Factors associated with spontaneous oxidized flavour in cow's milk

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2010

Acta Universitatis agriculturae Sueciae
2010:20

Cover photo: J. Wåreus (Mjölkfrämjandet)

ISSN 1652-6880
ISBN 978-91-576-7497-5

Print: SLU Service/Repro, Uppsala 2010

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Abstract

Spontaneous oxidized flavour (SOF) is an off-flavour in milk that has received attention in only a few countries, primarily due to lack of monitoring. The poly unsaturated fatty acids in milk are considered to make it more prone to oxidize, with effects like shorter shelf life of dairy products and a reduction in consumer acceptance of milk. On the other hand, unsaturated fatty acids have a reputation of being healthier than the saturated fatty acids. Means to optimize the fatty acid composition of milk include breeding measures, why acyl-CoA:diacylglycerol acyltransferase1 (*DGAT1*), a major gene for milk fat content, has lately attracted a lot of research efforts.

The risk of milk to develop SOF showed a strong and positive association with milk concentrations of both the well known prooxidant copper and its substrate the poly unsaturated milk fatty acids. The observed statistical interaction between copper and fatty acid composition suggests that SOF will not develop as easily in milk with high copper content unless sufficient amounts of substrate is available to promote the oxidation process. Breed and selection for fat content had an effect on the composition of milk fat where milk from Swedish Holstein cows generally had larger amounts of poly unsaturated fatty acids compared to the Swedish Red cows in the two selection lines in the SLU research herd. Selection for high milk fat content was associated with lower concentration of conjugated linoleic acid (CLA). The selection for fat content created differences in allele frequencies of the *DGAT1* gene between selection lines, with a higher frequency of the 232A allele within the low fat line. *DGAT1* genotype was associated with both composition of milk fat and occurrence of SOF, with the A allele being associated with a higher proportion of the polyunsaturated fatty acid CLA and an increased susceptibility of milk to develop SOF. The observed genetic variation in occurrence of SOF indicates that it may become a milk quality issue should bulls become selected that happen to carry genotypes predisposing for milk sensitive to oxidation.

Keywords: spontaneous oxidized flavour (SOF), Acyl-CoA:diacylglycerol acyltransferase1 (DGAT1), fatty acid composition, copper, α-tocopherol, bovine milk

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Till min familj

Ingen kan vara nere med en ballong.
Nalle Puh

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Juhlin, J., Fikse, W.F., Örde-Öström, I-L., Barrefors, P. and Lundén, A. (2010). Factors relating to copper in cow's milk and incidence of spontaneous oxidized flavour. (Submitted to *Acta Agriculturae Scandinavica, Section A - Animal Science*)

II Juhlin, J., Fikse, W.F., Lundén, A., Pickova, J. and Agenäs, S. (2010). Differences in fatty acid composition and contents of copper and α-tocopherol in milk from cows on different diets and its effect on oxidized flavour in milk. *Journal of Dairy Research (accepted)*

III Näslund, J., Fikse, W.F., Pielberg, G.R. and Lundén, A. (2008). Frequency and effect of the bovine acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*) K232A polymorphism in Swedish Dairy Cattle. *Journal of Dairy Science* 91, 2127-2134

IV Juhlin, J., Fikse, W.F., Pickova, J. and Lundén, A. (2010). Spontaneous oxidized flavour in cow's milk and its association with fatty acid composition, *DGAT1* genotype and concentration of copper in milk. (manuscript to be submitted)

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Abbreviations

CLA	Conjugated linoleic acid
FA	Fatty acid
h^2	Heritability
LCFA	Long chained fatty acid
MCFA	Medium chained fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SCFA	Short chained fatty acid
SFA	Saturated fatty acid
SOF	Spontaneous oxidized flavour

1 Introduction

1.1 Milk

The composition of milk differs considerably between mammals and is determined by the nutritional demands of the young. Cow's milk has been part of the human diet for thousands of years and it has been consumed as drinking milk and processed into dairy products such as cheese, cream, butter, ice cream and fermented products like yoghurt. In Sweden the consumption of drinking milk has since the seventies, when it was the leading dairy product, shown a decreasing trend and has now been surpassed by both cheese and fermented milk products when measured as consumption in kg/capita (Swedish Dairy Association, 2008).

1.1.1 Milk composition

Milk consists mainly of water, fat, protein, lactose with small amounts of organic acids and minerals. In cow's milk the contents of the major constituents protein, fat and lactose, and also the detailed protein and fat composition have been thoroughly studied and have been shown to vary both due to genetic and environmental factors. Selective breeding in order to improve milk yield has been a major success whereas less improvements have been made regarding the composition.

Both the breeding companies and the dairy industry in Sweden has for many decades been favouring milk volume rather than the dry matter content. This led to a decrease in milk protein and fat content. The breeding objective has now been shifted towards increasing weights on dry matter content and since 2002 a positive trend in both fat and protein has been observed (Figure 1). What has not been taken into consideration is

how the milk composition is affected by the shift in breeding strategies, and particularly, what happens to the milk fat composition.

Milk products are appreciated for their characteristic flavour which originates from its various components particularly the fat.

Figure 1. Changes in milk fat and protein content in Swedish milk (Swedish Dairy Association, 2008)

Milk fat

Milk is an emulsion of globules, where each fat globule is surrounded by a membrane consisting of phospholipids and proteins whereby the fat is maintained in an aqueous phase. The fat soluble vitamins A, D, E, and K are found within the fat portion of the milk.

Milk fat is composed primarily of triglycerides (98%), which are found within the globule, and other milk lipids which include diacylglycerides, monoacylglycerides, phospholipids, cholesterol, glycolipids and free fatty acids. The fatty acids (FA) in milk show considerable variation and over 400 different FA have been indentified in milk, varying in chain length and number, position and geometry of double bonds (Jensen, 2002). However, there are only a few of these fatty acids that are present at levels of interest (Table 1). Fatty acids consist of carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end.

Milk fat - Nomenclature

Fatty acids are frequently represented by a notation such as C18:2 indicating that the FA consists of an 18-carbon chain and 2 double bonds. Greek letters can also be used to identify the carbon atoms. The carbon next to the carboxyl carbon is called α, and the following carbon atoms are called β, γ, δ and so forth. However, the last carbon atom is always called omega (ω); the letter n is often used instead of ω.

Fatty acids lacking double bonds are classified as saturated fatty acids (SFA) and FA with one or more double bonds as unsaturated FA. Unsaturated FA with one double bond are called monounsaturated fatty acids (MUFA), whereas those with more than one double bond are referred to as polyunsaturated fatty acids (PUFA).

The unsaturated FA are grouped into the three families omega-9 (*n-9*), omega-6 (*n-6*) and omega-3 (*n-3*), where the names are given according to the position of the double bond nearest the omega (n) carbon atom.

Double bonds are said to be "conjugated" when they are separated from each other by one single bond, e.g., (-CH=CH-CH=CH-). The term "conjugated linoleic acid" (CLA) refers to several C18:2 linoleic acid variants such as 9,11-octadecadienoic acid and 10,12-octadecadienoic acid.

The principal isomer of CLA, *cis-9,trans-11* CLA is also known as rumenic acid. Since this is the isomer that is found in highest concentrations the name CLA will hereafter refer to *cis-9, trans*-11 C18:2.

Composition of bovine milk fat

Bovine milk fat contains on average 70 % SFA, 25 % MUFA and 5 % PUFA (Grummer, 1991). The most abundant fatty acids are C16:0 palmitic acid and C18:1 oleic acid (Table 1). Compared to milk from other species bovine milk also contains a large proportion of short chained fatty acids (SCFA) of which C4:0 and C6:0 are unique to ruminant milk. The milk fat composition is essential to many of the properties of milk products. The balance between SCFA and unsaturated and saturated long chained fatty acids (LCFA) can affect the processing properties, product quality and organoleptic characteristics of milk.

Table 1. *Fatty acids in bovine milk (Jensen, 2002).*

Fatty acid	weight %
4:0	2 - 5
6:0	1 - 5
8:0	1 - 3
10:0	2 - 4
12:0	2 - 5
14:0	8 - 14
15:0	1 - 2
16:0	22 - 35
16:1	1 - 3
17:0	0.5 – 1.5
18:0	9 - 14
18:1	20 - 30
18:2	1 - 3
18:3	0.5 - 2

The milk fat composition varies due to factors like feeding regime, stage of lactation, season, breed and genetic background of cow. How cow's diet plays a role in determining milk fat composition has been extensively studied (Palmquist, 2006) and efforts have been made to increase the proportions of FA that have a positive effect on human health.

Stage of lactation has a significant impact on FA composition, especially the early stage after parturition. At the onset of lactation cows are in negative energy balance and a mobilization of adipose tissue reserves increases the incorporation of C18:1 into the milk fat. Previous studies have shown that unsaturated FA are available at higher concentrations early in the lactation, whereafter it decreases to be followed again by an increase starting at around week 30 (Karijord *et al.,* 1982; Stoop *et al.,* 2009).

Season has been associated with variation in milk fat composition. This effect is probably to a large extent of dietary origin where fresh grass from pasture compared to silage has been shown to increase MUFA and PUFA content in milk. It is however, not only the type of forage but also the choice of lipid supplement that is influencing the FA profile (Chilliard *et al.,* 2000).

Milk fat synthesis and regulation

The FA in milk have different origin; being either synthesized *de novo* in the mammary gland, or absorbed from the circulating lipid pool in the blood plasma. The latter group originates from circulating plasma lipids, coming directly from the dietary lipids taken up in the small intestine or via storage fat, rumen microbial metabolism and from endogenous lipids. The SCFA (C4-C14) and some of the C16 are *de novo* synthesized while LCFA with 18 or more carbon atoms and part of the C16 are absorbed from the circulating lipid.

Milk fat synthesis involves numerous enzyme mediated pathways in which complex elongation and desaturation steps are involved. The precursors of milk FA in ruminant milk are acetate and β-hydroxybutyrate.

Enzymes that catalyze the addition of double bonds into the carbon chain are known as fatty acid desaturases. Several different desaturases are specific for the location on the carbon chain recognizing the stereochemistry of the acid to be desaturated. The enzyme Δ^9-desturase is an extensively studied desaturase in milk synthesis that converts saturated C10 - C18 acids into *cis*-9 monounsaturated acids (Pereira *et al.,* 2003). There are more than a dozen isomers of CLA in milk and body fat from ruminants. They originate from the biohydrogenation in the rumen and *cis-9 trans-11* C18:2, which is the major CLA form in ruminant fat, is synthesized from the rumen derived substrate *trans-11* C18:1 (Griinari *et al.,* 2000; Corl *et al.,* 2001). *Trans-11* C18:1 is also an intermediate in the biohydrogenation of linoleic acid (Bauman *et al.,* 2001).

During the triglyceride synthesis FA are first attached to positions sn-1 and sn-2 (according to the stereospecific numbering) on the glycerol molecule, under the catalyzing effects of enzymes. When a FA is attached to the third position the enzyme DGAT1 acts as a catalyst. A mutation was found in the gene encoding DGAT1 leading to an amino acid substitution, where a lysine (K) residue is replaced by an alanine (A) residue at position 232 (Grisart, *et al.,* 2002). The mutation, denoted K232A, was shown in a gene expression study to have a marked effect on the activity level of the enzyme, where the lysine variant showed a higher enzyme activity level (Vmax; Grisart *et al.,* 2004). An effect of the amino acid substitution is a reduction in milk fat content and an increase in milk yield (Grisart *et al.,* 2002; Spelman *et al.,* 2002; Thaller *et al.,* 2003; Schennink *et al.,* 2007). Evidence has been presented for multiple alleles at an additional polymorphism caused by a Variable Number of Tandem Repeats (VNTR) located in the promoter region of the *DGAT1* gene (Kuhn et al., 2004).

The effect of the VNTR, however, not as pronounced as the *DGAT1* K232A polymorphism.

Genetic variation in milk fat composition

Estimates of genetic variation and heritability (h^2) which is a measure on the proportion of the total variation in a trait that is due to heritable factors, in milk fat composition are limited. It has been argued that SCFA and some of the MCFA generally receive higher estimates of heritability due to their origin. Those FA that are synthesized *de novo* in the mammary gland may be influenced by genetics to a larger extent than the longer acids with 18 carbon atoms of dietary origin (Palmquist, 2006).

The variation in Δ^9-desaturase activity is another enzymatic process in milk fat synthesis that has been analysed. The steoryl-CoA desaturase (SCD) enzyme is responsible for catalyzing the insertion of a double bond specifically between carbon 9 and 10 in the fatty acid chain (Pereira *et al.,* 2003). In a study by Peterson *et al.* (2002) it was shown that variation in desaturase activity between individual animals exists and that this variation contributes to the variation in CLA content in milk fat. Estimators of genetic variation in unsaturation and genetic parameters for unsaturation indices are limited. However, Royal and Garnsworthy (2005) and Schennink *et al.* (2008) reported medium to high heritabilities for the unsaturation indices. Schennink *et al.* (2008) also found evidence that polymorphisms in the gene coding for the SCD enzyme, and the *DGAT1* K232A polymorphism affect saturation level of milk fat and largely explain genetic variance on the desaturase indexes. The *SCD* polymorphism was found to explain part of the variation in unsaturation and more so on medium/long-chain FA in milk. However, *DGAT1* had a greater effect on long-chain FA (18-carbon) than *SCD*.

The *DGAT1* allele that encodes the lysine (K) variant of the enzyme was found to be associated with a larger fraction of C16:0, and smaller fractions of C14:0, unsaturated C18 and CLA (Schennink et al., 2007). The A allele was shown to be associated with a higher total unsaturation index with the indices for unsaturated C18 and CLA being particularly high (Schennink *et al.,* 2008).

'Improving' milk fat composition

The high proportion of saturated FA in milk fat has during several decades caused concern regarding human health. The ratio of saturated to unsaturated FAs has been linked to the risk for cardiovascular disease, insulin resistance and hyperlipedemia (Vessby *et al.,* 2001; Sacks and Katan,

2002; Rasmussen *et al.,* 2006). Efforts have been made in order to improve the nutritional qualities of dairy products. Feed supplementation is the most common way to try to improve the FA profile in milk and thereby making it more beneficial for human health (Palmquist *et al.,* 1993; Chilliard *et al.,* 2000). However, more recent efforts have focused on exploring the genetic variation in FA profile to achieve the improvement through breeding measures (Soyeurt *et al.,* 2007; Stoop *et al.,* 2008).

More recently some FA found in ruminant-derived products have been shown to have a health-promoting potential, increasing the appreciation of dairy products as a functional food (Bauman *et al.,* 2006).

Other aspects of milk fat composition are its effect on processability and product properties, e.g. whipability of cream, and spreadability of butter but also on the characteristic flavours of milk and dairy products that are to a large extent dependent on the fat component.

1.2 Milk quality

Milk and milk products have a reputation of high quality among consumers. Yet the raw milk varies widely in its composition and quality due to several factors like feed, environmental condition and the genetic makeup of the animal.

Milk quality has many dimensions and some of the major ones are listed below:

- Nutritional value; high content of protein, omega-3 FA and vitamins are important for the nutritional value of milk.
- Human health; FA found in ruminant-derived products have been shown to have a health-promoting potential.
- Milk processing; yield and shelf life of processed products are influenced by composition of milk.
- Organic production; different production systems can increase the perceived quality of the product from an ethical point of view.
- Flavour, odour and texture; sensory perception affects consumer acceptance.

1.2.1 Off-flavour

The production of high quality dairy products begins at the farm and troubleshooting a milk flavour problem can both be a time-consuming and difficult task.

There are a variety of off-flavours with various origins that can be present in milk and dairy products. The cause of off-flavour can roughly be divided into substances that are formed from chemical reactions and substances that are transferred to the milk from the environment.

To eliminate the off-flavour it is important to determine the origin of an off-flavour and to classify which type of off-flavour it is. The off-flavours that are described in the Swedish quality tests are sour, unclean, oxidized, rancid, chemical and feed flavour. These off-flavours are defined by descriptive and associative terms to facilitate their classification. The classification of off-flavours differs somewhat between countries and regional differences can sometimes complicate the comparison of data between studies. Shipe *et al.* (1978) described a classification of common off-flavours with categories that has been commonly referred to internationally (Table 2).

Table 2. *Categories of off-flavours in milk (Shipe et al.1978).*

Causes	Descriptive or associative terms
Heated	Cooked, caramelized, scorched
Light-induced	Light, sunlight, activated
Lipolyzed	Rancid, butyric, bitter, goaty
Microbial	Acid, bitter, fruity, malty, putrid, unclean
Oxidized	Papery, cardboard, metallic, oily, fishy
Transmitted	Feed, weed, cow, barny
Miscellaneous	Absorbed, astringent, bitter, chalky, chemical, flat, foreign, lacks freshness, salty

Off-flavours arising from milk fat hydrolysis or oxidation of milk fat are the most common in Swedish raw milk (Lindberg et al., 2004). The hydrolysis of milk fat by an enzyme called the lipoprotein lipase (LPL) causes a characteristic off-flavour denoted as 'rancid'. The LPL can be indigenous or of bacterial origin and it gains access to its substrate when the fat globule membrane becomes damaged or weakened. Milk fat oxidation will produce volatile substances giving an off-flavour characterized by a cardbord, metal flavour (Shipe *et al.,* 1978).

1.2.2 Assessment of flavour

Sensory analysis

Sensory analysis refers to methods for milk quality testing with high sensitivity and good validation. Methods are commonly divided into

objective and subjective tests where the subjective tests often are performed by non-trained consumers expressing like or dislike for the product tested. Objective tests, however, are conducted by judges trained and calibrated for testing a specific product following a well defined protocol. In the quality systems approved by the Swedish dairies an objective method is used routinely whereby the milk flavour is compared with a set norm.

The drawback of sensory testing is that the repeatability can be low. This can be compensated for by joining judges together for 'calibration' tests and by letting two or more judges test the product and evaluate the result from their combined score.

Other methods to assess off-flavour compounds

There have been discussions on whether a so called 'electronic nose' could replace sensory analysis. Ampuero *et al.* (2002) described the development of an 'electronic nose' for the detection of fishy off-flavour. Previous studies have indicated strong correlations between chemical- and sensory analysis. For example, Hedegaard *et al.* (2006) found high correlations between sensory evaluation and concentration of hexanal, which is the quantitatively most important product from oxidation, lipid hydroperoxides as the primary oxidation products and also with tendency of formation of radicals early in the oxidative process.

1.3 Oxidized off-flavour

Oxidation in milk is a phenomenon which is not fully understood and the problem often arise in herds that are well managed, high producing and in other respects without problems (Nicholson and Charmley 1991; Barrefors *et al.,* 1995)

Lipid oxidation has been described thoroughly in a review by Frankel (1984). The process is an autooxidation reaction consisting of initiation, propagation and termination. The oxidation is dependent upon the production of free radicals from lipid molecules and it is an accelerating process which can be terminated by the destruction of the free radical by an antioxidant. Milk fat oxidation is catalyzed by copper and certain other metals in combination with oxygen. Propagation then occurs in triglycerides, primarily double bonds of unsaturated FA. During propagation, peroxide derivatives of FA accumulate. These undergo further reactions to form carbonyls, of which some, like aldehydes and ketones, have strong flavours.

1.3.1 Induced or spontaneous?

Oxidation in milk can be categorized into three subgroups: oxidation induced by metals, oxidation induced by UV-light and spontaneous oxidation. Spontaneous oxidation and oxidation induced by metals give rise to the same course of events and give the same characteristic off-flavour to the milk. Consequently these are not differentiated in the Swedish quality control system when sensory testing is concerned (Alnås and Bergwik, 1994). Upon exposure of UV-light additional chemical reactions apart from fat oxidation, like degradation of proteins set off, giving a characteristic off-flavour (van Aardt *et al.,* 2005).

Metal induced oxidation was a problem decades ago and was given much attention. It was mainly copper from pipes and containers for storage that contributed to the contaminating copper. Today, however, these sources of contamination have been eliminated. Although light can induce oxidation, this is not a problem in milk delivered to the dairy plant.

Spontaneous oxidation occurs when unsaturated FA oxidize in a reaction where neither light nor contaminating metals are contributing factors.

1.3.2 Factors influencing milk susceptibility to develop SOF

Milk fat varies considerably in its susceptibility to oxidation and Barrefors *et al.* (1995) speculated that fat oxidation results from the joint action of several contributin factors, rather than a single one. One of the most important contributing factor is milk fat composition and especially the proportion of PUFA. Other factors that influence the development of SOF are concentration and distribution of pro- and antioxidants.

Oxidation of milk fat

The phospholipids in the fat globule membrane are susceptible to oxidation as they contain unsaturated FA and are exposed to the main catalyzator, copper (Nicholson, 1993). Lipid oxidation and the development of SOF are highly influenced by the content and composition of the long chain PUFA, which are particularly susceptible to oxidation (Barrefors *et al.,* 1995; Granelli *et al.,* 1998; Timmons *et al.,* 2001). It is suggested that the more unsaturated milk fat the faster the oxidation (Walsta and Jenness, 1984; Barrefors *et al.,* 1995). The oxidative process is initiated already in the raw milk, with an acceleration of the process during storage and processing (Hedegaard *et al.,* 2006).

Prooxidants

Milk contains several types of metal ions, some of them acting as pro-oxidant such as copper, zinc, manganese and iron. Copper is considered to be the most potent of the prooxidants in milk and high copper concentrations have been shown to increase the susceptibility of the milk fat to oxidize and SOF to develop (King and Dunkley, 1959; Bruhn and Franke, 1975; Granelli *et al.,* 1998; Timmons *et al.,* 2001). Copper content in milk is normally found at levels around 20-25 µg/kg. It can, however, vary considerably during lactation and is markedly higher during the early stages of lactation when it can rise up to levels around 100 µg/kg.

Most of the digested copper is secreted through the faeces and only about 0.5 % is secreted through the mammary gland into the milk (Chase *et al.,* 2000). The correlation between the copper concentration in feed and what is later retrieved in milk is believed to be low. Several studies have found no correlation (King and Dunkley, 1959; Timmons *et al.,* 2001) while others have found a relationship between intake and copper source with what is later found in milk, like Ford *et al.* (1986). They found that cows housed indoors and fed silage produced milk with a higher copper content which was more prone to oxidize compared to milk from cows on pasture.

Milk sensitivity to copper induced oxidation varies and this might be due to the significant variation in oxidative substrate (i.e. PUFAs) between seasons in combination with the finding that milk containing a high amount of substrate requires less of the prooxidant for SOF to develop (Timmons *et al.,* 2001).

Reports of the heritability of copper content in milk is rather scarce. However, a study from 1973 by Neimann-Soerensen *et al.* (1973) reported a heritability of 0.44 ± 0.06. There exists an individual variation in the capacity to secrete copper and the ability to store copper in body tissues and the high heritability should reflect a significant genetic control of copper metabolism. In other species genetic disorders have been identified that result in disturbances in the copper metabolism, e.g. Menkes and Wilson disease in human and Copper toxicosis in the Bedlington terrier (Shim and Harris, 2003).

Antioxidants

Milk fats are protected from oxidation by several different compounds in milk that exhibit an antioxidative effect e.g. α-tocopherol (E vitamin), β-carotene, ascorbic acid, lutein, and uric acid. The mechanism by which these act as antioxidants and prevent oxidation in milk has been debated,

and the effect and relative importance of the main antioxidants α-tocopherol, β-carotene and ascorbic acid in preventing SOF varies. It is quite common to prevent SOF in affected herds by supplying extra α-tocopherol in feed, with varying results. Improvement of milk flavour scores as a result of varying rates of vitamin E supplementation have been reported by several authors (Atwal *et al.,* 1991; Focant *et al.,* 1998; Al-Mabruk *et al.,* 2004). Other studies have, however, failed in finding a relationship between α-tocopherol content and oxidative stability (Schingoethe *et al.,* 1979; Charmley and Nicholson 1994; Havemose *et al.,* 2004).

As a fat soluble vitamin α-tocopherol is transported with lipids and absorbed in the small intestine. When increasing the amount of PUFA in feed the amount of absorbed α-tocopherol has been seen to decrease. Also an increased level of roughage has been reported to increase the loss of E-vitamin in the rumen (Knudsen *et al.,* 2001).

Although only a small amount of the α-tocopherol present in the feed is transferred into the milk, 2-4 % according to Nicholson (1993), Thompson *et al.* (1964), Schingoethe *et al.* (1978) and Hidiroglou, (1989) concluded in their studies that the α-tocopherol content in milk was correlated with the content in feed. However, the efficiency of transfer into milk decreases with higher α-tocopherol intake (Weiss and Wyatt, 2003) and the secretion into milk is also believed to have an upper limit (Yeargan *et al.,* 1979).

Other factors

There are additional factors that are suggested to affect the oxidative stability of milk fat, such as water activity, temperature, pH, free fatty acids, and salt level. The effect of these factors will not be discussed further in this thesis.

1.3.3 Monitoring of SOF

Oxidative off-flavour is together with lipolysis the most common off-flavour in Swedish milk. In a report by Lindberg *el al.* (2004) statistics regarding off-flavours in Swedish milk were collected and a frequency of 0.39 % SOF affected milk was reported. These data were collected from tests performed on herd level. A study by Nicholson and Charmley (1991) showed that SOF is detectable in bulk tank milk only when more than 30 % of the cows in a herd are affected. Hence one would expect that the incidence among individual cows is considerably higher than the incidence on herd level.

In Sweden, several dairy companies perform sensory tests on milk from each milk producer. However, some companies store milk samples from

each bulk tank and only perform tests on these if there is any off-flavour detected in the silo milk in the dairy plant (Inger Andersson, Swedish Dairy Association, personal communication).

Internationally monitoring of milk flavour is uncommon. Denmark and Norway have previously had systems with sensory tests performed on milk, however, these have recently been taken out of the quality control.

2 Aims

- Investigate sources of variation in copper, α-tocopherol and fatty acid composition in milk
- Determine significance of copper, α-tocopherol and fatty acid composition in milk for the development of SOF
- Analyse the effect of genetic and systematic environmental effects on SOF.
- Investigate the role of inheritance, particularly *DGAT1* genotype in milk fat content and composition.
- Estimate the allele frequencies regarding *DGAT1* in the Swedish Red and Swedish Holstein breeds.
- Examine if the selection for different milk fat content is associated with differences in allele frequencies in *DGAT1*.
- Investigate if the selection for different fat content has led to a difference in milk composition.
- Investigate if *DGAT1* genotype has an effect on SOF development.

3 Summary of the investigations presented

3.1 Materials and Methods

3.1.1 Animal material

In paper I, III and IV milk was collected from cows of the Swedish Red breed (SR) and Swedish Holstein (SH) cows belonging to the Jälla experimental herd at the Swedish University of Agricultural Sciences, Uppsala. For paper III samples were collected from the Kungsängen experimental dairy herd at the Swedish University of Agricultural Sciences. Since 1985 the SR cows in the herds have been selected for either high (HF) or low (LF) fat content, at equal levels of total milk energy production.

DGAT1 genotype

For paper III and IV cows were genotyped for the *DGAT1* gene. DNA was extracted from blood according to standard protocol (Higuchi, 1992). Primers were designed using the NBI design software Oligo v5.0 and the following primers were used for the amplification of a 182 bp PCR fragment containing the *DGAT1* K232A mutation: F: 5'-AAG GCC AAG GCT GGT GAG-3' and R: Biotin- 5'-AGG TCA GGT TGT CGG GGT AG-3'. The sequence was picked from Genebank (NCBI), accession number AJ318490. Primers were selected so that a single PCR could be performed and with one primer biotinylated to allow capture of the PCR products onto avidin coated solid supports.

PCR amplification was carried out on a PTC-200 DNA Engine in 25 µl reaction volume using the AmpliTaq Gold (Applied Biosystems) PCR kit.

The reactions were performed with the addition of Betaine to improve the PCR reaction (SIGMA). To distinguish between the two variants a single PCR was performed where part of exon 8 was amplified.

The samples were analyzed with the method pyrosequencing (Ronaghi et al., 1998). The biotinylated PCR product (20 µl) was immobilized onto streptavidin-coated paramagnetic beads (Dynal AS, Oslo, Norway) using binding buffer (5 mM Tris-HCl, 1 M NaCl, 0.5 mM EDTA, 0.05% Tween 20, pH 7.6) in a total volume of 80 µl during a 10 minutes incubation on an vortex mixer (Vortex Genie 2, Scientific Industries, NY, USA). Biotinylated single-stranded (ss) DNA was obtained by washing the immobilized PCR product in 0.2 M NaOH and washing the beads in washing buffer (10 mM Tris-Acetate pH 7.6). A total of 15 pmol of detection primer, designed with its 3' end immediately upstream of the polymorphic site 5'-GCT CGT AGC TTT GGC AGG TA-3', was allowed to hybridize onto ssDNA in 40 µl of annealing buffer (20 mM Tris-Acetate, 2 mM MgAc2, pH 7.6) at 80°C for 2 min with subsequent cooling down to room temperature. Pyrosequencing was carried out on the PSQ96 Pyrosequencer instrument using the PSQ96 SNP Reagent kit (Biotage AB, Uppsala, Sweden) containing dATPαS, dCTP, dGTP, dTTP, enzyme mixture (DNA polymerase, ATP sulfurylase, luciferase, and apyrase), and substrate mixture (APS and luciferin).

3.1.2 Milk analyses

Gross composition

For the analysis of milk composition milk samples were treated with bronopol (Boots Microcheck, Nottingham, England) immediately after milking and samples of fresh milk were analyzed for somatic cell count (SCC) by flow cytometry (Fossomatic 5200, A/S Foss Electric, Hillerød, Denmark). Infrared technique was used to determine the contents of fat, protein, and lactose (Milko Scan 93; A/S Foss Electric, Hillerød, Denmark).

Copper content

For the analysis of copper in milk combustion of milk samples (5 g) was performed by automatic wet digestion according to a standard program. A mixture of 65 % supra pure nitric acid, 70 % perchloric acid and 95 % supra pure sulphuric acid was used. The digestion was performed in quartz-glass tubes overnight, using an automated system for control of time and temperature (Foss Tecator Digestion System, Model 40, Foss Tecator, Höganäs, Sweden).

The acid residue in the digestion tube was diluted with 1 M nitric acid to 10 ml. Analysis of copper was performed using an inductively coupled plasma atomic emission spectrometer, ICP-AES, (model JY 238, JY Horiba, division Jobin Yvon, Longgjumeau, France). Analytical copper line used was 324.75 nm. Four different concentrations of copper were used for preparation of the calibration curve, i.e. blank, 0.05, 0.10 and 0.20 mg/ml. The limit of detection (3s) in 5 g sample for copper was 0.002 mg/kg.

Quality control was regularly performed using Community Bureau of Reference Certified Reference Material 063R skim milk powder. The mean ±SD for N=28 was 0.58 ±0.02 mg/kg dry mass. The certified value was 0.602 ± 0.032 mg/kg dry mass. The evaluation of uncertainty was performed according to EURACHEM/CITAC Guide, 2000. The uncertainty values are reported as ± the expanded uncertainty calculated, using a coverage factor k=2, which gives a level of confidence of approximately 95%. Typical values of uncertainties at three different copper levels in milk (low, medium and high) are 0.01 ± 0.005, 0.05 ± 0.007, and 0.36 ± 0.034 mg/kg.

Sensory analysis

In paper I, II and IV aliquots of milk were tested for sensory quality by trained judges. In this test each sample was evaluated according to an instruction manual which carefully describes the sensory parameters to be considered, how to handle samples, and also how judges should be trained. Two judges tested each milk sample, independently of each other. Odour and taste were scored according to a standard of the expected quality characteristics of normal Swedish milk and deviations from the standard as described in the protocol/instruction manual (personal communication Gerd Virdeskog, Eurofins Steins Laboratory AB).

The milk samples were classified as either 'normal', 'moderate off-flavour' (class 1B), or 'pronounced off-flavour' (class 2). To classify a milk sample as belonging to class 1B, one of the two judges must scent and/or taste an abnormal odour/flavour in the milk, whereas if both judges characterize the off-flavour as pronounced the milk was assigned to class 2. The judges are trained in recognizing the off-flavours considered in the Swedish test system. Statistics regarding the tests performed throughout the year are analyzed in order to investigate the judges' performance and sensitivity to the off-flavours. Throughout the year the laboratory organizes sessions with all judges using known samples of different grades of off-flavour, in order to re-calibrate the judges and to harmonize the judges' assessments.

Milk fat composition

For paper II aliquots of morning milk were stored at +4°C for analysis of FA composition. Lipids were extracted within 24 h of sampling, using hexane and isopropanol (Nourooz-Zadeh and Appelqvist, 1988). The extracts were then stored frozen at -20°C until methylation of FA (Sukhija and Palmquist, 1988) and separation with a temperature-programmed gas chromatograph (Chrompack CP9001 GC, Chrompack, The Netherlands) equipped with a split injection system and a capillary column (Chrompack, CP-sil 88,50 m x 0.25 mm i.d.). Identification of individual FA was based on standards (Larodan Fine Chemicals, Sigma Chemical Co, Malmö, Sweden) and published data (Precht and Molkentin, 1996).

For paper IV aliquots of morning milk were stored at -80°C before analysis of FA composition. Lipids were extracted according to the method described by Nourooz-Zadeh and Appelqvist (1988). Preparation of FA methyl esters (FAME) was done using the protocol by Appelqvist (1968). FAME analysis was carried out with a HP5890 series II gas chromatograph (Hewlett Packard Co), fitted with a flame ionization detector and a capillary column DB-23 (Agilent Technologies) length 30 m. i.d. 0.25 mm, 0.25 μm film thickness. Column temperature was programmed at 2°C/min from 40°C to 220°C. Injector and detector temperatures were 250°C. Identification of FA was performed by comparing the obtained peaks with those of standards (Larodan Fine Chemicals, Sigma Chemical Co, Malmö, Sweden). Peak areas were integrated using HP ChemStation. The carrier gas was helium and make-up gas was nitrogen.

Statistical analysis

Paper I: After testing variables for normality using procedure UNIVARIATE the variable copper content in milk was log transformed (with base e). Data was analysed in procedure MIXED using a mixed animal model to identify effects contributing to the variation in copper content in milk. The model included the fixed effects of stage of lactation divided into four groups according to the shape of the lactation curve, housing divided into the effect of either loose or tied up housing systems, season and year of the test, and group which was the effect of the combination of breed and selection line of the cows. The model also contained the covariate fat content.

To account for the categorical nature of the data when analyzing the variation in occurrence of SOF a generalized linear mixed model was applied using the GLIMMIX procedure. Analyses were done with both a multinomial ordinal response variable with three levels (0, 1, and 2) and a

binomial response variable with two levels (0, 1). The model included the fixed effects of stage of lactation divided into four groups according to the shape of the lactation curve, housing divided into the effect of either loose or tied up housing systems, season and year of the test, and group which was the effect of the combination of breed and selection line of the cows. The model also contained the covariate of the natural logarithm of copper content.

For both the analyses in procedure MIXED and procedure GLIMMIX the correlations between repeated observations within a cow's lactation were handled by adding a correlation structure which assumes a decline in correlations with increasing time between observations.

Paper II: After testing variables for normality using procedure UNIVARIATE the variable copper content in milk was log transformed (with base e). Data was analysed in procedure MIXED by using a mixed animal model to identify effects contributing to the variation in copper and α-tocopherol content in milk. The model for copper content included the effect of period, i.e. whether cows were housed indoors, were transferred gradually to pasture or were at pasture. The model for α-tocopherol content included an effect of feed, seperating cows who received a dietary treatment of soya oil from those not receiving this supplement nested within the effect of period. This model also contained the covariate of fat content in milk. The model for α-tocopherol yield also included the effect of period nested within feed and an additional effect of the covariate fat yield.

Analyses of SOF were performed using procedure GLIMMIX considering SOF as a multinomial ordinal response variable with three levels (0, 1, and 2). The model included the effect of the covariates copper (natural logarithm) and fat components (PUFA, PI, C18:2 *n-6*,C18:3 *n-3* and CLA) analysed one at a time.

Correlations between the variables were computed with procedure CORR and were further analysed using procedure PRINCOMP. From this analysis six principal components describing the input variables and their correlations were chosen based on their eigenvalues and the cumulative proportion of variance explained. These components were further analyzed in procedure GLIMMIX in order to identify factors that contributed significantly to the risk of developing SOF.

For both the analysis in procedure MIXED and procedure GLIMMIX effects with P-value < 0.05 were considered statistically significant and were kept in the model. A relationship matrix containing two generations of ancestors was applied and the correlations between repeated observations within a cow's lactation were handled by adding a correlation structure

which makes no assumptions regarding the correlations between observations within an animal.

Paper III: Data were analysed using the procedure MIXED of SAS System for repeatability mixed models. An animal model was applied to investigate the effect of *DGAT1* genotype on the milk traits studied. Effects with P-value < 0.05 were considered statistically significant and were kept in the model. The model included the fixed effects of year-season, parity, group (which included information on breed and selection line) and *DGAT1* genotype. The model also included a function of the milk trait plotted against weeks in milk (WIM) in order to describe the average shape of lactation curve of cow. This was done for lactation 1 separately whereas lactation 2 and 3 were pooled together. To account for the structure of the data, with repeated observations on each cow, the variables parity and WIM were used as repeated measures variables. This was done by applying a multivariate correlation structure with a declining correlation between WIM and no assumptions regarding the correlation between parities.

To estimate the genotypic values (Falconer and Mackay, 1996) for the *DGAT1* the effect of *DGAT1* genotype was included as a fixed effect with three levels (AA, AK and KK). The dominance effect was estimated by testing the deviation of the heterozygote from the mean of the two homozygotes (0, 1). Average allele substitution effects (α; Falconer and Mackay, 1996) of the K and A alleles were obtained as regression coefficients of trait value on the number of copies of the respective allele.

Paper IV: After testing variables for normality using procedure UNIVARIATE the variable copper content in milk was log transformed (with base e). Data were analysed in procedure MIXED using a mixed animal model to identify factors contributing to the variation in copper and FA-composition in milk. The model included the fixed effects of season, parity, group (including information on breed and selection line) and *DGAT1* genotype. The model also included a function of the milk trait plotted against days in milk (DIM) in order to describe the average shape of a lactation curve of cows.

Analyses of SOF were performed using procedure GLIMMIX considering SOF as a multinomial ordinal response variable with three levels (0, 1, and 2).

For both the analysis in procedure MIXED and procedure GLIMMIX effects with P-value < 0.05 were considered statistically significant and were kept in the model. A relationship matrix containing two generations of ancestors was applied. To account for the structure of the data with repeated observations on each cow the variables parity and DIM were used as repeated measures variables by applying a covariance structure assuming

that correlations between observations within animal nested within parity decreases with time.

3.2 Summary of studies

3.2.1 Paper I

The effect of copper content and systematic effects on milk susceptibility to develop spontaneous oxidized flavour was analyzed in milk collected monthly (n = 893) from first lactation cows from two selection lines selected for either high or low milk fat content of the Swedish Red breed (n =87) and cows of the Swedish Holstein breed (n = 55).

3.2.2 Paper II

The effect of the milk components α-tocopherol and copper together with the FA profile in milk on the development of spontaneous oxidized flavour was assessed in milk (n = 131) from cows from two selection lines selected for either high or low milk fat content of the Swedish Red breed (n =44) fed different roughage types and different amounts of dietary fat.

3.2.3 Paper III

Swedish Red cows (n =143) from two selection lines for either high or low milk fat content but with similar total milk energy production, and Swedish Holstein cows (n = 96) were genotyped for the K232A polymorphism in the *DGAT1* gene. Allele and genotype frequencies were estimated and the effect of *DGAT1* genotype on milk composition and yield was estimated in individual milk samples (n = 16866) from genotyped cows.

3.2.4 Paper IV

The effect of *DGAT1* genotype on milk fat composition was estimated in individual milk samples (n = 1091) from genotyped cows. The cows were of the Swedish Red breed (n = 86) from two selection lines selected for either high or low milk fat content and of the Swedish Holstein breed (n = 50). The cows included in this study were a subsample of the cows in paper III. The effects of milk copper concentration, FA profile in milk, and *DGAT1* genotype on the development of spontaneous oxidized flavour were assessed.

3.3 Main results

3.3.1 Allele and genotype frequencies

Of the total number of genotyped cows (n = 239) the A allele and the AA genotype were the most frequent. In total there were only four cows that were homozygous for the K variant, of which three were of the SH breed and one in the SR/HF group (Figure 2).

Within the HF cows the K variant occurred at a higher frequency ($P < 0.0001$) than in the group of LF cows (0.18 and 0.01, respectively).

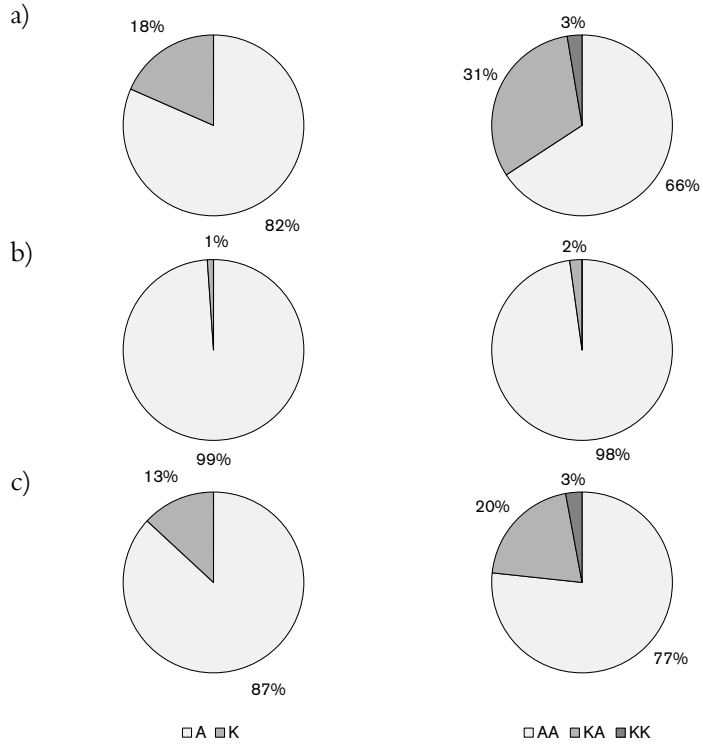

Figure 2. Allele and genotype frequencies of the *DGAT1* K232A polymorphism in two selection lines of the Swedish Red breed; a. High fat line, b. Low fat line; and c. in the Swedish Holstein breed

3.3.2 *DGAT1* effect on milk composition, yield and milk fat composition

The additive effect of *DGAT1*, expressed as genotypic value, was highly significant for fat content both in the SH and SR/HF groups of cows for which the increase in fat % units was 0.52 and 0.51, respectively, for each copy of the K variant. The genotypic value of the KK genotype was

positive also for protein content in the SH group. Due to very low frequency of the K-allele for the LF cows, there were no reliable estimates of *DGAT1* effects obtained for the LF line. The allele substitution effect of changing an A allele for a K allele were in accordance with the estimated allele effects based on genotypic values. However, the standard errors of the estimates were lower (Table 3).

There was no significant dominance effect observed for any of the traits. As regards yield and content of lactose, and SCC in milk there were no effects of the *DGAT1* polymorphism.

Table 3. *Effect of the DGAT1* K232A *polymorphism on milk composition and milk yield in the Swedish Holstein breed (SH) and two selection lines of the Swedish Red breed (SR)*

	Trait		
	Fat content (%)	Protein content (%)	Milk yield (kg)
a[3]			
SH	0.52 ± 0.10★★★	0.14 ± 0.05★★	-0.89 ± 1.05
SR/HF[1]	0.51 ± 0.17★★	0.11 ± 0.09	-0.13 ± 1.84
SR/LF[2,5]			
AA-AK	0.16 ± 0.23	-0.06 ± 0.12	-2.51 ± 2.50
α[4]			
SH	0.49 ± 0.07★★★	0.12 ± 0.04★★	-0.61 ± 0.75
SR/HF[1]	0.62 ± 0.08★★★	0.11 ± 0.04★★	-1.13 ± 0.86

[1,2] Cows from selection lines for high fat content (HF) or low fat content (LF), respectively, but with similar total milk energy production.

[3] Half of the difference between the genotypic values of the KK and AA genotypes.

[4] Allele substitution effect of changing A to K.

[5] Contrasts between homozygous and heterozygous genotypes.

★ = $P < 0.05$, ★★ = $P < 0.01$, ★★★ = $P < 0.001$.

The effect of *DGAT1* genotype on FA composition was reported in paper IV. A weak association between *DGAT1* polymorphism and contents of the unsaturated LCFA was observed ($P < 0.10$), where especially the CLA content was found to be higher in cows carrying the A allele ($P = 0.01$). The A allele was also associated with a lower proportion of C16 in milk.

3.3.3 Spontaneous oxidized flavour

Effect of copper as prooxidant

Results from paper I showed a marked effect of stage of lactation on variation in copper concentration and also on the occurrence of SOF when copper concentration was not included in the model (Figure 3). Other factors of significance for the variation in copper concentration were season, where the lowest values were observed during spring and highest during autumn and winter, and housing system where milk from tied cows in the SR/HF group had higher copper concentrations compared to cows of the same group in loose housing.

Results in paper II indicated that the copper content was highest during the indoor period, lower during transition to pasture whereafter it increased again; 84.9 ± 7.2, 52.7 ± 5.6 and 71.4 ± 4.6 µg/kg (LSM ± SE), respectively, with significant differences between all three levels ($P < 0.05$).

The heritabilities found for copper were 0.42 in paper I and 0.26, 0.17 and 0.44 for the three periods in paper II.

In paper I we wanted to investigate if SOF during early lactation was mainly a consequence of the high copper concentration in milk during the first three weeks of lactation. For this analysis we added a copper parameter adjusted for a variety of environmental effects, including stage of lactation. The effect of stage of lactation on SOF shifted from being most pronounced during the first three weeks of lactation when copper was not in the model, to a very low odds ratio for the same period if the model included the adjusted copper concentration (Figure 3).

The strong dependency of the risk of developing SOF on copper concentration observed in paper I is illustrated in Figure 4.

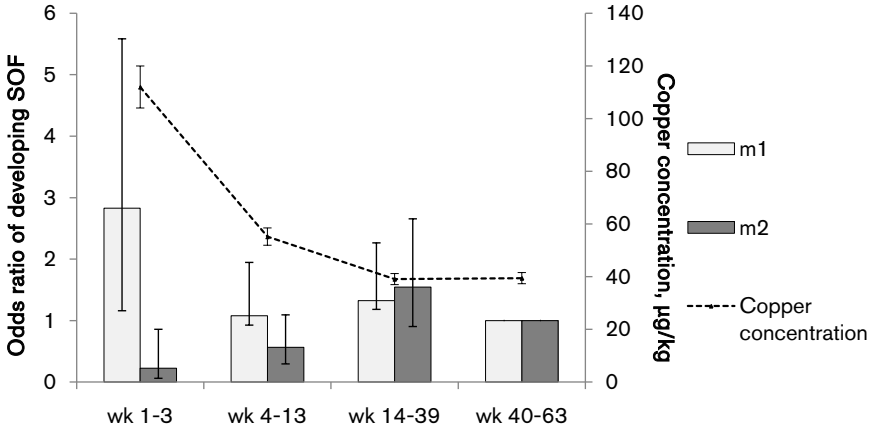

Figure 3. Odds ratio and 95 % confidence interval of milk developing spontaneous oxidized flavour (SOF) during lactation. Analysis was performed using two different models: m1: without copper as covariate, and m2 with the copper concentration adjusted for fixed effects as covariate. Both analyses used week 40-63 as reference. Secondary vertical axis shows least squares means (± SE) of milk copper concentration.

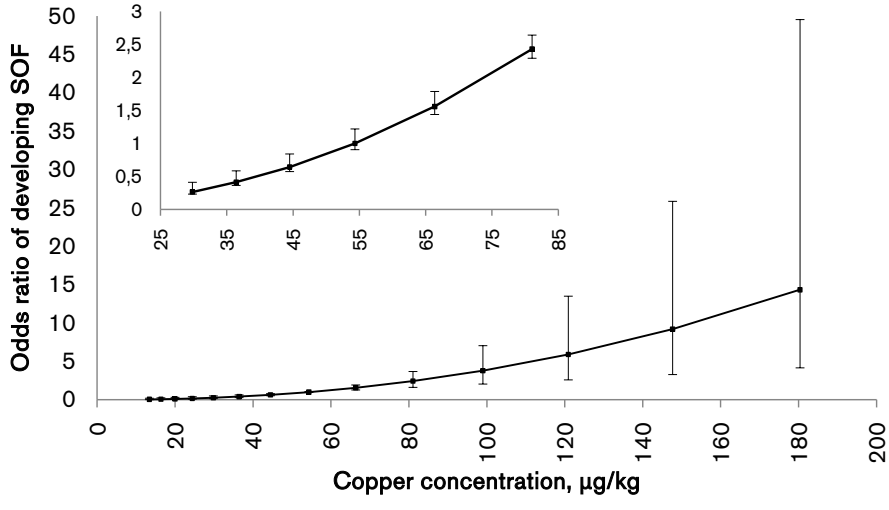

Figure 4. The association of copper content with the risk of milk developing spontaneous oxidized flavour (SOF). Estimates are expressed as odds ratios with 95 % confidence intervals using the mean copper concentration of 54.3 µg/kg as reference. The interval between copper concentration 30 – 80 µg/kg is zoomed in

The marked effect of copper content in milk on SOF development was confirmed in paper II. In paper IV we detected an interaction between copper content in milk and the FA profile for the risk of developing SOF.

Effect of α-tocopherol as antioxidant

In paper II we found that the highest α-tocopherol concentrations were obtained in milk from cows at pasture especially in the group that received the high fat diet. In the same paper heritabilities of 0.48, 0.62, and 0.54 were found for the three treatment periods.

In paper II we examined the role of α-tocopherol as antioxidant. In the principal component analysis the weights for the fourth principal component (significant in the model describing the variation in SOF, P-value < 0.05) showed high values but with opposite sign for α-tocopherol yield (weight: -0.48) on one hand and copper concentration and copper/PUFA (weights: 0.58 and 0.51, respectively) on the other hand. The weights of the different fat components were small and positive, ranging between 0.07-0.17.

Effect of fatty acid composition

In paper IV we found that the proportion of individual FAs differed between the SH and the SR groups of cows, where milk from the SH cows had higher amounts of all C18 PUFA analyzed compared with the SR cows. The only exception was CLA for which both the SR/LF and the SH cows showed higher amounts than the SR/HF cows. The SFA C12, C14 and C16 all showed a tendency of being lower in the LF compared to the HF cows but the difference was only significant for the C14 FA.

Heritabilities for the FA analyzed in paper IV were around 0.2 (0.15-0.23) for the LCFA, except for CLA and C18:3 n-3 for which the heritabilities were quite low (0.05 and 0.09 respectively). PUFA and PI showed heritabilities around 0.15. C16, which originates from both *de novo* synthesis and directly from blood plasma lipids had a heritability of 0.27, which could be compared to the heritability for C14 (0.45), a FA that is entirely synthesized in the mammary gland (Table 6).

In paper II we observed a strong relationship between FA composition and the occurrence of SOF. When limiting the analysis to just one fat variable at a time the variables PUFA and PI were the most significant (P-value 0.003 and 0.002, respectively). Figure 5 illustrates the marked effect of PUFA content in milk on SOF development.

Table 4. *Differences in fatty acid composition of test-day milk between the Swedish Holstein breed (SH) and two selection lines of the Swedish Red breed (SR) and heritabilities of fatty acids*

Trait[1]	SR/HF[2]	SR/LF[3]	SH	h^2
C 14	0 [a]	-0.51 ± 0.29 [b]	-0.96 ± 0.25 [c]	0.45
C 16	0 [a]	-0.628 ± 0.546 [a]	-0.748 ± 0.472 [a]	0.27
C 18	0 [a]	-0.293 ± 0.307 [a]	0.323 ± 0.268 [a]	0.15
C 18:1 *cis* 18:1 *n*-9	0 [a]	-0.292 ± 0.300 [a]	-0.320± 0.268 [a]	0.23
C 18:2 n-6	0 [a]	0.056 ± 0.064 [a]	0.250 ± 0.056 [b]	0.20
C 18:3 n-3	0 [a]	0.000 ± 0.023 [a]	0.036 ± 0.020 [b]	0.05
CLA	0 [a]	0.046 ± 0.029 [b]	0.049 ± 0.025 [b]	0.09
PUFA	0 [a]	0.086 ± 0.110 [a]	0.260 ± 0.090 [b]	0.15
PI	0 [a]	0.069 ± 0.135 [a]	0.278 ± 0.112 [b]	0.14

[1] Fatty acids and groups of fatty acids expressed as g/100g fatty acids; PUFA = polyunsaturated fatty acids (C18:2 *n*-6 + C18:3 *n*-3 + CLA); PI = polyunsaturated index (C18:2 *n-6* + (C18:3 *n*-3 x 2); CLA = conjugated linoleic acid, refers to C18:2 *cis-9, trans-11*

[2, 3] from selection lines for high fat content (HF) or low fat content (LF), respectively, but with similar total milk energy production

[a–c] Values within a row with different superscript differ (P<0.05)

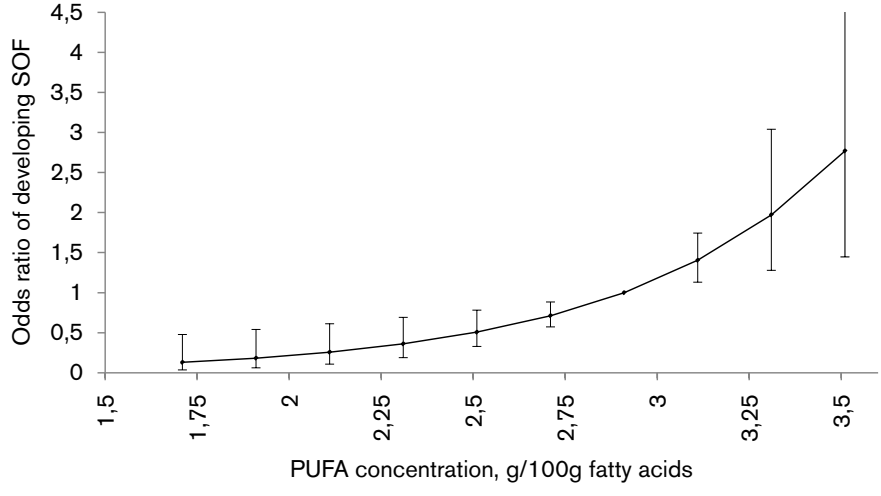

Figure 5. The association of PUFA content with the risk of milk developing spontaneous oxidized flavour (SOF) in milk from 44 multiparous cows of the Swedish Red breed. Estimates are expressed as odds ratios with 95 % confidence intervals using the mean concentration as reference.

Effect of DGAT1 genotype

In paper IV the model for describing the variation in occurrence of SOF that included *DGAT1* genotype and copper content was significant. When both *DGAT1* genotype and FA content were included in the models, the effect of FA content were reduced in significance. Such analyses are statistically cumbersome, because of the collinearity of explanatory variables (e.g. between *DGAT1* genotype and FA profile).

Residual values (adjusted for the effect of *DGAT1* genotype) for each observation were used as explanatory effects in the model instead of the corresponding unadjusted phenotypic values. By doing so, the variation in each (index of) FA resulting from *DGAT1* polymorphism and affecting SOF is attributed to the fixed effect of genotype in the model. As expected, in all analyses of SOF variation the effect of *DGAT1* genotype increased in significance when residuals were use instead of the phenotypic values. For C18:3 *n-3* and PI, using the residuals the effect of FA was still significant (apart from the interaction between C18:3 *n-3* and copper, P-value = 0.0509), suggesting that there is variation in C18:3 *n-3* and PI beyond the effect of the *DGAT1* genotype that is associated with the variation in SOF. For PUFA, CLA, and C18:2 *n-6*, using the residuals rather than actual phenotypic values reduced the level of significance below the significance threshold ($P > 0.05$), suggesting that the effect of FA profile on SOF development in those parameters is largely due to *DGAT1* genotype.

Interactions

In paper II there was no significant interaction found between FA composition and copper content on the risk of milk developing SOF. However, in paper IV a relationship between copper and FA-composition in determining SOF risk was established. Here our results indicated that those FA significant for SOF (i.e. PUFA, PI, C18:2 *n-6*, C18:3 *n-3* and CLA) are constituting the main risk factor for SOF development. We observed that at high contents of these FA the level of copper in milk did not affect odds ratios to the same extent as when the concentrations of FA were low (results not shown).

Other effects

The estimated heritability of SOF from paper II ranged from 0.12-0.21. The heritability found for the same trait in paper I was 0.15

4 General Discussion

4.1 Allele and genotype frequencies

Consequences of applying different selection strategies were illustrated in paper III, where selection for fat content in the SR cows was associated with a concomitant change in allele frequencies at the *DGAT1* K232A mutation.

When selecting for a higher fat content in milk there seems to be an indirect selection for the K variant. In Sweden the milk price to the farmer has for many decades been favoring milk volume rather than the dry matter content. Consequently the *DGAT1 A* variant has been given a selective advantage, which may be the reason for the high frequency of this variant in the present material in which the frequency of the A variant in the SH breed (0.86) was considerably higher than in other Holstein populations (Grisart *et al.,* 2002; Spelman *et al.*, 2002; Thaller *et al.,* 2003; Weller *et al.,* 2003; Kaupe *et al.,* 2004).

4.2 Milk composition

In paper III we found that selection towards either high or low milk fat content in the SR cows has clearly affected the contents of fat, protein and lactose in the milk whereas the difference in the corresponding yields was not as pronounced. The accuracy of the estimates regarding the effect of the K allele suffers from the small number of KK homozygous individuals in the material. Apart from this, the estimated effects of the *DGAT1* K232A polymorphism in this study were similar to previously published results (Grisart *et al.,* 2002; Spelman *et al.,* 2002; Thaller *et al.,* 2003; Sanders *et al.,* 2006). The allele substitution effects for fat and protein content were,

however, larger in this study than those reported by Thaller et al., (2003) but were similar to the allele substitution effects in the daughter design by Grisart *et al.* (2002). The lack of dominance effects were in accordance with previous results (Grisart *et al.,* 2002).

The *DGAT1* K232A effect on fat content has been reported to differ between breeds, (Spelman *et al.,* 2002; Thaller *et al.,* 2003) and the estimated allele substitution effect found in paper III was indeed higher for the SR/HF than for the SH cows. On the other hand, the fact that the genotypic values for fat content in this material were similar for the SR/HF and SH cows does not support the existence of breed differences regarding the *DGAT1* effects. Differences regarding effect on fat content may be due to the interaction with background genes which might differ between breeds. *DGAT1* plays a crucial role in the formation of triglycerides, but other genes can be of importance in the various pathways involved in the milk fat synthesis. However, including a relationship matrix to the statistical model should partly account for effects of the background genome.

4.3 Milk fat composition

In paper IV we noted that the proportion of individual FAs differed between the SH and the SR groups of cows. Milk from the SH cows had higher amounts of all analyzed C18 PUFA compared with the SR cows, the exception being CLA for which both the SR/LF and the SH cows had higher amounts than the SR/HF cows. Differences between breeds concerning milk FA composition have been reported previously and Holstein cows have generally been found to produce milk containing larger proportions of the C18 unsaturated acids (Krukovsky, 1961; Stull and Brown, 1964; Palmquist and Beaulieu, 1992; Arnould and Soyeurt, 2009).

A previous study on a limited number of cows in the two selection lines has indicated that the applied selection has altered not only the fat content of the milk but also the fat composition (Agenas *et al.,* 2003). That study showed that the HF cows had a higher content of C16 and lower contents of C18 FA compared with the LF cows. This result was not reproduced in paper IV except for CLA where a significantly higher content was found in milk from LF cows. The SFA C12, C14 and C16 all showed a tendency of lower concentrations in the LF cows but this difference was only significant for the C14 FA. These results are in agreement with previously published heritabilities and genetic correlations concerning FA composition indicating that selection towards high milk fat content would lead to an increased

proportion of saturated FAs of medium length and a lower proportion of unsaturated C18 FAs (Renner and Kosmack, 1974; Karijord *et al.,* 1982; Soyeurt *et al.* 2007; Stoop *et al.* 2008).

In paper IV we found that *DGAT1* genotype contributed to variation in FA composition and that the 232K allele was associated with a larger proportion of the C16 FA. This result is supported by the findings of Schenninck *et al.* (2007). They also reported that the K allele was associated with a smaller proportion of C14, which was however not the case in our study. Schenninck *et al.* (2007) also found an association of the K allele with a lower proportion of unsaturated C18 FA, and in paper IV the K allele showed the same tendency of being associated with low proportions of all unsaturated FA included in the analysis.

The theory that SCFA and some of the MCFA generally receive higher estimates of heritability than LCFA due to its origin from mammary gland synthesis (Palmquist, 2006) is supported by studies by Renner and Kosmack (1974) and Stoop *et al.* (2008) were a decreasing trend in heritability associated with length of the carbon chain was found. Schennink *et al.* (2007) also found high heritabilities for SCFA and MCFA (0.43-0.59) but lower heritabilities for the LCFA (around 0.25) except for CLA which had a heritability of 0.42. The heritabilities found in paper IV were around 0.2 (0.15-0.23) for the LCFA analyzed except for CLA and C18:3 n-3 where the heritabilities were quite low (0.05 and 0.09 respectively). C16 with its dual origin had a heritability of 0.27, lower than for C14 (0.45) which is solely synthesized in the mammary gland.

4.4 Spontaneous oxidized flavour

Describing the risk factors for SOF development was difficult due to the large set of correlated variables involved. In paper II proc CORR was used to describe the correlations between variables to be tested (results were not shown). After finding high correlations between the various milk constituents being analysed in separate models in paper II, proc PRINCOMP was employed to create uncorrelated variables to be used in the analysis of SOF. The results from this approach were not straight forward to interpret and for paper IV we chose to stay with the more simple strategy where individual FA and indices of FA were analyzed in separate model one at a time.

4.4.1 Copper

In paper I, II and IV we found evidence of a strong association of copper content in milk and SOF development. In paper I we observed that SOF was most common during early lactation partly due to the elevated levels of copper in milk after parturition. Previous studies have also reported a higher incidence of SOF at earlier stages of lactation (Kratzer *et al.* 1967; Bruhn and Franke, 1975)

The observed increased risk of developing SOF in mid/late lactation might also be due to the changes in copper distribution in milk over lactation. King and Williams (1963) found that only 15 % of copper in milk was associated with the fat globule membrane in week 2 and 4 of lactation compared with 35 % after 10 weeks. Copper that is associated with the fat globule membrane has been proposed to have a higher oxidative capacity (Chen and Nawar, 1991).

4.4.2 α-tocopherol

The principal component regression in paper II suggested an association of α-tocopherol with the development of SOF, but it did not reach significance when it was included as a main effect in the GLIMMIX analyses.

While some studies have failed to show an antioxidative capacity of α-tocopherol in milk (Timmons *et al.,* 2001; Havemose *et al.,* 2006), others conclude that α-tocopherol is one of the most important antioxidants in cow's milk and supplementation of α-tocopherol improved milk oxidative stability in several cases (Lundin and Palmquist, 1983; St-Laurent *et al.,* 1990; Focant *et al.,* 1998; Al-Mabruk *et al.,* 2004). However, dietary supplementation of vitamin E did not linearly increase the α-tocopherol in milk and only a small proportion of the α-tocopherol in feed is secreted into milk (Hidiroglou, 1989; Jensen *et al.,* 1999; Schingfield *et al.,* 2005). Jensen *et al.* (1999) also found an increase of α-tocopherol in milk fat concentration in cows at pasture.

It is important to note that antioxidants in a medium such as milk are often multifunctional and their properties might differ widely in different systems and conditions (Frankel and Meyer, 2000). One might argue that analyzing one antioxidant such as α-tocopherol is not sufficient in trying to establish the antioxidative capacity of milk. Chen *et al.* (2003) suggested that looking at the total antioxidant capacity without distinguishing the individual contributors may give a better evaluation of oxidative stability of milk.

4.4.3 Fatty acid composition

The role of FA composition in determining risk of SOF developing in the milk was analysed in paper II and IV. In paper II we had access to a small set of milk samples on which we also had information regarding α-tocopherol content in addition to copper, FA composition, and SOF. In this material we found a positive association between the occurrence of SOF and PUFA for all PUFA categories when analysed one at a time. The highest significances were obtained for the fat indices PUFA and PI (P-values 0.003 and 0.002, respectively). Also in paper IV we observed a positive association between all our analysed PUFAs and risk for SOF. These results are in agreement with previous studies in which lipid oxidation and the development of SOF have been shown to be highly dependent on the content and composition of the PUFA in milk (Barrefors *et al.,* 1995; Granelli *et al.,* 1998; Timmons *et al.,* 2001).

In a study by Timmons *et al.* (2001) a dietary supplement expected to increase proportions of C18:2 and c18:3 in milk was tested with regards to its effects on milk SOF. For this purpose they used roasted soybeans whereas in paper II soy oil was tested. Timmons *et al.* (2001) argued that feeding roasted soybeans or other fat sources that could increase the concentrations of C18:2 and C18:3 in milk fat should be restrictive in case the milk contains high concentrations of copper, to prevent development of SOF. Previous results by Agenäs *et al.* (2002), obtained on the same material as in paper II, had shown that grazing cows being fed soya oil produced milk with a lower content of *de novo* synthesized FA (C4:0 to C14:0) and a higher content of C18:0, C18:1, and CLA. The effect of feeding soya oil was, however, not significant regarding the risk of SOF in our study.

4.4.4 Interactions

No clear interaction regarding occurrence of SOF between copper, being an initiator of oxidation, and milk FA, which act as its substrate, was found in the relatively small dataset in paper II. However, a trend indicating that SOF will not develop as readily in milk with high copper concentrations unless the substrate level is sufficiently high was observed. This trend was confirmed in paper IV, a study where we had access to a larger set of data. For all fat components that were tested in this study (PUFA, PI, C18:2 *n-6,* C18:3 *n-3* and CLA) we found a significant interaction with copper. The interaction between concentrations of PUFA and copper for the occurrence of SOF found by Timmons et al. (2001) indicated that SOF would not develop as easily in milk with high copper content unless there is PUFA available, and similarly that high milk PUFA

would not lead to SOF if copper levels were low. However, our results indicate that the polyunsaturated FA (i.e. PUFA, PI, C18:2 *n-6*, C18:3 *n-3* and CLA), rather than copper are the main risk factor for SOF development. We observed that at high concentrations of these FA the level of copper in milk did not affect odds ratios to the same extent as when the concentrations of these FA were low.

4.4.5 Genetics

The estimated heritability for SOF from paper I was 0.15 and in paper II it ranged between 0.12-0.21, depending on the component of fat entered into the model. Kratzer *et al.* (1967) reported a heritability of 0.26 ± 0.17 for SOF and in a study by Neimann-Soerensen *et al.* (1973) the repeated estimates of the heritability of thiobarbituric-value, which is an estimate of oxidation in milk, was in the range of 0.17 ± 0.04 to 0.47 ± 0.06. The heritability estimates suggest that occurrence of SOF is partly under genetic control.

Copper, acting as an important catalyst for SOF, was found to have a heritiability of 0.42 in paper I whereas in paper II the estimates differed somewhat between the three different treatments, with 0.26, 0.17, and 0.44 for indoor, transition, and pasture, respectively. The estimate obtained in paper I, and from cows on pasture in paper III were comparable with the heritability of 0.44 ± 0.06 reported by Neimann-Soerensen *et al.* (1973). The relatively high heritability suggests a significant genetic control of the copper metabolism and that ignoring milk copper concentration in breeding decisions may result in undesirable changes in the level of milk copper concentration and thereby the occurrence of SOF.

In paper IV we estimated heritabilities for the LCFA known to act as substrate in the oxidative process and found moderate to low estimates for these FA. Another important contributor to the genetic variation in FA composition is the *DGAT1* K232A polymorphism, which also came out as a significant factor for SOF development in paper IV. There are other genetic polymorphisms being investigated with respect to their role in FA composition. One that might contribute to the variation in SOF is SCD, which in a study by Schennink *et al.* (2008) was shown to explain more of the variation in degree of unsaturation than the *DGAT1* genotype. However, the *DGAT1* polymorphism had a larger effect on the concentration of LCFA (18-carbon), where the A allele was associated with increased susceptibility of milk to develop SOF.

4.5 Avoiding SOF

The results presented in this thesis show that the amount of PUFAs clearly affects SOF development, as do copper content in milk. Results from paper IV also indicate an interaction between PUFA and the amount of natural copper in milk for the development of SOF. This should offer several means of preventing SOF; either by decreasing the amount of PUFA, decreasing copper content, or both.

Low fat diets to cows leading to a negative energy balance in the cow and feeding supplemental fat rations containing large amounts of unsaturated fatty acids should thus be avoided from a milk quality perspective, since they result in increased concentration of SOF substrate. On the other hand, efforts to increase the amount of unsaturated fatty acids in milk have been undertaken to improve the milk fat from a human health aspect. In this perspective more efforts should be given to SOF prevention through decreasing the content of prooxidants and increasing the antioxidants. The requirement of antioxidants has been proposed to increase with increasing proportions of PUFA (Granelli et al., 1998). However, the relationship is complex and there are several antioxidants that play different roles in the milk, e.g. α-tocopherol has its effect in preventing oxidation in the milk fat globules whereas β-carotene acts in the neutral lipids inside the globules. Focant *et al.* (1998) concluded in their study that the increased PUFA content in milk that was observed when feeding oil seeds did not increase susceptibility to develop SOF if supplementary α-tocopherol was given.

The role of antioxidants in the prevention of SOF is complex. Several studies have shown that both intra muscular as well as dietary supplements with α-tocopherol will lead to increasing levels of α-tocopherol in plasma and milk and also to reduce the intensity of SOF (Ericsson *et al.,* 1963; St:Laurent *et al.,* 1990; Charmely and Nicholson, 1993; Focant *et al.,* 1998). However, the results regarding the effect of dietary supplementation of vitamin E on α-tocopherol concentration in milk are not consistent, and also only a small proportion of the α-tocopherol in feed is secreted into milk (Hidiroglou, 1989; Jensen *et al.,* 1999; Schingfield *et al.,* 2005). There are also contradicting studies that failed to show an antioxidative capacity of α-tocopherol in milk (Timmons *et al.,* 2001; Havemose *et al.,* 2006).

Fresh grass is rich in vitamins A, E, and β-carotene and higher intake of α-tocopherol has been shown to result in a higher output of α-tocopherol in milk (Thompson *et al.,* 1964, Schingoethe *et al.,* 1978; Focant *et al.,* 1998), In paper II we observed that α-tocopherol in milk increased when cows were turned out to pasture, which could be considered as a natural

consequence of what was stated above. This increase was, however, not found to be sufficient to prevent milk oxidation. The relationship between levels of α-tocopherol and the development of oxidative flavour is not clear and there are also other antioxidants present in milk which in combination with various prooxidants may add to the complexity of PUFA oxidation.

Supplementation of dietary fat should be done with caution and with consideration to the copper concentration in milk. It was found in paper II that copper concentration in milk was markedly increased after parturition. To counteract this rise in SOF initiatior one should consider not to feed dietary fat known to increase PUFA concentrations at this stage. Another strategy to reduce the occurrence of SOF in a herd could be to modify the calving pattern.

4.5.1 Routine testing or not?

Even if problems with SOF seem quite rare, both farmers and advisors signal that when a problem arises it is generally difficult and time consuming to solve. Since SOF development is such a complex phenomenon the underlying factors might differ between herds, making the problem even more problematic to solve.

One important reason for monitoring SOF is the moderately high heritabilities that has been reported in our own and in previous studies, suggesting that occurrence of SOF is partly under genetic control. This indicates that milk quality may be compromised if breeding bulls are selected that carry genotypes predisposing for milk prone to develop SOF.

If no monitoring is done the problem with SOF might go undetected until the off-flavour rise above the detection level. If the problem is allowed to proceed this far it will most likely be both expensive and time consuming to manage. A serious consequence could be that the consumer acceptance may be compromised. An example indicating the importance of flavour tests is the mutation causing trimethylamine to accumulate in milk, giving it a fishy off-flavour (Lundén *et al.,* 2002). It was found that breeding bulls carrying the mutated genotype had been frequently used in herds affected by the problem. Today most breeding bulls of Ayrshire origin are genotyped for this mutation. With no monitoring such a defect might have further accumulated in the population causing severe problems.

Because the oxidative reaction is a chain-reaction where each free radical being formed sets off new reactions, milk in which the oxidative process has been initiated will contaminate milk in the bulk tank and in the silo at the dairy plant. This is the reason why SOF is the most common off-flavour at

bulk tank level, whereas when tests are performed at herd level lipolysis is somewhat more frequent.

Studies have been undertaken to explore the possibilities to test for SOF by a so called 'electronic nose', something that has been developed for the detection of fishy off-flavour (Ampuero *et al.,* 2002). This might facilitate the routine testing of large amounts of samples. High correlations have been found between chemical measurements and sensory analyses if the appropriate compounds have been selected. However, it is important to note that most likely no single analysis will cover all volatile compounds attributing to a perceived flavour. In order to get a more complete picture several analyses might be needed. However, methods that give a rough assessment of milk flavour may be adequate if they are applicable for routine measures on a large scale (Drake *et al.,* 2006).

5 Main findings and conclusions

- *DGAT1* genotype has a large effect on milk yield and composition, especially pronounced on fat content where the allele substitution effect was 0.49 % units and 0.62 % units for cows of the SH and the SR/HF breed, respectively. The 232A allele was associated with high milk fat content.
- When selecting for a higher fat content in milk there seems to be an indirect selection for the K variant of *DGAT1,* illustrated by the higher frequency of the K allele within the group of HF cows than among the LF cows of the SR breed (0.18 and 0.01, respectively).
- Breed and selection for fat content had an effect on the composition of milk fat where milk from SH cows generally had larger amounts of PUFA compared to the SR cows in the two selection lines. Selection for high milk fat content was associated with lower concentration of CLA.
- *DGAT1* genotype had an effect on FA composition, where milk content of unsaturated LCFA, especially CLA, was higher in cows carrying the 232A allele.
- There was a marked effect of concentration of naturally occurring copper in milk on SOF development, where a rise in copper content increased the risk of milk to develop SOF. As an initiator of the oxidative process copper can accelerate the auto-oxidation of the PUFA present in milk.
- There was no clear association between increased levels of the antioxidant α-tocopherol in milk and a lower risk of SOF. There was a marked effect of FA composition on SOF development, where high concentrations of PUFA were associated with an increasing risk of SOF. Copper also showed a marked effect on

SOF where it interacts with PUFA, but where PUFA rather than copper, constitutes the main risk factor.

- In order to decrease the risk for SOF the feeding of soy oil or other diet supplements known to increase the content of PUFA in milk should be limited at times when copper content is high, e.g. early in the lactation when copper is up to three times as high. Conversely, mineral supplement, containing copper, should be administered with caution at times when PUFA levels in milk are high.
- The *DGAT1* 232A allele was associated with an increased risk of SOF. Also, the observed effect of some PUFA on occurrence of SOF may to a large extent be an underlying effect of *DGAT1* genotype.
- Heritability estimates for the FA analyzed indicate that selective breeding can be applied in order to alter FA composition in milk.
- Copper content as well as α-tocopherol in milk were found to have a moderate to high heritability and could consequently be altered through breeding.
- SOF was found to have a moderate heritability, suggesting that it may become a milk quality issue should bulls become selected that happen to carry genotypes predisposing for milk sensitive to oxidation.

6 Future Research

- Our results indicate that there is a conflict between objectives to improve the nutritional quality of milk fat and to decrease the risk of SOF. Further studies are needed on how breeding should be applied in order to safeguard milk quality while increasing the nutritional value of milk.

- More detailed knowledge about the genetic background of SOF is warranted, to identify which and how many genes contribute to the genetic variation in SOF. Depending on the genetic architecture of SOF, the possibility to reduce the occurrence of SOF through genomic selection could be evaluated. Identified genes that affect FA composition could be evaluated with regards to their effect on SOF.

- The antioxidative processes present in milk need further investigation. Synergy effects between the antioxidants and their antioxidative capacity in milk should be evaluated as well as methods to assess the total antioxidant capacity.

- Further investigations are warranted in order to evaluate the actual incidence of SOF. Our studies indicate that the problem could be underestimated and that there is a potential risk in not monitoring this trait. Development of instrumental measurements to detect SOF is highly demanded and could increase the possibilities for routine monitoring but needs further investigation.

7 Populärvetenskaplig sammanfattning

Jämte proteinet är fettet den viktigaste komponenten i mjölk. Mjölkfettets sammansättning och dess innehåll av pro- och antioxidanter är av betydelse för såväl konsumenter som mejeriindustri och mjölkproducenter. För konsumenten är närings- och hälsoaspekter viktiga, liksom produktegenskaper som lukt och smak, konsistens, etc. Mejeriindustrin är främst intresserad av egenskaper som processbarhet och produktutbyte, medan den enskilde mjölkbonden drabbas genom sänkt mjölklikvid i de fall mjölkfettet bidrar till uppkomst av smakfel.

Mjölkråvarans fettsammansättning påverkas av fodertyp och utfodringssystem, men även till stor del av ärftliga faktorer. Eftersom kons genuppsättning är ett resultat av det avelsarbete som bedrivs är det viktigt att känna till hur avelsurvalet bör ske för att mjölken ska få önskad fettsammansättning. Från kokontrolldata kan vi skaffa oss relativt goda kunskaper om mjölkens halter av fett och protein hos den enskilda kon, medan motsvarande kunskap om fettets och proteinets sammansättning saknas. Under den senare delen av 90-talet såg vi hur fetthalten stadigt sjönk. Avelsprogram och betalningssystemet för mjölkråvara har under 2000-talet anpassats för att förhindra ytterligare sänkning av mjölkfetthalten och sedan 2002 har vi sett en liten ökning. Frågan är dock hur fettets sammansättning påverkats av dessa anpassningar och vilka förändringar av mjölkfettet vi kan förvänta oss i framtiden utifrån dagens avelsprogram.

Mjölkfett utgörs i huvudsak av triacylglycerol-molekyler, uppbyggda av en glycerolmolekyl med tre fettsyror bundna till sig. Vid syntesen av triacylglycerol krävs medverkan av enzymet acyl-CoA:diacylglycerol acyltransferas (DGAT). Genen som kodar förenzymet (*DGAT1*-genen) förekommer i två funktionellt sett olika varianter vilka skiljer sig vad gäller förmågan att binda fettsyror till glycerol-molekylen. Den”ursprungliga”

genvarianten ger högre halter av både fett och protein samt har en effekt på fettets sammansättning.

Mjölkens lukt och smak är av avgörande betydelse för konsumentens förtroende för mjölkprodukter, men är samtidigt egenskaper som är svåra att kontrollera i stor skala. Endast ett fåtal länder smaktestar mjölken på leverantörs/gårdsnivå. Det finns följaktligen i de flesta länder en dold variation i mjölkens lukt och smak. På grund av svårigheterna att rutinmässigt analysera mjölken från individuella kor för förekomst av smakfel vore det önskvärt att identifiera gener som inverkar på mjölkens lukt och smak för att möjliggöra ett effektivt avelsarbete för att minska problemen.

– inverkan av fettsyrasammansättning(?)

En intressant frågeställning är hur fettsyrasammansättningen inverkar på uppkomsten av smakfel i mjölk. De två vanligaste smakfelen, 'oxidationssmak' och 'härsken smak', är båda förknippade med fettkomponenten i mjölk. Det är av stor vikt att utröna vilken roll fettsyrasammansättningen har i sammanhanget och arvets betydelse för dess variation.

– inverkan av mjölkens α-tocoferol- och kopparkoncentration(?)

Mjölkfettets benägenhet att oxidera är avhängigt mjölkens innehåll av olika komponenter och deras inbördes samspel, där vissa fungerar som prooxidanter och andra som antioxidanter. En av de kraftfullaste prooxidanterna i mjölk är koppar, som katalyserar fettoxidationen. α-tocoferol (vitamin E) å andra sidan, anses vara en av de viktigaste antioxidanterna i mjölk genom sin förmåga att på olika sätt bryta kedjereaktionen.

Man har observerat stora individuella skillnader vad gäller överföring av tillsatt α-tocoferol i fodret till mjölken och denna variation har man funnit vara ärftligt betingad. Man har även funnit att med ökade mjölkvolymer per ko minskar koncentrationerna av antioxidanter och vitaminer. Om aveln fokuseras på ökad mjölkavkastning riskerar koncentrationen av vitaminer och antioxidanter att minska som en effekt av utspädning.

Slutsatser

- Mjölkfettets sammansättning påverkas av olika avelsstrategier där det inbördes förhållandet mellan olika fettsyror i mjölken påverkas av avel för hög respektive låg fetthalt.

- Kandidatgenen *DGAT1* har en signifikant effekt på mjölkens fettsyrasammansättning.
- Olika avelsstrategier påverkar frekvensen av de två varianterna av *DGAT1* där selektion för ökad fetthalt ökar frekvensen av den 'ursprungliga' varianten.
- Fettsyrasammansättningen och mjölkens innehåll av koppar påverkar risken för oxidationssmak, ju mer fleromättat fett och koppar desto högre risk för SOF.
- Fettsyrasammansättningen och mjölkens innehåll av koppar påverkas i hög grad av ärftliga faktorer.
- *DGAT*1 genotypen påverkar genom sin effekt på fettsyrasammansättningen mjölkens benägenhet att utveckla oxidationssmak.
- SOF hade en arvbarhet som visade att avel kan påverka mjölkens benägenhet att utveckla oxidationssmak.

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Acknowledgements

The work for this thesis has been carried out at the department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences. Data for paper I, III and IV was from the departments research herd at Jälla Agricultural High School and data for paper II was from the university herd at Kungsängen research centre. The Swedish Farmers' Foundation for Agricultural Research is gratefully acknowledged for funding the project.

There are many people who have supported me during these years whom I would like to express my gratitude towards. I would like to thank you, past and present colleagues for support and for making Hgen a nice place to work at! In addition a special thanks to some of my colleagues, friends and family:

Mina handledare, Anne Lundén, Freddy Fikse and Jana Pickova, tack för allt ni lärt mej och för det stöd och engagemang ni visat under min tid som doktorand!

Mina medarbetare, Lars Johnsson och Toomas Allmere, många stunder med GC trassel får man väl hoppas att det gav lärdom för livet. Gerli Pielberg, tack för att du lärde mej labba på BMC. Sigrid, det blev svettigt på sluttampen och utan dej hade det kanske blivit helt annorlunda, tack för fint teamwork!

Hgens administrativa personal, särskilt tack till Jörgen, Monica, Harriet och såklart Bijan och Siw. Tack för att ni alltid ställer upp när det behövs!

Dan och Stickan, tack för all hjälp med datorkrångel.

Gudrun och Lena, tack för all hjälp med data från besättningen på Jälla!

Alla doktorander på Hgen, nuvarande och före detta, det har varit jätteroligt att vara en av er, kommer sakna er!

Tack även ni medarbetare på LMV som jag träffat på under mina år. Särskit tack till Elin och Anna på mjölklabbet och Lotta som alltid hjälpt mej med smått och gott.

Mams och paps, bror, söstrar, Görre, U-B, gulliga svärisar. Helt enkelt ett stor tack till hela La familia, det finns inte ord nog, utan er hade inget varit möjligt!

Ebbe och Bacchus mina fyrbenta kompanjoner under dessa år, tack för underbart sällskap, utan våra promenader hade min hjärna kokat över.

Helmer och bebishen, tack för att ni ger mej perspektiv på tillvaron, och för den oändliga lycka som jag upplever varje dag med er.

Erik, tack för allt stöd och kärlek, nu börjar nästa äventyr!