.

The present study investigates both the genetic impact and the effect of different diets on the quality of goat milk for cheese making. Paper I covers the genetic impact and is a comparison between goats that have a double deletion gene for α_{S1}-casein (α_{S1}-CN) and heterozygote goats with genes that code for α_{S1}-CN. The aim of the study was to investigate the effect of the different synthesis of α_{S1}-CN genotypes on ripened cheese since only the coagulation properties of the Norwegian goat milk has been analysed in previous experiments.

Paper II covers the diet experiment, which was included in the interdisciplinary project on quality milk for rennet coagulated goat cheese. This project was a collaboration between The Department of Chemistry, Biotechnology and Food Science (IKBM) and The Department of Animal and Aquacultural Sciences (IHA) at the University of life science (UMB), Ås, Norway. The objective of this project was to examine how carbohydrate based concentrate compared with concentrate added different fat supplementations would influence the energy status, fat metabolism and milk quality trough lactation. By this project the aim was to come to an increased understanding of the correlation between the fat metabolism in the goat and modifications in the milk quality, and how the composition of the milk fat influence lipolysis and the frequent taste deficiency which occurs in Norwegian goat milk.

In addition to the mentioned two parts of the study a product development was also carried out as a supplement to the principal study. Paper III covers the development of a semi-hard

blue veined cheese made of goat milk. In contrast to the rest of the study, the milk used in the product development was collected from the bulk tank.

2. A BRIEF THEORETICAL REVIEW

2.1. Differences in goat and bovine milk

Lactation

The composition of bovine milk that enters the dairy has minimal changes during the year. This is due to the fact that most farmers practice a year-round breeding program that leads to stabile milk in the bulk tank, independent of the lactation (Park, Juarez et al. 2007). This is different in the goat milk production, where farmers primarily practice seasonal breeding. Changes in the bulk tank goat milk occur as a consequence of the seasonal breeding during lactation. In general the content of fat, protein and minerals is higher early and late in lactation than at mid lactation (Fekadu B 2005).

Lipids

The milk fat is present as globules of triglycerides surrounded by a complex milk fat globule membrane (MFGM) (Cebo, Caillat et al. 2010). Figure 1 describes the composition of the MFGM with the trilayer structure, the lateral organisation of polar lipids and the heterogeneous distribution of proteins (Christelle 2011).

The fat content of goat milk is similar to bovine milk but the fat globules in goat milk are in general smaller. In goat milk the fat globules are only present in sizes less than 3,5 μm (Park, Juarez et al. 2007). The small fat globules and the absence of agglutinin, which causes clustering of the globules in bovine milk, result in slow creaming of goat milk compared to bovine milk (Haenlein 2001; Park, Juarez et al. 2007).
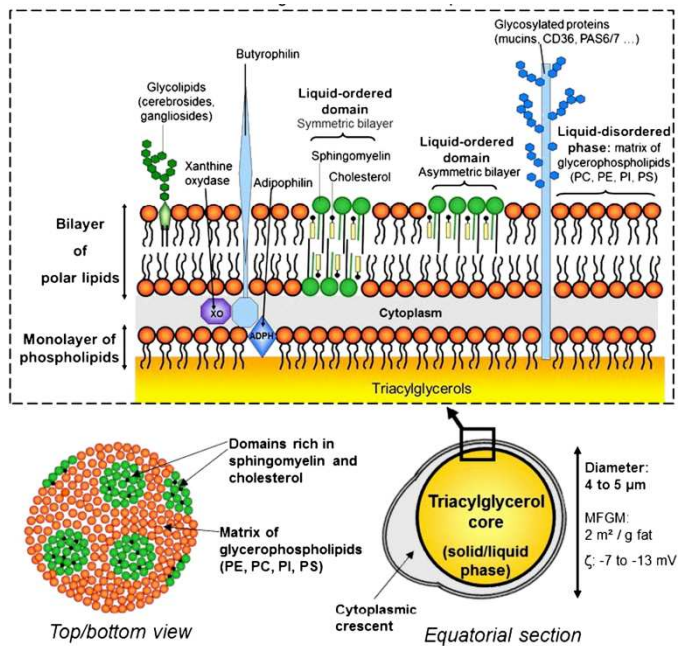
Figure 1 The milk fat globule: triglycerides surrounded by the complex MFGM (Christelle 2011).

The abundance of short (SCFA) and medium (MCFA) chain fatty acids are higher in goat milk. The content of caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), and lauric ($C_{12:0}$) are significantly higher in goat milk (Alonso, Fontecha et al. 1999). Especially the caprylic acid and the branched capric acids are most probably the main contributors to the goat like flavour in goat milk and both caprylic and butyric ($C_{4:0}$) acid can give a rancid and tart taste (Chilliard, Ferlay et al. 2003; Collins, McSweeney et al. 2003; Park, Juarez et al. 2007).

Proteins

The content of protein is in average similar in goat and bovine milk but the proportions of the major caseins are different (Park, Juarez et al. 2007). Goat milk, in general, contains less α_{S1}-CN and more α_{S2}-CN, β-CN and κ-CN, in relative amounts, than bovine milk (Mora-Gutierrez, Kumosinski et al. 1997). In addition goat milk has a higher content of non-protein nitrogen and less casein nitrogen (Park, Juarez et al. 2007).

The interaction between the serine-phosphate groups in α_{S1}-CN and calcium ions (Ca^{2+}) in milk is strong and this interaction contribute, along with hydrophobic bonds between the caseins, to the presence of the casein as casein micelles (Walstra, Wouters et al. 2006). The high relative content β-CN in goat milk compared with bovine milk appears to affect the properties of the casein micelles in goat milk. The β-CN is very voluminous and hydrophobic and probably leads to entrapment of water in the increased spatial structure of the casein micelle (Mora-Gutierrez, Kumosinski et al. 1997). The lower relative content of α_{S1}-CN and

higher β-CN leads to larger micelles that entrap more water than the casein micelles in bovine milk.

Minerals and vitamins

Goat milk has in general a higher content of Ca, P, K, Mg and Cl and less Na and S than bovine milk (Park, Juarez et al. 2007). The white colour of goat milk is caused by the lack of β-carotene. β-carotene is the precursor of vitamin A. In goat milk all β-carotene is converted and the content of vitamin A is therefore higher than in bovine milk (Park, Juarez et al. 2007). Goat milk has deficiencies in folic acid and vitamin B_{12} and the content is five times higher in bovine milk. Folate is nessesary for the synthesis of haemoglobin and a deficiency of Folate and B_{12} can cause a megaloblastic anaemia in infants (Park, Juarez et al. 2007).

2.2. Genetic impact on goat milk and cheese quality

Several studies have revealed the differences in milk quality between different goat livestock's and genotypes. It is obvious that the α_{S1}-CN genotype has a major influence on the milk composition and sensory quality of the milk and cheese (Ambrosoli, di Stasio et al. 1988; Delacroix-Buchet, Degas et al. 1996; Pierre, Le Quere et al. 1998; Pierre, Michel et al. 1998; Tziboula and Horne 1999; Vegarud, Devold et al. 1999; Clark and Sherbon 2000; Ådnøy, Vegarud et al. 2003; Chilliard, Rouel et al. 2006; Devold, Nordbo et al. 2011). The milk with high amounts of α_{S1}-CN is characterized by a high content of protein, fat, Ca, lower pH, smaller casein micelles and are less susceptible to lipolysis and off-flavours. A common agreement exits, that the milk from the strong α_{S1}-CN genotypes has a favourable quality for cheese making. The genotypes are divided into variants according to the amount of α_{S1}-CN in the milk; high variants with approximately 3,6 g/L (A, B (1,2 and 3) and C), the intermediate variant with approximately 1,6 g/L (E), low variants with approximately 0,6 g/L (D, F and G) and the "null" variant with absence of α_{S1}-CN (0) (Grosclaude, Mahe et al. 1987; Martin, Addeo et al. 1996).

2.3. The impact of diet on goat milk and cheese quality

The genetic impact on the quality of goat milk appears to explain the major differences in the milk quality of individual goats. Nevertheless research has also shown improvement of the quality of goat milk by favourable diets (Chilliard, Ferlay et al. 2003; Morand-Fehr 2005; Eknæs and Skeie 2006; Morand-Fehr, Fedele et al. 2007; Eknæs, Havrevoll et al. 2009). Alongside the problem of casein composition in goat milk there is also a frequent rancid and

tart flavour. This defect, in goat dairy products, is decreasing with supplementation of diets rich in fat fed to lactating goats. In contrast to studies of bovine milk it appears that a fat supply in correct proportions do not reduce the protein content of the goat milk.

In Norway, the typical farming practice is to keep the goats indoor in the winter and at pasture during the summer season. In addition, the lactation changes the quality of the milk during the year. Eknæs et al. (2009) also observed a higher milk protein content at mountain pasture compared to the period of indoor feeding. This could be explained by a favourable composition of nutrients in the mountain pasture. The same study also showed that the frequency of rancid and tart taste increased during the mountain pasture, which is considered to be a negative property of the milk quality.

Rouel et al. (2002) found that a supply of linseed oil decreased the goat flavour of fresh cheeses but they also detected a fish flavour, which is considered as a negative characteristic. A supply of oleic sunflower oil decreased the tart flavour of ripened cheeses. The same study also found that a diet rich in hay led to a decrease in oxidised and bitter flavours, while a diet rich in maize had the opposite effect.

Soryal et al. (2004) studied the influence of different levels of concentrate fed to grazing goats on ripened Domiati cheese produced of milk from these goats. The level of concentrate supply did not affect the composition of the cheese, but the cheese from goats without a supply of concentrate had a lower SCFA content and a higher flavour score.

2.4. Goat's lactation

The content of milk fat and protein is always high after kidding and decreases during the major part of lactation. In the end of the lactation there is an increase in the fat and protein content due to a decrease in milk yield. The initial is both due to the increase in milk yield until the lactation peak and to a decrease in fat mobilization for mammary lipid synthesis (Chilliard, Ferlay et al. 2003)

Goat milk lipolysis and LPL activity are low during the first four weeks of lactation and after week 30 of lactation. While the lipolysis and the *lipoprotein lipase* (LPL) activity are highest after the lactation peak in mid lactation. (Chilliard, Ferlay et al. 2003)

2.5. Lipolysis in goat milk

Lipolysis occurs when the bond between the glycerol and fatty acids on the milk fat triglycerides are hydrolyzed and free fatty acids (FFA) are formed. In milk the native lipolytic enzyme LPL is responsible for the spontaneous lipolysis (Walstra, Wouters et al. 2006). Since

goat milk has a high content of SCFA and MCFA lipolysis will result in release of $C_{8:0}$, branched $C_{10:0}$ and $C_{4:0}$ which will give the milk a typical goat flavour and a rancid and tart taste (Chilliard, Ferlay et al. 2003). Lipolysis in milk can be induced by agitation, pumping, air inclusion, temperature changes (cooling and heating), churning and homogenisation (Christelle 2011). This is due to the mechanical damage of the milk fat globules, which expose the triglycerides. The resistance to damage of the milk fat globules relies on the function of the MFGM. It has been considered that the MFGM of goat milk was similar to bovine milk. But a recent study have observed fundamental differences between the species (Cebo, Caillat et al. 2010). The analysis of the MFGM proteins from goat milk revealed the presence of CN in the goat MFGM, whereas practically no CN were detected in the MFGM of bovine milk. Further, the abundance of CN was higher in the MFGM protein fraction in α_{S1}-CN "null" genotypes than in high α_{S1}-CN genotypes. Cebo et al. (2010) suggested that an alternative pathway for milk fat secretion is present in the goats that are α_{S1}-CN defective. It is not revealed if this particular composition of the MFGM will lead to a weaker membrane, but it could explain why milk from α_{S1}-CN "null" genotypes is highly susceptible to lipolysis.

In goat milk the LPL is most likely situated on the milk fat globules rather than bound to casein micelles as in bovine milk (Chilliard, Ferlay et al. 2003). This can explain why lipolysis of milk fat is well correlated to LPL activity in goat milk and not in bovine milk. The LPL activity is low during early and late lactation and when goats are underfed or are fed a diet supplied with unsaturated fat (Chilliard, Ferlay et al. 2003).

2.6. Biochemical changes during cheese ripening

Lipolysis

The fat in cheese can be hydrolysed to FFA by lipolysis. The SCFA and MCFA can either contribute positive to the flavour of the cheese or to a rancidity defect depending on the concentration and perception threshold (Collins, McSweeney et al. 2003). The lipases and esterases originate mainly from lactic acid bacteria (LAB) since naturally occurring LPL in milk is inactivated by pasteurisation. The enzymes are located inside the bacteria cells and the extent of autolysis of the cell is determining for the lipolysis to occure (Fox, Guinee et al. 2000). Compared with other bacteria and mould species *Lactococcus* spp. and *Lactobacillus* spp. have low lipolytic activity, but the bacteria occurs in such large numbers in the cheese that they thereby account for the main lipolysis during ripening (Fox, Guinee et al. 2000).

Free fatty acids can further act as a precursor leading to the formation of aromatic compounds (Collins, McSweeney et al. 2003). The oxidation of FFA leads to formation of β-ketoacids. The β-ketoacids can further be decarboxylated to methyl ketones (Alkan-2-ones),

which in turn can be reduced to the corresponding alakan-2-ol (Collins, McSweeney et al. 2003). Free fatty acids can react with the alcohol formed by the fermentation of lactose or by the decomposition of amino acids and form ethyl esters. Thioesters can be formed when FFA react with free sulphydryl groups (-SH) (Collins, McSweeney et al. 2003).

Proteolysis

During the cheese ripening the α_{S1}-CN is degraded by splitting of the Phe23-Phe24 bond by the rennet into large peptides. Clevage of this bond is believed to cause a softer texture of the ripened cheese (Fox, Guinee et al. 2000). It is mainly enzymes from the LAB that degrade the large peptides further down to small peptides and amino acids. The proteinases from the LAB exist on the bacterial cell wall while the peptidases are intracellular, and autolysis of the cell is necessary for their action. *Lc.* spp. and *Lb.* spp. are the bacteria strains that primarily contribute to the degradation of the proteins. Proteinase from *Lc* spp. hydrolyzes large peptides to small peptides. While small peptides are degraded by peptidases from both *Lc* spp. and *Lb* spp. into free amino acids (FAA) (Sousa, Ardö et al. 2001).

Large peptides contribute to the texture of the cheese and small peptides and amino acids contribute to the flavour. Amino acids are an energy source for LAB and the composition of amino acids in cheese changes during ripening. Amino acids can further be degraded to volatile aroma compounds of different enzymes in the cheese (Sousa, Ardö et al. 2001).

The catabolism of amino acids often begins with aminotransferase of the amine group on the amino acid to an α-ketoacid, resulting in a new amino acid and a new α-ketoacid. α-ketoacids are decarboxylated to aldehydes, which further can be reduced to alcohols by alcohol dehydrogenases. Aldehydes can also be oxidized to carboxylic acids by aldehyde dehydrogenase. Carboxylic acids and alcohols formed from aldehydes can then react and form esters by alcohol acyltransferase (Walstra, Wouters et al. 2006).

The major part of the products from proteolysis are aromatic compounds that contribute to the flavour of the cheese (Ardö 2006).

3. BRIEF SUMMARY OF PAPERS I-III

3.1. PAPER I

<u>The influence of the α_{S1}-casein genotype of goats on the quality of cheese milk and cheese quality</u>
The objective of this study was to investigate the differences on the cheese making properties and cheese ripening between milk from heterozygote 01-goats and from homozygote 00-goats for α_{S1}-CN.

The differences in milk quality between the α_{S1}-CN genotypes of goats have been studied in different goat breeds in several countries. It is in general accepted that the cheese making properties of the high α_{S1}-CN variants are preferable. But few studies have investigated the effect in ripened cheese. The cheese making was standardized so that the cheese was comparable from production to production without any other experimental factors than genotype. The study included analysis of FFA, FAA, organic acids, dry matter, microbiology, pH and sensorial analysis. In addition coagulation properties (formagraph) and composition of milk from each goat was analysed one week after each cheese making. The composition (fat and protein) and the coagulation properties (K_{20} and A_{30}) of milk from goats of the high α_{S1}-CN variant were preferable for cheese making. Further it appeared like the milk from the high α_{S1}-CN variant leaded to a better and more stabile cheese quality during lactation. The cheese from the low α_{S1}-CN variant milk had lower % dry matter content, which implied that the cheese matrix trapped more water, and had a frequent rancid flavour.

3.2. PAPER II

<u>Effect of supplementation of saturated and unsaturated fat in the goat diet on the quality of cheese milk and cheese quality</u>
The objective of this study was to investigate the effect of diets added a supplement of saturated or unsaturated vegetable fat on the goat milk and its cheese making properties.

Due to a high frequency of off-flavours and an unfavourable composition a great deal of the Norwegian goat milk has been poor for production of cheese. This has led to a large quantity of discarded milk. Different diets can affect the quality of the milk and especially a supply of fat in correct proportions in the diet has shown to contribute to an increase in

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quality. The cheese making was standardized so that the cheese was comparable from production to production with only the feed as an experimental factor. The study included analysis of FFA, FAA, organic acids, dry matter, microbiology, pH and sensorial analysis. In addition coagulation properties (formagraph) and composition of milk from each goat was analysed one week after each cheese making. The fat content of the milk was higher in the groups that were fed a supply of saturated and unsaturated fat. The milk from goats fed a supply of unsaturated fat had a higher protein content then the other groups. The cheese from the control group had the best sensory attributes and the cheese from the group fed a supply of saturated fat had a high abundance of rancid taste. The cheese from the group fed a supply of unsaturated fat appeared to ripen slower than the other cheeses.

3.3. PAPER III

Development of a semi-hard blue veined cheese of goat milk
The objective of this study was to develop a recipe for a semi-hard cheese of goat milk ripened with *Penicillium roqueforti*.

    With an increasing quality of the goat milk in Norway there is a market for developing new products. A hindrance in the development of new cheese varieties has been poor cheese making properties of the milk and a frequent rancid flavour. The taste defect is a result of lipolysis of milk FFA. Blue veined cheese, ripened with *Penicillium roqueforti*, gain a high pH, which will to some extent neutralize the FFA and cover up the taste defects of the milk. The sensory quality of the cheese was satisfactory but the interior structure was too dense and hindered a proper development of *P. roqueforti*. Further development of the cheese making technology is needed to create the desired structure.

4. GENERAL DISCUSSION

    In most studies of milk quality for production of cheese, the research focuses on the composition of the milk and coagulation properties. These studies will conclude about how suitable the milk is for the manufacture process but not about what occurs during the ripening of the cheese. During ripening a series of complex biochemical processes occur, which will determine the final quality of the cheese. This study included consequently analysis of the ripened cheese. The results will then better describe how the different α_{S1}-CN genotypes of goats and different diets affect the final product. Altogether the cheese made of milk from the

high α_{S1}-CN genotype (Paper I) and cheese made of milk from the goats given a supplement of unsaturated fat (Paper II) gained the best quality.

The cheese made of milk from the high α_{S1}-CN genotype (Paper I) was made of milk that had most favourable composition (protein and fat) and best coagulation properties. The cheese had a firmer texture (high DM) and better sensory quality.

The cheese made of milk from the goats given a supplement of unsaturated fat (Paper II) was made of milk that had most favourable composition (protein and fat) and best coagulation properties. The cheese ripened slower but had better sensory quality than the cheese made of milk from the goats given a supplement of saturated fat.

The blue veined cheese (Paper III) had satisfactory sensory quality but in all trials the cheese making did not result in cheeses with sufficiently open structure.

5. FURTHER PERSPECTIVES

The present study was merely a master thesis and was therefore limited in time and costs. Nevertheless there were several analyses that gave results with statistical significance, which makes further studies interesting. The limited time resulted in few replications of each part of the study (Paper I and II). Further investigations of the effect of α_{S1}-CN genotype trough the hole lactation, including the mountain pasture, would be interesting. Little research has also been done on the effect of different diets on the α_{S1}-CN genotypes. The cheese made in the main part of the study (Paper I and II) was not a commercial cheese and it was therefore difficult to evaluate it sensorial. In further studies it would be interesting to make a cheese of superior quality, which could in addition be evaluated with a consumer survey.

In addition to the already accomplished results, the content of FFA and the composition of caseins in the cheese will be analysed. These results will most likely give an even better understanding of the observed differences between the cheeses.

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PAPER I

The influence of the α_{s1}-casein genotype of goats on the quality of cheese milk and cheese quality

Lise Brunborg Jakobsen

The influence of the α_{S1}-casein genotype of goats on the quality of cheese milk and cheese quality

Abstract

The differences in milk quality between the α_{S1}-CN genotypes of goats have been studied in different goat breeds in several countries. It is in general accepted that the cheese making properties of the high α_{S1}-CN variants are preferable. But few studies have investigated the effect in ripened cheese. The objective of this study was therefore to investigate the differences on the cheese making properties and ripening between milk from a high α_{S1}-CN genotype and from the "null" genotype with absence of α_{S1}-CN of the ordinary Norwegian goat breed. The cheese making was standardized so that the cheese was comparable from production to production without any other experimental factors than genotype. The study included analysis of free fatty acids (FFA), free amino acids (FAA), organic acids, dry matter, microbiology, pH and sensorial analysis. In addition coagulation properties (formagraph) and composition of milk from each goat was analysed one week after each cheese making. The composition (fat and protein) and the coagulation properties (K_{20} and A_{30}) of milk from goats of the high α_{S1}-CN variant were preferable for cheese making. Further it appears like the milk from the high α_{S1}-CN variant leads to a better and more stabile cheese quality during lactation. The cheese from the "null" α_{S1}-CN variant milk had lower % dry matter content, which implies that the cheese matrix is trapping more water, and a frequent rancid flavour.

Sammendrag

Kvalitetsforskjellen mellom geitemelk fra ulike α_{S1}-CN-genotyper har blitt undersøkt i ulike geiteraser og i ulike land. Men få studier har undersøkt effekten av genotyper på modnet ost. Formålet med denne studien var derfor å undersøke ystingsegenskapene og modingen av ost ystet av melk fra geiter med gener som koder for et høyt innhold av α_{S1}-CN og av geiter med "null" gener som har fravær av α_{S1}-CN i melka. Ysteprosessen var standardisert slik at osten skulle bli sammenlignbar fra produksjon til produksjon uten andre eksperimentelle faktorer enn genotype. Studien inkluderte analyser av frie fettsyrer (FFA), frie aminosyrer (FAA), organiske syrer, karbohydrater, tørrstoff, mikrobiologi, pH og sensorisk bedømmelse. I tillegg ble koaguleringsegenskaper (formagraf) og sammensetning

av ystemelka fra hver enkelt geit analysert. Sammensetningen (protein og fett) og koaguleringsegenskapene (K_{20} og A_{30}) til melka fra geitene med gener som koder for et høyt innhold av α_{S1}-CN egnet seg best til ysting. Videre viste det seg at denne melka førte til en mer stabil kvalitet på osten gjennom laktasjonen. Osten ystet av melk fra "null"-genotypen hadde et lavere tørrstoffinnhold, som tyder på at ostematriksen holder på mere vann, og en hyppig forekomst av harsk smak.

6. Introduction

The demand for rennet coagulated goat cheese in Norway is increasing. Cheese of good quality is imported, especially from France, while for cheese produced in Norway varying milk quality has been a major problem with a rancid and tart flavour of the milk. Essentially the goat milk was used for production of brown whey cheese. Whey cheese is not a cheese by definition but is made of concentrated whey added milk and cream in the final processing step (CodexAlimentarius 1971; CodexAlimentarius 1978). The goat stock was breed towards a high milk yield and a strong goat flavour of the milk. The goaty flavour can be a negative characteristic if it is to dominant but in the whey cheese the sweetness will to some extent balance the flavour defect.

The direction of breeding did as well lead to goats with a double deletion (00) gene for α_{S1}-casein (CN) (Ådnøy, Vegarud et al. 2003) and a frequent rancid and tart taste of the milk (Eknæs and Skeie 2006). Vegarud et al. (1999) compared goat stocks from different parts of Norway and found a frequency of 58% of the special deletion in the exon 12 D allele of the α_{S1}-CN gene in the north, 75% in the southeast and 79% in the west of Norway. These goats are further denoted as 00-goats. Ådnøy et al. (2003) genotyped goats from two farms in northern Norway and found the frequency of the 00-goats to be 86% on these farms.

Analysis has shown that the protein and fat content of the milk from the 00-goats are significantly lower than in the milk from goats with other α_{S1}-CN genotypes (Clark and Sherbon 2000; Ådnøy, Vegarud et al. 2003). A good correlation between a high content of good quality α_{S1}-CN in milk and good properties for cheese production has been shown (Ambrosoli, di Stasio et al. 1988; Vegarud, Devold et al. 1999; Clark and Sherbon 2000; Ådnøy, Vegarud et al. 2003). Ambrosoli et al. (1988) found that milk with a low content of α_{S1}-CN had a faster coagulation time whereas milk with high contents of α_{S1}-CN produced a firmer curd. The latter has been associated with a more advantageous composition of milk for cheese production. Clark and Sherbon (2000) found that milk lacking α_{S1}-CN had poorer coagulation properties than milk with high contents of α_{S1}-CN. However they found that the

% of dry matter, non-fat solids (SNF), and protein were more highly correlated with good coagulation properties than α_{S1}-CN. Vegarud et al. (1999) analysed milk from goats at three different locations in Norway and found that milk from the 00-goats coagulated later after rennet addition (rct), had longer coagulation time (K_{20}) and gave a less firm or not sufficiently firm coagulum after 30 min (A_{30}) than milk from goats with other α_{S1}-CN genotypes. The same analysis showed that the cheese curds of the 00-goats had lower syneresis than the cheese curds from goats with a high content of α_{S1}-CN in the milk. Ådnøy et al. (2003) analysed milk from two farms in northern Norway and found that the 00-goats gave a slower coagulation and a weaker coagulum than the mean of the other genotypes.

The result of the breeding strategy in Norway until 2007 (NSG 2011) was milk that was poor for production of rennet coagulated cheese but was acceptable for the sweet cooked whey cheese. However, with the increasing demand for rennet coagulated goat cheese the status of the Norwegian goat stock was discovered. At The University of Life Science in Ås, Norway, breeding towards heterozygote (01) goats with genes that code for α_{S1} casein started in 2008. The breeding regime is only including ordinary Norwegian dairy goats and the bucks used for breeding has a known genetic profile. Alongside with the breeding regime for 01-goats a few 00-goats has also been retained.

The objective of this study was to investigate the differences on the cheese making properties and cheese ripening between milk from heterozygote 01-goats and from homozygote 00-goats for α_{S1}-CN. The cheese making was standardized so that the cheese was comparable from production to production without any other experimental factors than genotype. The cheese was semisoft and ripened in plastic foil. An aromatic mesophilic DL starter culture was used and *Lactobacillus casei* was added as an adjunct culture to accelerate ripening. The cheese was analysed one day after production, after 2 and 4 months.

7. Materials and methods

7.1. Animals and diets

Two groups of goats of the Norwegian dairy breed were designed. One group with eight 00-goats and the other with twelve 01-goats. The date of kidding differed somewhat between the goats, but the groups were adjusted according to this. The average age was 3,5 years for the 00-goats and 2 years for the 01-goats. The young goats usually have a lower milk yield than the older ones, so the 01-group was added a few more goats to ensure enough milk for the cheese production. All goats were given the same diet during the experimental period. The diet consisted of grass silage and a concentrate based on barley.

7.2. Manufacture process

The goats were kidding during February 2011 and the first production of cheese was made in the second month in lactation (March, week 10). The second production was made in the third month in lactation (April, week 14), 2011. Two parallel cheese productions were carried out during these weeks, with one day between each cheese making. Milk was collected in the evening two days ahead of the production day, morning and evening the day before and in the morning the same day as production.

Pasteurised milk was tempered to 32°C and inoculated with 0,7 % CHN19 from Chr. Hansen A/S (Hørsholm, Denmark) and preacidified for 25 min, then the milk was added 10 mL *Lb. casei* (University of Life Science, Ås, Norway) per 100 L milk and further acidified 5 more minutes. The milk was then added 25 mL rennet (CHYMAX, Chr. Hansen A/S, Hørsholm, Denmark) per 100L milk. The coagulum was cut in 10 mm cubes at appropriate firmness with a clear cut. The coagulation time varied from 25 to 35 min during the experimental period. The cheese curds was let to rest for 5 min and thereafter stirred for 40 min keeping the temperature at 32°C. The whey was then diluted by 50% whey removal and 50% water (32°C) addition. The curds were heated to 39°C during 10 min and cooked for 30 min at this temperature. Most of the whey was removed and the curds were transferred to Camembert forms and pressed lightly by hand. The cheese was kept at 36°C and turned straight away, after 30 min and 1 hour. After two hours the cheese was cooled down to 10°C and salted in brine for 1 hour. The cheese dried over night in room temperature and was then vacuum packed in plastic foil. The ripening of the cheese took place at 16°C for 2 weeks and thereafter at 4°C for the remaining ripening period. An overview of the complete manufacture process is presented in figure 1.
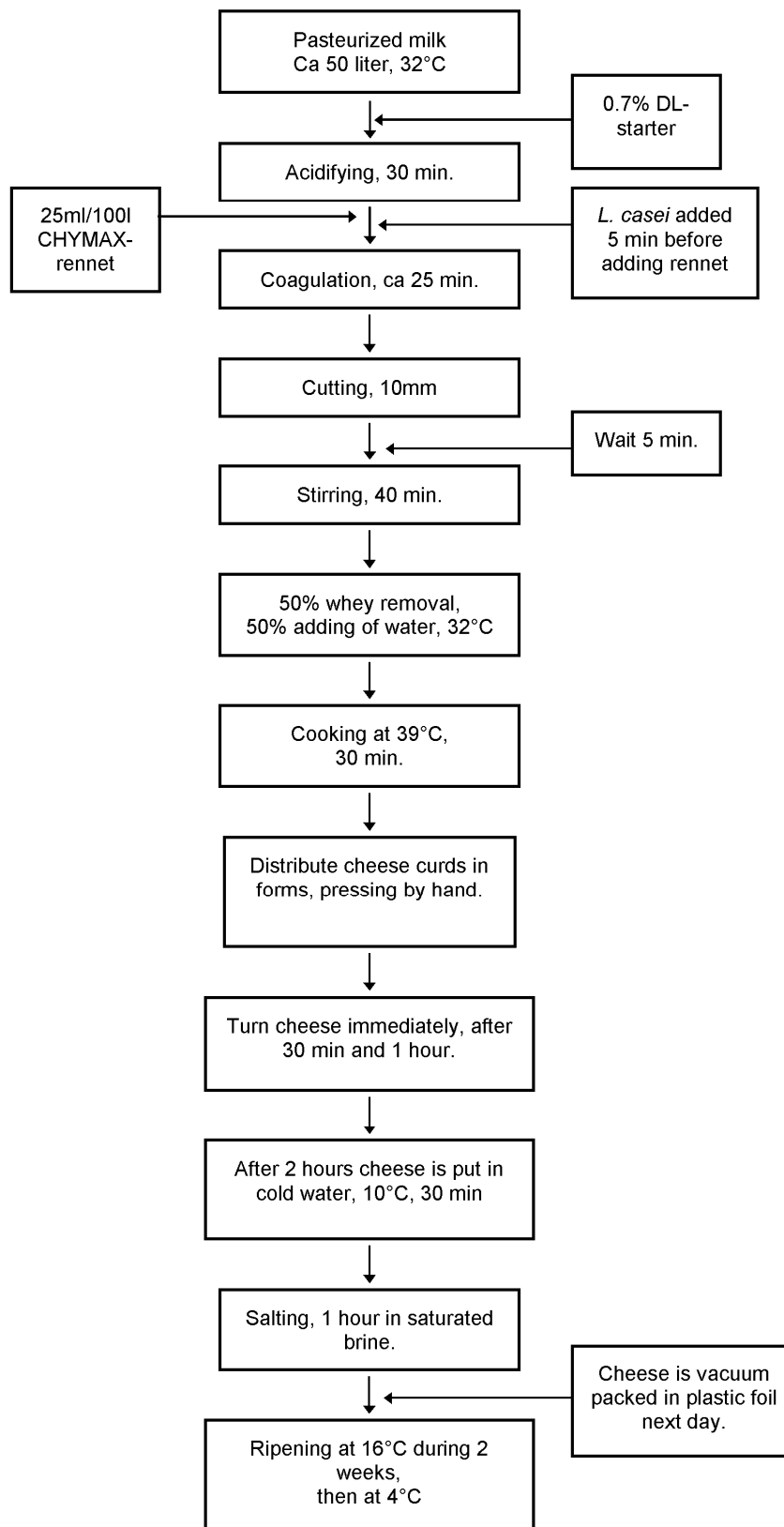
4

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┌─────────────────────┐
│ Pasteurized milk    │
│ Ca 50 liter, 32°C   │
└─────────────────────┘
           │                              ┌──────────────┐
           │◄─────────────────────────────│ 0.7% DL-     │
           ▼                              │ starter      │
┌─────────────────────┐                   └──────────────┘
│ Acidifying, 30 min. │
└─────────────────────┘
┌──────────────┐           │         ┌──────────────────┐
│ 25ml/100l    │──────────►│◄────────│ L. casei added   │
│ CHYMAX-      │           ▼         │ 5 min before     │
│ rennet       │   ┌─────────────────────┐  │ adding rennet │
└──────────────┘   │ Coagulation, ca 25 min. │ └──────────────────┘
                   └─────────────────────┘
                              │
                              ▼
                   ┌─────────────────────┐
                   │ Cutting, 10mm       │
                   └─────────────────────┘
                              │                  ┌──────────────┐
                              │◄─────────────────│ Wait 5 min.  │
                              ▼                  └──────────────┘
                   ┌─────────────────────┐
                   │ Stirring, 40 min.   │
                   └─────────────────────┘
                              │
                              ▼
                   ┌───────────────────────────┐
                   │ 50% whey removal,         │
                   │ 50% adding of water, 32°C │
                   └───────────────────────────┘
                              │
                              ▼
                   ┌─────────────────────┐
                   │ Cooking at 39°C,    │
                   │ 30 min.             │
                   └─────────────────────┘
                              │
                              ▼
                   ┌─────────────────────────────┐
                   │ Distribute cheese curds in  │
                   │ forms, pressing by hand.    │
                   └─────────────────────────────┘
                              │
                              ▼
                   ┌─────────────────────────────┐
                   │ Turn cheese immediately, after │
                   │ 30 min and 1 hour.          │
                   └─────────────────────────────┘
                              │
                              ▼
                   ┌─────────────────────────────┐
                   │ After 2 hours cheese is put in │
                   │ cold water, 10°C, 30 min    │
                   └─────────────────────────────┘
                              │
                              ▼
                   ┌─────────────────────────────┐
                   │ Salting, 1 hour in saturated │
                   │ brine.                      │
                   └─────────────────────────────┘
                              │                  ┌────────────────────┐
                              │◄─────────────────│ Cheese is vacuum   │
                              ▼                  │ packed in plastic foil │
                   ┌─────────────────────────────┐│ next day.          │
                   │ Ripening at 16°C during 2   │└────────────────────┘
                   │ weeks,                      │
                   │ then at 4°C                 │
                   └─────────────────────────────┘
```

Figure 1 Manufacture process of semi-hard goat cheese

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Samples were collected from pasteurised cheese milk, fresh cheese and from matured cheese ripened for two and four months. Microbiology, dry matter, pH and organic acids were analysed and measured at the time of sampling. Samples for analysis of FFA and FAA were stored at -20°C for later analysis.

The microbiological analysis included coliform bacteria, *Lactobacillus* ssp (NSLAB) and *Lactococcus* ssp. Coliform bacteria were enumerated on VRBA agar (OXOID, Hampshire, England) and incubated at 37°C for 24 hours. *Lactobacillus* ssp was enumerated on BBL™ LBS agar (Becton Dickinson and co., Le Pont de Claix, France) and incubated anaerobic at 30°C for 4 days. *Lactococcus* ssp was enumerated on M17 broth (MERCK, Darmstadt, Germany) and incubated at 30°C for 2 days.

Samples for measurement of dry matter were first dried in room temperature over night then dried at 120°C for 20 hours ((IDF) 1982). The pH of the cheese was measured with a Orion pH-meter model 320 with an Orion Ross 8155 electrode (Orion Research, Cambridge, USA) according to Skeie et al (2001).

The analysis of FAA were done according to a modified method described by Bütikofer and Ardö (1999) using high-performance liquid chromatography (HPLC) with OPA derivatisation. The samples were added an extraction solution based on 0.1M HCl added Piperidine-4-carboxylic acid (PICA) (Fluka, St.Louis, USA) and L-norvalin (Sigma, St. Louis, USA) as internal standards (IS). The HCl solution was diluted from 37% HCl with milliQ water. The samples were then homogenized by an Ultra-Turrax (Pro Scientific Inc, Monroe, USA) for 5 min at 20 000 rpm. Then the samples were placed in an ultrasound bath (Branson, Soest, The Netherlands) for 30 min and centrifuged for 40 min at 4°C and 3400 rpm (Beckman J2-MC, GMI Inc., Minnesota, USA). 1 ml of the supernatant was added 1 mL of 4% trichloroacetic acid (Merck, Darmstadt, Germany). The solution was mixed on a vortex (Gene 2, New York, USA) and put on ice for a minimum of 30 min. The samples were centrifuged for 5 min at 13 000 rpm (Eppendorf 5415 D, Hamburg, Germany). The supernatant was then filtered through a 25mm syringe filter with 0.2μm cellulose acetate membrane (VWR International) directly in HPLC vials. The samples were stored at -20°C until analysis.

The separation of the FAA was carried out using a *Perkin Elmer series 410 LC Pump* (Perkin Elmer, Conneticut, USA), an Agilent Technologies 1200 series autosampler (Agilent Technologies, Waldbronn, Germany), a Perkin Elmer 200 column oven and an Agilent Technologies 1200 series thermostat. The system was driven by an EZChrom Elite (Agilent Technologies). A XTerra RP 18 column with 150 x 4.6 mm (Waters, Massachusetts, USA)

was used and the injection temperature was 42 °C. Free amino acids were detected with a 1200 series fluoriscens detector from Agilent Technologies.

Organic acids and carbohydrates were analysed according to the method described by Skeie et al. (2008) with a *Perkin Elmer series 410 LC Pump* HPLC (Norwalk, USA) with a 300x7.8 mm Aminex HPX 87 H column (Biorad, Richmond, USA) and Perkin Elmer Applied biosystems 759A absorbance UV detector (Norwalk, USA). The samples were added 5 mL milliQ water, 0,7 mL 0,5 M H_2SO_4 and 20 mL acetonitril (CH_3CN) then mixed for 30 min and centrifuged. The supernatant was then transferred to a HPLC vial trough a 13 mm syringe filter with a 0.2 µm PTFE (Polytetrafluoroethylene) membrane (Acrodisc®). The samples were injected on the HPLC with a Perkin Elmer series 200 auto injector (Norwalk, USA). The injection volume was 25µl, with a 0.4 mL/min flow at 32°C. The mobile phase was 5mM H_2SO_4 in purified water. The wavelength of the detector was 210 nm.

The analysis of FFA were done using gas chromatography (GC). The procedure for the extraction of the FFA in the cheese was done as described by Dejong and Badings (1990). The samples were diluted in a 1:1 v/v solution of diethyl ether (Merck, Darmstadt, Germany) and heptane (Merck, Darmstadt, Germany), added sulphuric acid and dried with Na_2SO_4 (Merck, Darmstadt, Germany). All samples were added 1mL of a 1mg/mL C:19 (Nonadecanoic acid, Larodan AB) internal standard diluted in 1:1 v/v solution of diethyl ether and heptane . The solution was then washed trough a 3 mL 500 mg solid phase extraction (SPE) aminopropyl column (Thermo Fisher Scientific Inc) placed in a Vac Elut 20 vacuum manifold (Aglient Technologies, Waldbronn, Germany). Neutral lipids were removed from the column with 20 mL 3:2 v/v of hexane (Merck, Darmstadt, Germany) and 2-propanol (Merck, Darmstadt, Germany). FFA were isolated by washing the column with 5 mL diethyl ether added 2 % formic acid (Merck, Darmstadt, Germany).

The methylation of the FFA were done according to the method used by Ekeberg, IKBM, UMB (Personal communication). The solvent was evaporated using nitrogen gas. The isolated FFA were then methylated with boron trifluoride methanol (BF_3MeOH) in a 70°C water bath for 5 min and added hexane. The upper phase (hexane) was then transferred to the GC vial. FFA were analysed using a wall coated open tubular fused silica capillary column with an inner diameter (id) of 0,25 mm, 50 m length and 0,2 µm film thickness (Varian, Middelburg, The Netherlands) and a Carlo Erba AS V570 flame ionization detector (FID) (Milano, Italy). Helium at 75 kPa was used as carrier gas. The injector temperature was 250 °C and the FID temperature was 300 °C. The sample (1µl) was split with a ratio of 1:25 when injected. The temperature program during the detection of FFA was 60 °C for 3 min, then raised with 10

°C/min to 140 °C and held for 1 min, raised with 10 °C/min to 160 °C and held for 1 min and finally raised with 2 °C/min to 210 °C and held for 15 min.

The rennet clotting properties of the milk from individual goats were analyzed by formagraph (Lattodinamografo, Foss Italy, Padova, Italy) according to the method described by McMahon and Brown (1982). The milk was pasteurised (63°C/30 min) and cooled to 30°C before 10 mL was transferred to the formagraph sample cuvette and incubated at 32°C. The Rennet (200µl CHYMAX, Chr. Hansen A/S, Hørsholm, Denmark) was diluted in acetate buffer (1:50) and added to the milk 30 min after incubation. The samples were analyzed in the formagraph for 30 min at 32°C. The time (min) after addition of rennet before the milk started to coagulate (rct), the firmness (width between the curves in mm) of the coagulum 30 min after the addition of rennet (A_{30}) and the time (min) before the coagulum reached a firmness of 20mm (K_{20}) was measured. All samples were run in triplicates.

The sensory properties of the cheese was analysed after 2 and 4 months. The analysis made was hedonic and the cheese was evaluated for appearance, texture, aroma and taste. The cheese was evaluated on a scale from 0 points; really bad quality, to 5 points; excellent quality. The texture was compared with Gräddost, where the highest score was given a cheese with an open structure. Gräddost was chosen as a standard because of the similarities in the production process. However, the cheese produced in this experiment had in general a softer texture than Gräddost, the structure was less open and the average score would therefore be lower. If the cheese gained a score of 4 or lower, the extent of doughiness was graded from 1 to 5 points. The aroma and taste was not compared to a reference cheese and the grading differed between the individual preferences of the judges on what flavour a good goat cheese should have. Cheese with rancid and tart taste occurred frequently and the cheese was graded for rancidness from 1 to 5 points if it gained a score of 4 or lower for aroma and taste. The cheese was also given an overall score of the total quality of the cheese.

7.4. Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of Minitab® version 16 (MINITAB Inc., State College, PA, USA).

Multivariate data analysis were carried out using Unscrambler® X (CAMO Process AS, Oslo, Norway) using principal component analysis (PCA). A two-way analysis was done with 1; month in lactation and 2; genotype as classification variables. The statistical analysis was performed on data from each ripening step.

8. Results

8.1. Milk composition and coagulation properties

The mean values and standard deviation of milk composition in milk from 00- and 01-goats from the second (March) and third (April) month in lactation are summarized in table 1. The significant effect between different genotypes of goats and month in lactation is shown in the same table. The milk composition is the mean of the milk composition of each goat in each group.

The fat, protein and lactose content were significantly higher in the 01-goats in both months in lactation but was also significantly lower in April than in March for both genotypes.

Table 1 Composition of goat milk from different genotypes (00 and 01) from the second (March) and third (April) month in lactation. Results are given as mean values with standard deviation. Statistical significance of lactation and genotype is listed under the results.

Month in lactation		n	Milk composition (wt%)		
			Fat	Protein	Lactose
March	00-goats	8	5,0±0,66	3,1±0,22	4,8±0,17
	01-goats	12	5,4±0,49	3,5±0,21	4,9±0,18
April	00-goats	8	4,4±0,44	2,8±0,21	4,6±0,14
	01-goats	12	4,6±0,49	3,1±0,19	4,6±0,17
Statistical significance					
Lactation			***	***	***
Genotype			***	***	***

NS: not significant. * P<0,05; ** P<0,01; *** P<0,001
n: number of goats

The mean values and the standard deviation of the coagulation properties in milk from 00- and 01-goats from the second (March) and third (April) month in lactation are summarized in table 2. The significant effect between different genotypes of goats and month in lactation is also shown Table 2.

The time after addition of rennet until the milk started to coagulate (rct) was not significantly different between the genotypes but was significantly faster in April than in March. The firmness of the coagulum 30 min after addition of rennet (A_{30}) was significantly higher in milk from the 01-goats. The time before the coagulum reached a firmness of 20mm (K_{20}) on the formagraph was significantly shorter in the milk from the 01-goats. Both

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genotypes had milk samples that did not obtain a sufficient firmness; in March, 73% of the samples from the 00-goats and 11% from the 01-goats did not reach K_{20} and in April, 75% of the samples from the 00-goats and 29% from the 01-goats did not reach K_{20}. These samples were given a value of 40 min in statistical calculations (Devold, Nordbo et al. 2011).

Table 2 Rennet coagulation properties and composition of goat milk from different genotypes (00 and 01) from the second (March) and third (April) month in lactation. Results are given as mean values with standard deviation. Statistical significance of lactation and genotype is listed under the results.

Month in lactation		n	rct (min)	A_{30} (mm)	K_{20} (min)
March	00-goats	8	11,47±3,02	17,59±3,70	31,57±14,17
	01-goats	12	10,70±1,81	22,06±3,15	11,46±10,66
April	00-goats	8	10,48±2,37	15,98±2,75	31,93±14,29
	01-goats	12	10,51±2,07	19,80±3,31	17,51±14,43
Statistical significance					
Lactation			*	***	NS
Genotype			NS	***	***

NS: not significant. * P<0,05; ** P<0,01; *** P<0,001
n: number of goats
rct: the time after addition of rennet before the milk starts to coagulate
A_{30}: the firmness of the coagulum 30 min after added rennet
K_{20}: the time before the coagulum reaches a firmness of 20mm

8.2. Dry matter content of cheese

The mean value and standard deviation of the dry matter (DM) content in fresh cheese and cheese ripened for 2 and 4 months from two months in lactation are summarized in table 3. The significant effects between different genotypes of goats and months in lactation listed in the same table. In both fresh cheese and in ripened cheese there was a significantly higher dry matter content in the cheese from 01-goats. The cheeses made in April had a significantly higher content of dry matter in fresh cheeses and cheeses ripened for 4 months, however after 2 months of ripening no significant effect of lactation was found.

Table 3 Dry matter (%) in fresh cheese and in cheese matured for 2 and 4 months. Results are given as mean value of two parallel cheese productions with standard deviation. Statistical significance of lactation and genotype is listed under the results.

Month in lactation		Dry matter %		
		Fresh	2 months	4 months
March	00-goats	47,4±0,00	48,2±0,07	47,5±0,07
	01-goats	50,1±0,71	51,3±0,49	50,9±0,49
April	00-goats	48,85±0,49	48,2±0,42	48,3±0,35
	01-goats	51,15±0,35	50,8±0,71	51,4±0,42
Statistical significance				
Lactation		**	NS	*
Genotype		***	***	***

NS: not significant. *P<0,05; **P<0,01; ***P<0,001

8.3. Free amino acid composition

The result of the PCA of the FAA in cheese ripened for 2 and 4 months are presented in figure 2. The first factorial component, which explained 86% of the variation, explained the age of the cheeses with the 4 month old cheeses having the highest content of FFA. However the first component also showed a clear separation between months in lactation for the cheese made from the 00-goats while the cheese made from the 01-goats were randomly mixed between the lactation months. The second component, which explained 7 % of the variation, showed a clear separation of the cheese from the different genotypes of goats. The content of L-asparagine (Asn) was significantly (P<0,01) higher in cheese from 01-goats and the content of L-tryptophan (Trp) was significantly higher in cheese from 00-goats
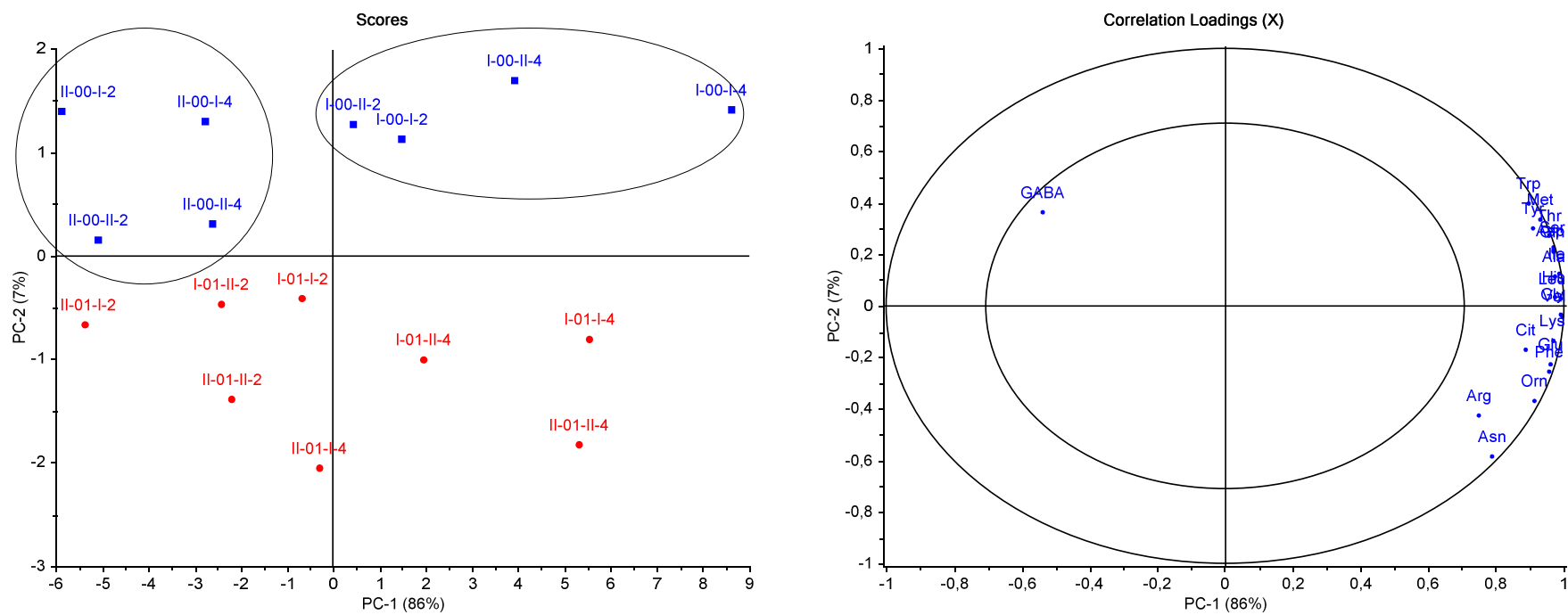
Figure 2 Score plot from the PCA of free amino acids during ripening of cheese from 00-goats and 01-goats. 86% and 7% of the variation is explained by the first two components. The cheeses are labelled according to the month in lactation (I; March and II; April), genotype (00 and 01) and age (2 and 4 months). Left plot: The left circle surround the 00-cheeses made in April. The right circle surround the 00-cheeses made in March. Right plot: Upper circle; L-tryptophan. Bottom circle; L-asparagine.

8.4. Sensory quality

The mean value and standard deviation of the scores from the sensory analysis of ripened cheese from two months in lactation are summarized in table 4. The significant effect of different genotypes of goats and month in lactation listed in the same table. The cheese from 01-goats got a significantly higher texture score while no statistical significance was found of the months in lactation on texture. The grade of doughiness was significantly higher in the cheese from 00-goats and in the cheese made in April. The cheese made from 01-goats got a significantly higher score for aroma and taste while there was no statistical significance between the months in lactation. The cheese made from 00-goats was significantly more rancid while it was no significant effect on months in lactation. The main score of the cheese, with an emphasis on aroma and taste, was significantly higher in the cheese from 01-goats while it was no significant on months in lactation.

Table 4 Sensory scores for cheese matured for 2 months. The cheese was evaluated on a scale from 0 points; really bad quality, to 5 points; excellent quality. Results are given as mean values with standard deviation. Statistical significance of lactation and geotype is listed under the results.

Month in lactation		n	Apperance	Texture	Doughiness	Aroma and taste	Rancidity	Main score
March	00-goats	5	2,7±0,48	2,8±0,79	3,1±0,88	2,9±0,88	2,2±1,40	2,7±0,59
	01-goats	5	3,2±0,42	3,1±0,76	2,7±0,95	3,2±0,79	1,4±0,70	3,0±0,55
April	00-goats	4	3,5±0,76	3,0±0,76	4,6±0,52	2,6±0,44	2,13±0,99	2,7±0,46
	01-goats	4	3,1±0,83	3,8±0,70	3,3±0,70	3,6±0,52	1,1±0,35	3,7±0,59
Statistical significance								
Lactation			NS	NS	**	NS	NS	NS
Genotype			NS	*	*	*	**	*

NS: not significant. *P<0,05; **P<0,01; ***P<0,001
n: number of judges

The PCA of the sensorial analysis is shown in figure 3. The first factorial component, which explained 64% of the variation, showed that cheese made from 00-goats had a more rancid taste and doughy texture than cheese made from 01-goats. The second component, which explained 20% of the variation, showed a clear separation between the months in lactation for the cheese made from 00-goats while the cheese made from 01-goats were randomly mixed between the lactation months.
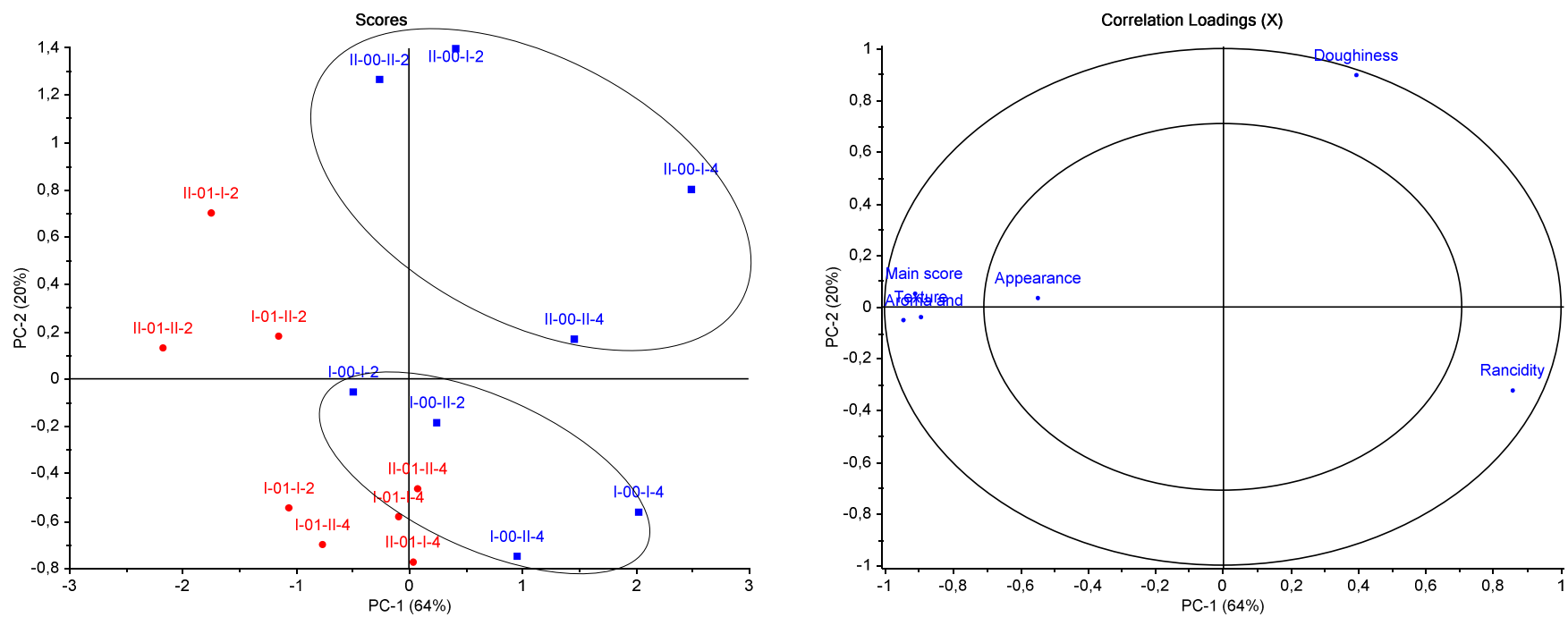
Figure 3 Score plot from the PCA of sensory scores during ripening of cheese from 00-goats and 01-goats. 64% and 20% of the variation is explained by the first two components. The cheeses are labelled according to the month in lactation (I; March and II; April), genotype (00 and 01) and age (2 and 4 months). The upper circle surrounds 00-cheese made in April. The bottom circle surrounds 00-cheese made in March.

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9. Discussion

Until now some studies of the cheese making properties (composition of milk, renneting properties, syneresis and whey drainage) of the milk from different genotypes of Norwegian goats have been made but this is the first study that actually examines the final product, cheese (Vegarud, Devold et al. 1999; Ådnøy, Vegarud et al. 2003; Devold, Nordbo et al. 2011). The advantage of studying the influence of the goat genotypes on the final product, is that the biochemical processes during ripening will change the cheese over time (Fox, Guinee et al. 2000). Cheeses made from milk with different composition and properties will therefore ripen in different manners, which will influence the quality of the final product.

As previously shown the coagulation properties (K_{20} and A_{30}) of milk from goats with genes that code for of α_{S1}-CN was in this study significantly better than of the milk from goats with double deletion gene for α_{S1}-CN (Ambrosoli, di Stasio et al. 1988; Vegarud, Devold et al. 1999; Clark and Sherbon 2000; Ådnøy, Vegarud et al. 2003; Devold, Nordbo et al. 2011). However, the time until the milk started to coagulate after addition of rennet (rct) was the same for milks from the two genotypes investigated in this present experiment. All previously published results have shown the same tendency of the influence of α_{S1}-CN on K_{20} and A_{30}, while both longer and shorter rct has been observed (Ambrosoli, di Stasio et al. 1988; Vegarud, Devold et al. 1999; Devold, Nordbo et al. 2011). Clark and Sherbon (2000) found that the content of dry matter, not fat solids (SNF) and protein were more highly correlated with good coagulation properties than high amounts of α_{S1}-CN. This study, in accordance with the present study, also showed a higher content of fat, protein and lactose in the milk from goats with genes coding for α_{S1}-CN. Since the analysis of casein composition in the milk and cheese in the present study had to be postponed it is difficult to conclude if the composition of the milk or the level of α_{S1}-CN in the milk had most impact on the coagulation properties. The casein composition will be analysed with capillary electrophoresis during the spring 2012.

The age of the experimental goats may influence the milk composition. The milk yield will increase with age and the milk composition will most likely differ between goats of different age, the group with double deletion gene for α_{S1}-CN had an average age of 3,5 years while it was 2 years for the group with genes coding for α_{S1}-CN.

The content of FAA in the ripened cheese can be used as a measure of the rate of ripening. FAA are products of proteolysis in cheese (Fox, Guinee et al. 2000). Proteolysis is considered to be the most important biochemical event that occurs in cheese during ripening. It is

responsible for textural changes and is also a major contributor to the flavour development of the cheese. In cheeses made of milk from the goats with genes coding for α_{S1}-CN the cheese ripened for 4 months had as expected a higher content of FAA than cheese ripened for 2 months. This was not the case for the cheese made of milk from goats with a double deletion gene for α_{S1}-CN. The cheeses made in March, independently of age of ripening, had a higher content of free amino acids than the cheese made April. These results indicate that the milk from goats with genes coding for α_{S1}-CN had a more stabile quality during lactation than the milk from goats with the double deletion gene for α_{S1}-CN.

The result also shows that the composition of the FAA in the ripened cheeses was different in the cheese from different genotypes. This is most likely explained by different casein composition of the cheeses.

The cheeses made of milk from goats with a double deletion gene for α_{S1}-CN had a lower dry matter content, which implies that the cheese matrix was trapping more water. The result could be explained by the presence of larger casein micelles in the milk with a low content of α_{S1}-CN (Pierre, Michel et al. 1998; Pierre, Michel et al. 1999; Tziboula and Horne 1999). In the surveys of the Norwegian genotypes of goats made by Ådnøy et al. (2003) and Devold et al. (2011) it was found that the goats with a double deletion gene for α_{S1}-CN had larger casein micelles than the other α_{S1}-CN genotypes.

The interaction between the serine-phosphate groups in α_{S1}-CN and calcium ions (Ca^{2+}) in milk is strong and this interaction contribute, along with hydrophobic bonds between the caseins, to the formation of the casein micelle (Walstra, Wouters et al. 2006). Compared to bovine milk, goat milk has a high content of β-CN (Mora-Gutierrez, Kumosinski et al. 1997). β-CN favours the formation of highly hydrated casein micelles. The β-CN is also very voluminous and hydrophobic and probably leads to entrapment of water in the increased spatial structure of the casein micelle (Mora-Gutierrez, Kumosinski et al. 1997). Mora-Gutierrez et al. (1997) found high amounts of β-CN in both goat milks with high and low content of α_{S1}-CN compared to bovine milk.

Tziboula and Horne (1999) found that the lack of α_{S1}-CN in the low α_{S1}-CN milk was not counterbalanced by an increase in the content of the other caseins. But the relative amounts of the caseins changed with a high % of β-CN relative to the other caseins in the low α_{S1}-CN milk. Pierre et al (1999) found that the relative content of β-CN in the low α_{S1}-CN milk was 59% of the total casein, compared to 48% in the high α_{S1}-CN milk. It also appears like the low content of α_{S1}-CN in the milk to some extent was counterbalanced with a higher relative amount of α_{S2}-CN (Pierre, Michel et al. 1999; Tziboula and Horne 1999). In theory α_{S2}-CN

would lead to an even more compact structure of the casein micelle than α_{S1}-CN by having more serine-phosphate groups (Walstra, Wouters et al. 2006).

The casein composition of the milk from Norwegian goat genotypes has not been surveyed, but will as previously mentioned, be analysed in the near future. This will be interesting especially since studies from different countries shows that there probably are differences in the casein composition between the high α_{S1}-CN genotypes and the low α_{S1}-CN genotypes in the different countries and breeds (Mora-Gutierrez, Kumosinski et al. 1997; Pierre, Michel et al. 1999; Tziboula and Horne 1999).

The cheese made in this study was not made as a commercial product for the market and got in general a low score in the sensorial evaluation. But either way, there was a significant difference between the genotypes. The most distinguished characteristic was the rancid taste of the cheese made of milk from goats with the double deletion gene for α_{S1}-CN. The frequent rancid and tart flavour of Norwegian goat milk (Eknæs and Skeie 2006) results from the lipolysis of milk fat into FFA (Collins, McSweeney et al. 2003). The Long chain fatty acids (LCFA) ($> C_{12:0}$) have a high perception threshold and are considered to be minor contributors to the cheese flavour (Molimard and Spinnler 1996). While the short and intermediate chain fatty acids ($C_{4:0}$-$C_{12:0}$) have a low perception threshold and will contribute to the characteristic flavour of a cheese. Caprylic acid ($C_{8:0}$) and branched capric acid ($C_{10:0}$) are most probably the main contributors to the goat like flavour in goat milk and both caprylic and butyric ($C_{4:0}$) acid can give a rancid and tart taste (Chilliard, Ferlay et al. 2003; Collins, McSweeney et al. 2003). The short chain fatty acids can either contribute positive to the flavour of the cheese or to a rancidity defect depending on the concentration and perception threshold.

Chilliard et al. (2006) analysed the fatty acid (FA) content of goat milk from high and low α_{S1}-CN genotypes and found that the high α_{S1}-CN genotype had a significant (P<0,01) higher content of $C_{8:0}$ and $C_{10:0}$ in fresh milk. This result contradicts the more pronounced rancid taste in the cheese made of milk from goats with double deletion gene for α_{S1}-CN in the present study. But during cheese manufacture and ripening the rate of lipolysis is provoked and more FFA will be released (Fox, Guinee et al. 2000).

Delacroix-Buchet et al. (1996) studied the influence of high and low α_{S1}-CN genotypes on the sensorial properties of cheese. They found a higher lipase activity and a higher content of total FFA in the milk with low α_{S1}-CN. The cheese made from the same milk had a less firm texture and a more pronounced goat flavour than cheese made from milk with high α_{S1}-CN content. This is in accordance with observations made in the present experiment, where the sensory analysis showed, like the analysis of FAA, a clear difference between the quality of

the cheese, made of milk from goats with double deletion gene for α_{S1}-CN, in March and April. While the quality of the cheese made of milk from the high α_{S1}-CN variant was rather constant. Soryal et al. (2005) studied the effect of goat breed on the sensory quality of soft cheese during 6 months (May-October) of lactation. The goat breeds were Alpine, with a low content of casein, and Nubian, with a high content of casein. The cheese from Alpine milk showed a significantly lower flavour and texture score in May and August while the Nubian cheese had no significant changes during lactation.

The analysis of FFA of fresh and ripened cheese had to be postponed because of problems with the GC, the samples will be analysed as soon as the instrument is repaired. These results will hopefully contribute to a better understanding of the difference in quality between the cheeses from the α_{S1}-CN genotypes.

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PAPER II

Effect of supplementation of saturated and unsaturated fat in the goat diet on the quality of cheese milk and cheese quality

Lise Brunborg Jakobsen

Effect of supplementation of saturated and unsaturated fat in the goat diet on the quality of cheese milk and cheese quality

Abstract

Due to a high frequency of off-flavours and an unfavourable composition a great deal of the Norwegian goat milk has been poor for production of cheese. This has led to a large quantity of discarded milk. Different diets can affect the quality of the milk and especially a supply of fat in correct proportions in the diet has shown to contribute to an increase in quality. The objective of this study was to investigate the effect of diets added a surplus of saturated or unsaturated vegetable fat on the cheese making properties of the goat milk and on ripened cheese. The cheese making was standardized so that the cheese was comparable from production to production with only the feed as an experimental factor. The study included analysis of free fatty acids (FFA), free amino acids (FAA), organic acids, dry matter (DM), microbiology, pH and sensorial analysis. In addition coagulation properties (formagraph) and composition of milk from each goat was analysed one week after each cheese making. The fat content of the milk was higher in the groups that were fed a supply of saturated and unsaturated fat. The milk from goats fed a supply of unsaturated fat had a higher protein content then the other groups. The cheese from the control group had best sensorial attributes and the cheese from the group fed a supply of saturated fat had a high abundance of rancid taste. The cheese from the group fed a supply of unsaturated fat appeared to ripen slower than the other cheeses.

Sammendrag

På grunn av en høy frekvens av harsk og besk smak og en uegnet sammensetning av den norske geitmelka har den vært dårlig egnet for ysting. Dette problemet har ført til at mye melk har blitt kassert. Ulike dietter har vist seg å kunne påvirke melkekvaliteten, spesielt har dietter med et tilskudd av fett i riktige proporsjoner ført til en økt kvalitet. Hensikten med denne studien var å undersøke effekten av dietter med et tilskudd av umettet og mettet vegetabilsk fett på melkas ysteegenskaper og på modnet hvit geitost. Ysteprosessen var standardisert slik at osten skule bli sammenlignbar fra produksjon til produksjon uten andre eksperimentelle

faktorer enn genotype. Studien inkluderte analyser av frie fettsyrer (FFA), frie aminosyrer (FAA), organiske syrer, karbohydrater, tørrstoff, mikrobiologi, pH og sensorisk bedømmelse. I tillegg ble koaguleringsegenskaper (formagraf) og sammensetning av ystemelka fra hver enkelt geit analysert. Fettinnholdet i melka fra geiter med et tilskudd av fett i dietten var høyere enn i melka fra geiter fôret med kontrolldiett. Melk fra geiter med et tilskudd av umetta fett i dietten hadde et høyere innhold av protein. Ost laget av melk fra geitene fôret med kontroll fôr hadde best sensoriske egenskaper og ost fra geitene som ble fôret et tilskudd av metta fett hadde høy forekomst av harsk smak. Ost fra geitene som ble fôret et tilskudd av umetta fett synes å modne seinere enn de andre ostene.

10. Introduction

The production of goat milk has a long tradition in Norway. The milk was essentially used for production of whey cheese. Whey cheese is not a cheese by definition but is made of concentrated whey added milk and cream in the final processing step (CodexAlimentarius 1971; CodexAlimentarius 1978). The goat stock has traditionally been breed towards a high milk yield and a strong goaty flavour of the milk. This particular taste results from lipolysis of the milk fat into free fatty acids (FFA). The short (SCFA) and medium (MCFA) chain FFA ($C_{4:0}$-$C_{12:0}$) have a low perception threshold and octanoic acid ($C_{8:0}$) is most probably the main contributor to the goat like flavour (Collins, McSweeney et al. 2003). Both octanoic and butanoic ($C_{4:0}$) acid will also contribute to a rancid and tart taste of the milk (Collins, McSweeney et al. 2003). The goaty flavour can be a negative characteristic if it is too dominant but in the brown whey cheese the sweet taste will to some extent balance the goaty flavour.

In recent years the demand for rennet coagulated goat milk cheese has been increasing in Norway. Cheeses made from goat milk are imported, but both small-scale cheese farmers as well as TINE SA have started to produce goat milk cheese for the Norwegian market.

The goat milk produced in Norway has been of varying quality and rancid and tart taste as well as bad coagulation properties has been a major problem for the cheese producers. This problem has led to between 10 and 50 % discarded goat milk and thereby lack of goat milk for cheese production (Skeie, Personal communication).

The quality of the goat milk varies trough the lactation and between different stocks (Fekadu, Soryal et al. 2005; Soryal, Fekadu et al. 2005; Eknæs and Skeie 2006).

Recent research has shown high frequencies of goats with a double deletion gene (00-goats) for α_{S1}-casein in the Norwegian stocks (Vegarud, Devold et al. 1999; Ådnøy, Vegarud et al. 2003; Devold, Nordbo et al. 2011).

 Since 2008 the direction of breeding at The University of Life Science in Ås, Norway, has favoured goats which produce high quality milk for rennet coagulated cheese. These goats are denoted as 01-goats. The characteristics of the bucks used for breeding is genes that code for alpha$_{S1}$-CN. Research has shown a correlation between high amounts of good quality alpha$_{S1}$-CN in goat milk and good properties for cheese making (Ambrosoli, di Stasio et al. 1988; Clark and Sherbon 2000; Chilliard, Rouel et al. 2006).

Although breeding seems to be the long-term solution to the problem, different diets have been proven to contribute to a better quality of the milk as a short-term solution (Morand-Fehr 2005; Eknæs and Skeie 2006; Morand-Fehr, Fedele et al. 2007; Eknæs, Havrevoll et al. 2009).

Morand-Fehr (2005) reviewed the recent developments in goat nutrition and stated that the quality of the cheese flavour and the quality of the cheese coagulum tended to be reduced when the milk protein content was lower than the fat content. These quality defects were observed when the diets of the goats lacked fat or fibre. Balancing the fat or fibre content of the diet may increase the fat content of the milk and reduce these defects.

Morand-Fehr (2005) also claimed that in contrast to dairy cows, a fat supply in correct proportions did not reduce the protein content of the goat milk. Then the fat supply should then not be too rich in unsaturated fatty acids and be limited to less than 5% of the dry matter (DM) content of the diet.

Chilliard, Ferlay et al. (2003) compared different studies of fat supply to lactating goats. The studies included supplements of saturated FFA, in the form of calcium salts or triglycerides, animal fat, vegetable oils rich in C18:1, C18:2 or C18:3, both free and encapsulated and whole, crushed, extruded or formaldehyde-treated oilseeds. In all the experiments the content of fat in the milk increased significantly (+ 5,7 g/kg in average of 23 supplemented groups). Even the milk fat content from goats fed vegetable oils rich in polyunsaturated FA as a supply to a low-forage diet increased, which is contrary to what has been clearly observed in dairy cows.

Eknæs and Skeie (2006) studied the effect of feeding low and high amounts of concentrate to Norwegian goats. The two groups were divided in one group grazing freely while the rest had limited periods of roughage restriction and free supply of hay. The goats grazed on cultivated pasture in the spring and on mountain pasture during the summer. Milk from the goats with roughage restriction and free supply of hay had a higher fat content and a lower

content of FFA than the milk from goats that grazed freely. However, during the mountain grazing a low level of concentrate led to more pronounced off-flavours in the milk. The former typical Norwegian goat management with low levels of concentrate and free access to pasture gave the milk with strongest off-flavours. However Dønnem, Randby et al. (2011) observed that increasing the energy intake and the energy balance during the first four months of lactation did not reduce the FFA content in goat milk.

Eknæs et al. (2009) studied the effect of feeding concentrates with a supplement of unsaturated and saturated fat sources during the grazing season (June-October) on Norwegian goats. The results revealed that saturated long chain fatty acids (LCFA) reduced the frequency of rancid and tart flavour of the milk. The authors suggested that the positive effect of a supply of saturated LCFA in the diet was due to a direct effect on the fatty acid composition in the milk. In addition they proposed that the higher content of $C_{16:0}$ in the milk could have improved the stability of the milk fat globule membrane (MFGM) and thereby decreased the level of lipolysis and the tart and rancid flavour.

Several publications have been made on the effect of different diets on the sensorial and nutritional properties of goat milk cheese as well as on the fatty acid profile of the cheese. However, very few publications have covered the effect of feeding of the goats on the cheese making properties of the milk (Rouel, Gaborit et al. 2002; Soryal, Fekadu et al. 2005).

The objective of this study was to investigate the effect of diets added a surplus of saturated or unsaturated vegetable fat on the cheese making properties of the goat milk. The cheese making was standardized so that the cheese was comparable from production to production with only the feed as an experimental factor. The cheese was semisoft and ripened in plastic foil. An aromatic mesophilic DL starter culture was used and *Lactobacillus casei* was added as an adjunct culture to accelerate ripening. The cheese was analysed one day after production, and after 2 and 4 months. The study included analysis of FFA, FAA, organic acids, dry matter, microbiology, pH and sensorial analysis.

11. Materials and methods

11.1. Animals and diets

The experimental animals were divided in three groups of 10 goats. All goats were of the ordinary Norwegian dairy goat breed. The goats were divided between the different groups as to create three groups with the same average properties (body mass index, age, lactation years, genotype of α_{S1}, milk yield, date of expected kidding and weight) as to create three

groups, in each group 3 of the goats were 00-goats and 7 were 01-goats. All goats were fed grass silage and a control concentrate the first 60 days after kidding. The control concentrate was a glycogenic concentrate that consisted mainly of barley added sugar beet cuts and soy and rapeseed flour. In the rest of the experimental period, one of the groups continued on the control concentrate while one group was fed a concentrate with a supplement of saturated fat and one group a supplement of unsaturated fat. In this experiment the goats were fed the same diet during the whole experimental period and there were no interchange between the groups. Table 1 shows the composition of the different concentrates. The feed with saturated fat consisted of somewhat less barley than the control concentrate and was added Akofeed Giant 60 Veg fat as the source of saturated fat. The concentrate with unsaturated fat did also consist of less barley and was added rapeseed oil as the source of unsaturated fat. During the indoor period the goats were additionally fed grass silage. During the mountain period the goats had free supply of mountain pasture.

Table 1 Composition of raw materials (wt%) in the three different concentrates.

	Control	Saturated	Unsaturated
Barley	54	46	46
Rapeseed flour, expro 00SF	9	9	9
Soy flour, extracted	16	18	18
Sugar beet cuts	12	10	10
Molasses	5	5	5
Akofeed Giant 60 Veg fat	-	8	-
Rapeseed oil	-	-	8
Mineral and vitamin premix	4	4	4

11.2. Manufacture process

The Goats were kidding during February 2011 and the first production of cheese was made in the fourth month in lactation (May, week 19) approximately 60 days after the new experimental feeding was applied. The second production was made in the fifth month in lactation (June, week 23). Two parallel cheese productions were carried out during these weeks with one day between each cheese making. Milk was collected in the evening two days ahead of the cheese making day, morning and evening the day before and in the morning the same day as cheese making.

In addition a third cheese making was performed in the end of the mountain pasture in the seventh month in lactation (August, week 33). Milk for the cheese produced at the mountain farm was collected in the evening the day before and in the morning the same day as cheese making.

The cheese made was a semi-soft cheese, acidified with CHN19 from Chr. Hansen A/S and added *Lb. casei* as an adjunct culture, ripened in plastic foil. The complete manufacturing process was made as described by Jakobsen (2011).

11.3. Sampling, measurements and analysis

Samples were collected from fresh cheese and from cheese ripened for two and four months. Microbiological analysis, dry matter, pH and analysis of organic acids was analysed and measured at the time of sampling. Samples for analysis of FFA and FAA were stored at -20°C for later analysis. The methods used for the analysis are described by Jacobsen (2011).

11.4. Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of Minitab® version 16 (MINITAB Inc., State College, PA, USA). A two-way analysis was done with 1; month in lactation and 2; diets as classification variables. The statistical analysis was performed on data from each ripening step. Multivariate data analysis was carried out using Unscrambler® X (CAMO Process AS, Oslo, Norway) using principal component analysis (PCA).

12. Results

12.1. Milk composition and coagulation properties

Mean values and standard deviation of milk composition in milk from goats given diets with different fat sources (control, saturated and unsaturated) from the fourth (May), fifth (June) and seventh (August) month in lactation is summarized in table 2 with the significant effects between different diets and months in lactation listed in the same table. The milk composition is the mean of the individual composition of the milk from each goat in each group. The fat content of the milk was significantly influenced by the fat composition of the diet with the highest content in milk from goats fed saturated fat and lowest in the milk from

the goats fed control diet. The fat content was also significantly influenced by month in lactation with the highest content in May and the lowest in August. The protein content of the milk was significantly influenced by the fat composition of the diet with a higher content in milk from goats fed unsaturated fat than in milk from goats fed the control diet or the diet with saturated fat. The protein content was also significantly influenced by month in lactation with the highest content in August and the lowest in June. The lactose content was significantly influenced by the fat composition of the diet with the highest content in milk from goats fed unsaturated fat and the lowest in the milk from goats fed saturated fat. The content of lactose was significantly influenced by month in lactation with a higher content in May and June than in August.

Table 2 Composition of goat milk from goats given different diets (C, U and S) from the fourth (May), fifth (June) and seventh (August) month in lactation. Results are given as mean values of individual measurements of the goats in each group with standard deviation. Statistical significance of lactation and diet is shown under the results.

Month in lactation		n	Milk composition (wt%)		
			Fat	Protein	Lactose
	Control	10	3,6±0,47	2,8±0,12	4,3±0,12
May	Unsaturated	11	4,3±0,61	2,9±0,31	4,5±0,27
	Saturated	10	4,4±0,76	2,8±0,17	4,3±0,21
	Control	10	3,1±0,47	2,7±0,16	4,3±0,20
June	Unsaturated	11	3,6±0,44	2,8±0,27	4,5±0,23
	Saturated	10	3,9±0,84	2,8±0,17	4,3±0,29
	Control	10	3,3±0,52	3,2±0,15	4,1±0,20
August	Unsaturated	11	3,9±0,62	3,2±0,27	4,1±0,40
	Saturated	10	3,9±0,62	3,1±0,21	4,0±0,27
Statistical significance					
Lactation			***	***	***
			M>A>J	A>M>J	JM>A
Diet			***	***	***
			S>U>C	U>CS	U>C>S

NS: not significant. * P<0,05; ** P<0,01; *** P<0,001
n: number of goats

Mean values and standard deviation of the coagulation properties of milk from goats fed diets with different fat sources (control, saturated and unsaturated) from the fourth (May), fifth (June) and seventh (August) month in lactation are summarized in table 3. The significant effect between different diets and months in lactation listed in the same table.

The time after addition of rennet until the milk started to coagulate (rct) was not significantly influenced by the different diets but was significantly influenced by the month of lactation. Milk produced in August had a longer onset (rct) of gelation than milk produced in May and June. The firmness of the coagulum 30 min after the addition of rennet (A_{30}) was significantly influenced by the fat composition of the diet and was higher in the milk from the goats fed unsaturated fat than in milk from goats fed saturated fat or the control diet. A_{30} was significantly influenced by the month of lactation and was higher in August than in May and June. The time before the coagulum reached a firmness of 20mm (K_{20}) on the formagraph was significantly influenced by the fat composition of the diet and was shorter in the milk from goats fed unsaturated fat than milk from goats fed saturated fat. K_{20} was significantly influenced by the month of lactation and was shorter in August than in May and June.

Table 3 Rennet coagulation properties of goat milk from different diets (C, U and S) from the fourth (May), fifth (June) and seventh (August) month in lactation. Results are given as mean values with standard deviation. Statistical significance of lactation and diet is listed under the results.

Month in lactation		n	rct (min)	A30 (mm)	K20(min)
	Control	10	10,53±1,46	15,69±5,07	29,83±14,49
May	Unsaturated	11	10,11±2,65	17,98±5,47	23,09±16,04
	Saturated	10	10,08±3,56	15,67±4,15	25,21±16,85
	Control	10	9,16±1,16	15,26±5,38	24,62±16,29
June	Unsaturated	11	9,55±2,18	17,96±5,49	21,33±16,65
	Saturated	10	9,78±2,93	16,86±6,11	27,77±16,40
	Control	10	13,06±3,54	21,47±6,64	16,29±15,32
August	Unsaturated	11	12,63±4,28	20,54±7,46	18,17±16,69
	Saturated	10	15,54±6,17	19,08±10,17	22,01±17,12
Statistical significance					
Lactation			***	***	**
			A>MJ	A>JM	MJ>A
Diet			NS	**	*
				U>SC	S>U

Rct: the time after addition of rennet before the milk starts to coagulate
A_{30}: the firmness of the coagulum 30 min after added rennet
K_{20}: the time before the coagulum reaches a firmness of 20mm.
NS: not significant. * P<0,05; ** P<0,01; *** P<0,001
n: number of goats

Within milk from goats fed all the different diets there were some samples that did not reach a sufficient firmness (table 4). These samples were given a value of 40 min (Devold, Nordbo et al. 2011). There was highest abundance of samples that did not achieve K_{20} in May, a bit fewer in June for all diets, and in August there were even less. The best progress was in the milk samples from the control group.

Table 4 Proportion of milk samples (given in %) in each diet in which gel strength (K_{20}) were insufficient during the formagraph measurement.

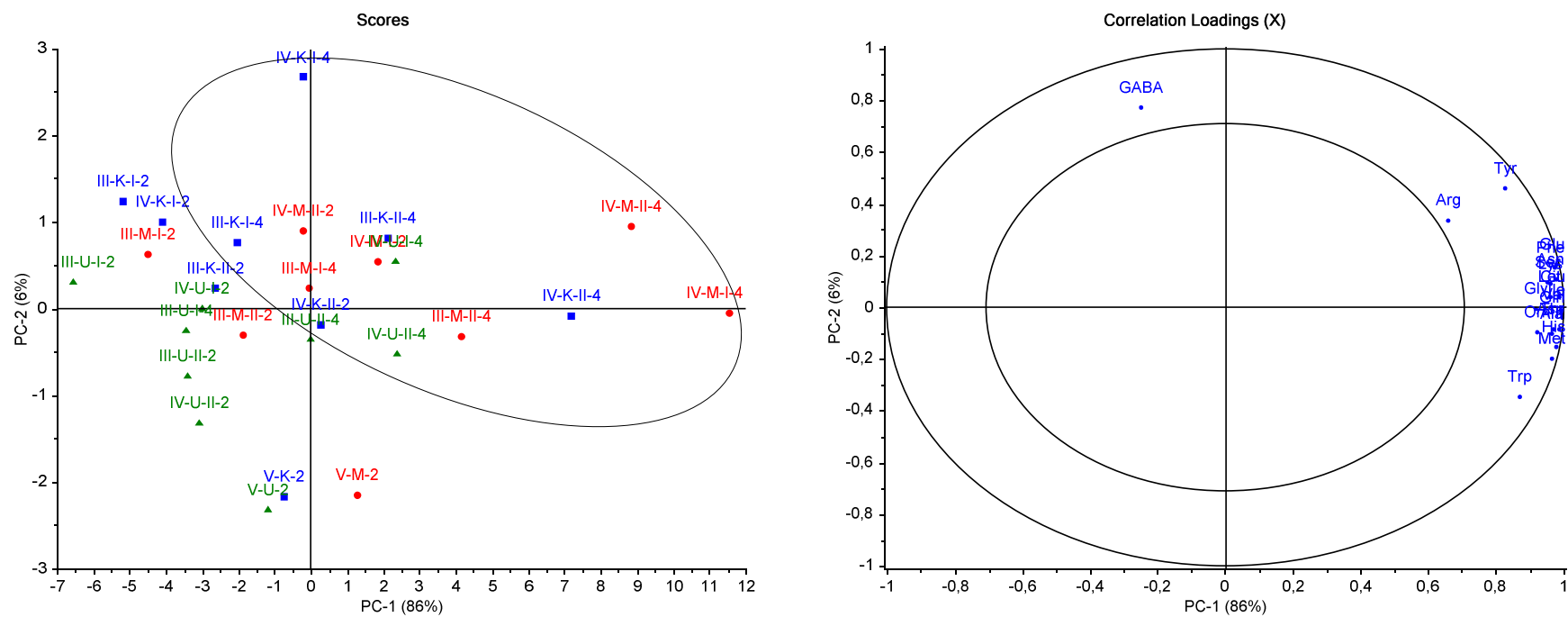| Month in lactation	Control	Unsaturated	Saturated
May	65%	45%	55%
June	52%	43%	63%
August	29%	36%	46%

Figure 1 Score plot from the PCA of free amino acids during ripening of cheese from goats given diet with different fat sources. 86% and 6% of the variation is explained by the first two components. The cheeses are labelled according to the month in lactation (III;May, IV;June and V;August), diet (C;control, U;unsaturated and S;saturated) and age(2 and 4 months). The cheeses are coloured according to diet (Blue;control, Green;unsaturated and Red;saturated). The circle surround the cheeses matured for 4 months.

12.2. Microbiology

The results of the microbiological analysis did only show significant effect of the different diets on cheese ripened for 4 months. The amounts of *Lactococcus* spp. was significantly higher in the cheese made of milk from the goats fed the control diet and the diet with unsaturated fat than in the cheese made of milk from the goats fed the diet with saturated fat.

12.3. Free amino acid composition

The PCA result from the analysis of FAA in cheese ripened for 2 and 4 months is shown in figure 1. The first and second factorial components, which explained 86% and 6% of the variation, showed that there is a lower abundance of free amino acids in cheese made from the milk of goats given a diet with a supply of unsaturated fat. When a circle is drawn around all cheeses that are ripened for 4 months, almost all cheeses ripened for 2 months from the saturated and control group is included while almost all the cheese ripened for 2 months from the unsaturated group are left outside.

12.4. Sensory quality

The PCA result from the sensorial analysis is shown in figure 2. The first and second factorial components, which explained 56% and 17% of the variation, showed that there was a high abundance of rancid taste in the cheese made from goats given a diet with a supply of saturated fat. Several of the cheeses from goats given the control diet obtained a high main score and a high score on flavour and texture. Even tough the PCA shows tendencies in the results from the sensorial evaluation there were no significant effect of the different diets on the sensory scores.
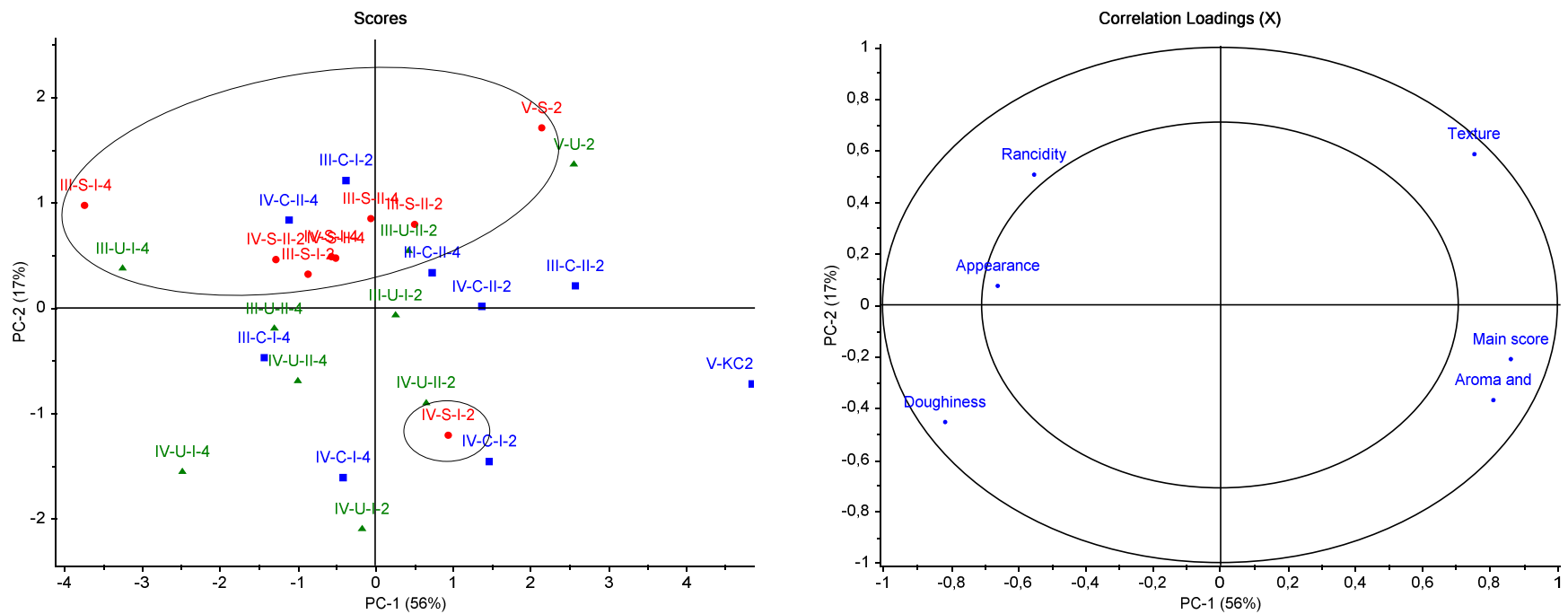
Figure 2 Score plot from the PCA of sensory scores during ripening of cheese made from the milk of goats given diet with different fat sources. 56% and 17% of the variation was explained by the first two components. The cheeses are labelled according to the month in lactation (III;May, IV;June and V;August), diet (C;control, U;unsaturated and S;saturated) and age (2 and 4 months). The cheeses are coloured according to diet (Blue;control, Green;unsaturated and Red;saturated). The circles surround the cheeses from the unsaturated group.

13. Discussion

Several studies have been made on the effect of different diets on the composition and quality of goat milk, but few studies have investigated the cheese making properties and the influence of the milk quality on the ripened cheese made thereof. Most of the goat milk produced is manufactured into cheese. The advantage of studying the final product is that the biochemical processes during ripening will change the cheese over time (Fox, Guinee et al. 2000). Cheeses made from milk with different composition and properties will therefore develop in different manners during ripening, which will again influence the quality of the final product.

The fat content of the milk was as expected higher in milk from goats given a supply of fat in the diet regardless of its saturation (Chilliard, Ferlay et al. 2003). Further the milk from goats given a supply of saturated fat had a significantly higher fat content than the milk from the goats feed unsaturated fat.

Contrary to the present study, Eknæs et al. (2009) found no significant effect on the content of milk fat, protein or lactose by feeding Norwegian goats concentrates with unsaturated and saturated fat supplements during the grazing season (June-October).

In the present study, milk from goats given a supplement of unsaturated fat had the highest protein content compared to milk from the two other groups. Sanz Sampelayo et al. (2002) studied the effect of concentrates with different contents of protected fat rich in poly unsaturated fatty acids (PUFA) on the composition of the goat milk and found, in contrast to the present study, no significant effect on the fat, protein or lactose content. Morand-Fehr (2005) claimed in his review that in contrast to dairy cows, a fat supplement in correct proportions did not reduce the protein content of the goat milk. But then the fat supplement should, in contrary to the results of the present study, not be too rich in unsaturated fatty acids.

The milk fat content was highest at the start of the experimental period (May, mid lactation) and the protein content was highest in the end of the experimental period (August, late mid lactation). The content of milk fat is always high after kidding and decreases during the major part of lactation. This is both due to the increase in milk yield until the lactation peak and decrease in fat mobilization for mammary lipid synthesis (Chilliard, Ferlay et al. 2003). The high content of protein in the milk in August could be due to the mountain pasture. Eknæs et al. (2009) also observed a higher milk protein content at mountain pasture compared to the period of indoor feeding. This could be explained by a favourable composition of nutrients in the mountain pasture.

The results of the present study also showed that the milk had better coagulation properties (rct, A_{30}, K_{20}) in August and these were also better in the group fed a supply of unsaturated fat. These results are most likely related to the higher milk protein content in August and in the group fed a supply of unsaturated fat.

The cheeses from the control group appear to have obtained the best score in the sensorial evaluation and the cheese from the group fed a supplement of saturated fat appear to have a high abundance of rancid taste. Rouel et al. (2002) studied the effect of lipid supplementation of oleic sunflower and linseed oil on milk fatty acid composition and sensory characteristics of different types of cheese. The supplement of linseed oil decreased the goat flavour of fresh cheeses and the supplement of oleic sunflower oil, decreased the pungent flavour of ripened cheeses. These results contradict the results of the present study but as there were no significant effects of the diets on sensory quality in the present study, no concluding remarks can be done.

The content of FAA in the ripened cheese is in the present study principally a measure of the rate of ripening. FAA are products of proteolysis in cheese (Fox, Guinee et al. 2000). Proteolysis is considered to be the most important biochemical event that occurs in the cheese during ripening. It is responsible for textural changes and is a major contributor to the flavour of the cheese. The cheese made of milk from goats given a diet with a supply of unsaturated fat appeared to have a slower proteolysis and thereby a later ripening than the cheese from the other groups. The main proteolytic enzymes in cheese with no surface ripening comes from the lactic acid bacteria (Fox, Guinee et al. 2000). In the present study the cheese made from milk from the goats fed the control diet and the diet with a supplement of unsaturated fat had a higher content of *Lc.* Spp in the ripened cheese than the cheese made from the milk of goats fed saturated fat. The tendency of a slower ripening of the cheese from the unsaturated group is therefore likely related to other factors. At present there is no studies on the effect of different diets on the rate of ripening in cheese.

The analysis of FFA of fresh and ripened cheese had to be postponed because of problems with the GC, the samples will be analysed as soon as the instrument is repaired. This analysis will probably give the most important results in this study and will hopefully contribute to a better understanding of the difference in quality between the cheeses from goats fed diets with different supplements of fat. In a study by Eknæs et al. (2009) it was shown that the content of SCFA ($C_{6:0}$-$C_{14:0}$) was significantly lower and the content of LCFA ($C_{16:0}$-$C_{18:3}$) was significantly higher in the milk from goats fed a supply of saturated LCFA.

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PAPER III

Development of a semi-hard blue veined cheese of goat milk

Lise Brunborg Jakobsen

Development of

a semi-hard blue veined cheese of goat milk

Abstract

With an increasing quality of the goat milk in Norway there is a market for developing new products. A hindrance in the development of new cheese varieties has been poor cheese making properties of the milk and a frequent rancid flavour. The taste defect is a result of lipolysis of milk fat into free fatty acids (FFA). Blue veined cheese, ripened with *Penicillium roqueforti*, gain a high pH, which will to some extent neutralize the FFA and cover up the taste defects of the milk. The objective of this study was to develop a recipe for a semi-hard cheese of goat milk ripened with *P. roqueforti*. The sensory quality of the cheese was satisfactory but the interior structure was too dense and hindered a proper development of *P. rogueforti*. Further development of the cheese making technology is required to create the desired structure.

Sammendrag

Med en økende melkekvalitet er det et marked for å utvikle nye produkter av geitmelk i Norge. Et hinder for utvikling av nye ostetyper av geitmelk har vært melk med dårlige ystingsegenskaper og en hyppig forekomst av harsk smak. Smaksdefekten er et resultat av lipolyse av melkefett til frie fettsyrer (FFA). Blåmuggost, som er modnet med *Penicillium roqueforti*, oppnår en relativt høy pH som vil til en viss grad nøytralisere FFA. Nøytraliseringen kan dermed dekke over noe av smaks defekten. Hensikten med denne studien var å utvikle en resept for en semi-hard hvit geitost modnet med *P. Roqueforti*. Den sensoriske kvaliteten av osten var tilfredsstillende men den indre strukturen ble for tett og hindret en tilstrekkelig utvikling av *P. Roqueforti*. Videre utvikling av ysteteknologien kreves for å skape den ønskede strukturen.

14. Introduction

In the recent years demand for rennet coagulated goat cheese in Norway has been increasing. On the Norwegian marked the French imported variations of Chèvre is the main contributor alongside the Norwegian produced (TINE SA) cream cheese Snøfrisk, Norwegian chèvre and Balsfjord which is a semi-hard cheese without surface ripening. There are also several small-scale producers of goat cheese in Norway, but these have a limited production quantity and area of distribution. The increasing quality of the Norwegian goat milk (Vegarud, Devold et al. 1999; Jakobsen 2011) for production of rennet coagulated cheese makes it interesting to develop new cheeses that can contribute to a wider selection of goat cheese on the market. A hindrance in the development of new cheese varieties from Norwegian goat milk has been its poor cheese making properties and the frequent rancid and tart flavour. The main problems with the Norwegian goat milk has been a high frequency of a deletion in the exon 12 D allele of the $alpha_{S1}$-casein in the goat stock (Vegarud, Devold et al. 1999) and a frequent rancid and tart taste (Eknæs and Skeie 2006). The taste defect is a result of lipolysis of milk fat into free fatty acids (FFA) (Collins, McSweeney et al. 2003). The Long chain fatty acids ($> C_{12:0}$) have a high perception threshold and are considered to be minor contributors to the cheese flavour (Molimard and Spinnler 1996). While the short and intermediate chain fatty acids ($C_{4:0}$-$C_{12:0}$) have a low perception threshold and will contribute to the characteristic flavour of a cheese. Octanoic acid ($C_{8:0}$) and branched decanoic acid ($C_{10:0}$) are most probably the main contributors to the goat like flavour in goat milk and both octanoic and butanoic ($C_{4:0}$) acid can give a rancid and tart taste (Chilliard, Ferlay et al. 2003; Collins, McSweeney et al. 2003). The short chain fatty acids can either contribute positive to the flavour of the cheese or to a rancidity defect depending on the concentration and perception threshold. This effect is regulated by the pH of the cheese. A high pH of the cheese will neutralize the FFA and the flavour effect may be negated (Molimard and Spinnler 1996).

The challenge of improving the quality of the casein composition in goat milk must be solved with directional breeding (Ådnøy, Vegarud et al. 2003; Jakobsen 2011). This solution to the problem has already been carried trough by the Norwegian breeding organization for sheep and goats (NSG). The frequent rancid and tart flavour can on short term be decreased by changes in diets fed the goats (Morand-Fehr 2005; Eknæs and Skeie 2006; Morand-Fehr, Fedele et al. 2007; Eknæs, Havrevoll et al. 2009) or by using an innovative cheese manufacture. In cheese production the milk is acidified with the help of lactic acid bacteria, this gives the cheese a relatively low pH. As stated above the flavour threshold of FFA is regulated by pH. A relatively high pH can contribute positive to the flavour of goat cheese with an initial high content of FFA. There is several ways to regulate the pH of the final

cheese in the manufacture process; amount of culture added, fermentation time, size of cheese curds, water addition and whey removal (Fox, Guinee et al. 2000). While these factors also will determine the texture and the dry matter content of the cheese an addition of mould or bacteria, to the surface of cheese, can regardless of the physical properties of the cheese increase the pH. The most utilized bacteria and mould for surface ripened cheese are *Brevibacterium linens*, the most important bacteria in smear-ripened cheese, *Penicillium camemberti*, for white mould cheese and *Penicillium roqueforti*, used in blue veined cheese. As mentioned Chèvre is a popular cheese, this cheese is most often ripened with *P. camenberti*. Chèvre is a soft cheese and the ripening will be completed during 2-3 weeks (Fox, Guinee et al. 2000). Garborit et al. (2001) compared the sensory quality of cheeses made of the same goat milk but with different ripening strains. The camembert-type cheese had a significant higher pH and scored higher on the sensory evaluation than the lactic cheese.

In production of blue veined cheese the mould will also grow in the interior of the cheese and this property will enable to ripen cheese with a higher content of dry matter for a longer time.

The objective of this study was to develop a recipe for a semi-hard cheese of goat milk ripened with *P. roqueforti*.

15. Development and manufacture

15.1. Development

The cheese recipe was a modification of the cheese made in the study of the quality of Norwegian goat milk for rennet coagulated cheese by Jakobsen (2011).

The development of the cheese started with a cheese making performed in the same manner as the original recipe but in addition spores of *P. roqueforti* was added to the cheese milk and small scale 15 L cheese vats were utilized. This cheese did not have a sufficiently open structure to be ripened as a blue veined cheese. In the further development cheese makings with minor changes of the recipe were carried out step by step to solve this problem.

The modification was made in two steps: In the first step, cheese making was made in two small-scale cheese vats containing 15 L milk, these were acidified, coagulated and cut in the same way. Further one vat was stirred continuously after cutting of the coagulum while the other vat was stirred in 10 min laps and left to rest for 10 min. The technique with stirring in laps will give the cheese curds a tough surface while the interior will remain soft and by this the cheese will gain a more open structure when it is moulded.

In the second step, the cheese making was done in 150 L Silkeborg vats with 100 L milk. This time the original recipe (Jakobsen 2011) was followed except that the curds were not pressed after moulding. The curds were just let to drain in the moulds and turned in the same manner as the original recipe. This technique will also enhance the open structure of the cheese.

The cheese was ripened in a ripening room that previously was contaminated by B. linens. The cheese therefore had a mixed surface of *P.roqueforti* and *B. linens* while *P. roqueforti* was dominating in the interior.

15.2. Manufacture process

The original recipe was as follows. Pasteurized milk was tempered to 32°C and inoculated with 0,7 % CHN19 from Chr. Hansen A/S (Hørsholm, Denmark) and preacidified for 25 min then added 10 mL *Lb. casei* (University of Life Science, Ås, Norway) per 100 L milk and spores of *P. roqueforti* (TINE SA) and the milk was preacidified for 5 more minutes. The milk was then added 25 mL rennet (CHYMAX, Chr. Hansen A/S, Hørsholm, Denmark) per 100L milk.

The coagulum was cut in 10 mm cubes when it had appropriate firmness and gave a clear cut. The coagulation time varied from 30 to 35 min during the research period. The cheese curds was let to rest for 5 min and thereafter stirred for 40 min keeping the temperature at 32°C. The whey was diluted by 50% whey removal and 50% water (32°C) addition. The curds were further stirred while heated to 39°C during 10 min and cooked for 30min at this temperature. Most of the whey was removed and the curds were transferred to Camembert forms and pressed lightly by hand. The cheese was kept at 36°C and turned straight away, after 30 min and 1 hour. After two hours the cheese was cooled down to 10°C and salted in brine for 1 hour.

The cheese was ripened for 1 week at 11°C and approximately 85% relative humidity (RH) to get a sufficient growth of *P. roqueforti* on the surface. The cheese was then pierced to allow air into the interior and CO_2 out of the cheese to create an optimal environment for *P. roqueforti*. The cheese was further stored for 2-3 weeks at 11°C at 85 % RH. After a sufficient growth of *P. roqueforti* in the interior the cheese was scraped and packet in aluminium foil and further ripened for 1,5 months at 4°C.

16. Result

16.1. Structure and growth of mould

The cheeses from the first cheese making, in the small-scale 15 L vats, are presented in figure 1. The cheese with stirring every 10 min during preacidification and scalding seems to have a more compact structure than the cheese with continuous stirring. The growth of *P. roqueforti* (dark blue/green) is quite limited in both cheeses with the best growth in the pierced holes. Both pictures also show that the orange smear layer from the surface of the cheese is clogging the pierced holes.

Figure 1 Cheese with stirring every 10 min and pressing (Upper). Cheese with continuously stirring and pressing (Bottom).

The cheese from the second cheese making, in the 150 L Silkeborg vat, without pressing is presented in figure 2. This cheese had large mechanical openings but they were quite few and unevenly distributed in the cheese. *P. roqueforti* (dark blue/green) is growing in almost all mechanical openings and in the pierced holes. The picture also shows that the orange smear

layer from the surface of the cheese is clogging the pierced holes and may possibly have limited the growth of *P. roqueforti*.

Figure 2 Cheese with continuously stirring without pressing.

16.2. Sensorial evaluation

The cheese made in the first and third cheese making was sensory evaluated by 4 judges. The analysis made was hedonic and the appearance, texture, aroma and taste and main score was evaluated on a scale from 0 points; really bad quality, to 5 points; excellent quality. The cheese was not compared to any similar cheeses but was evaluated at the same time as the goat milk cheese from a similar experiment (Jakobsen 2011). There was an overall positive feedback to the cheeses and the mean score of the evaluation was 4 out of 5 points.

17. Discussion

17.1. Present status

The sensory evaluation of the cheese was very satisfactory. Even though there was a limited growth of *P. roqueforti* in the interior of all cheeses in this study, the spores were added to the cheese milk and the cheese had therefore a profound taste of the mould throughout the interior of the cheese.

The curds of the cheese made with stirring every 10 min were too soft and the desired effect of a more open structure did not appear in the final cheese. Using this technique

apparently requires a prolonged stirring during scaling, Wilster (1951) suggests stirring every 10-15 min for 2 hours for Roquefort-type cheese.

The contamination of *B. linens* is very interesting. It seems like the secondary smear ripening contributes to the good sensory characteristics of the cheese but at the same time it may have hindered a good growth of *P. roqueforti*.

17.2. Further development

The interior structure of the cheese made in the first three productions was not satisfactory. Therefore in the further development, an extra step in the production should be introduced where the cheese curds are transferred to a sieving board to drain off the surplus whey, before the curds are transferred to the moulds. The stirring of the cheese curds every 10 min could then be reintroduced to create a softer cheese.

Another problem during ripening was the contamination of *B. linens,* which probably contributed to the nice flavour of the cheese, but made the surface of the cheese sticky and humid. This smear layer clogged the aerating holes and may have stopped the development of *P. roqueforti* in the interior of the cheese. *P. roqueforti,* as other moulds, need oxygen for growth and is deprived by carbon dioxide (Foster, Eugene Nelson et al. 1957). If the aerating holes are clogged the concentration of oxygen will decrease and carbon dioxide increase. Further experiments should be made where the cheese should also be stored in a ripening room not contaminated with *B. linens* to investigate the development of *P. roqueforti* and to which extent *B. linens* contributed to the flavour of the cheese. Morris et al. (1951) studied the relation of surface smear ripening in Minnesota Blue cheese and found that cheese with a slimy smear surface had higher pH, higher values of volatile acids, better body and texture and a finer flavour than waxed cheese.

If the cheese looses its good sensory properties without the smear ripening it would be interesting in further studies to store the cheese in a ripening room not contaminated with *B. linens*, for the first weeks as to get a good interior growth of *P. roqueforti,* and then inoculate it with B. linens for further ripening.

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APPENDIX I

APPENDIX II

APPENDIX III