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LIPOLYTIC CHANGES IN THE MILK FAT OF RAW MILK AND THEIR EFFECTS ON THE QUALITY OF MILK PRODUCTS

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Abstract

Lipolytic changes in milk fat affect sensory attributes and technological properties of milk and milk products. They are affected by physiological, thermal, and biochemical factors as well as by the mechanics of fluids. Lipolytic processes in milk are intensified by modern processing methods.

In this review, special attention has been paid to ruminant-related feeding of dairy cows, foaming of milk, mechanical and thermal influences, and the growth of psychrotrophic bacteria.

Feeds deficient in energy affect the chemical composition of the milk fat. Tests have shown that on an average, approximately 55% of free fatty acids in the raw milk pass into the cream and the rest passes into the skim milk. In the froth-churning process, approximately 0.15 mmol free fatty acids per 100 g of fat pass into buttermilk.

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Introduction

The quality of dairy products is determined by their sensory, chemico-physical and microbiological characteristics. It is the objective of the milk treatment and processing to preserve these characteristics.

Milk is a complex polydisperse biological system. When it is extracted from a healthy udder, all its constituents are in their native state. Any action of energy affects the equilibrium in the milk and may ultimately affect the properties of the milk products (60, 76).

Milk is particularly sensitive to factors influencing its odour and taste. Both properties are affected by the absorption of substances present in the feed, by absorption of alien odours and flavours from the barn air, by formation of metabolites of the milk components etc. Of all these factors, which influence the odour and taste of milk, lipolytic changes in the milk fat play one of the most important roles. In addition to the properties mentioned above, they affect technological properties of the milk, such as the separation of the milk fat.

Lipolytic changes which develop in the milk fat include the aggregation of milk fat globules, the formation of free fat, and the hydrolysis of glycerides that produces free fatty acids (Fig. 1) but do not include oxidative changes.

Fat present in the milk in the form of globules is protected by membranes from lipolytic and oxidative effects. Thus, lipolysis occurs only after the disruption of the membranes and the subsequent formation of free fat (96). Due to incomplete esterification in the udder, a small amount of free fatty acids is present in fresh milk (37, 62). Additional, post-secretory formation of free fatty acids is influenced by physiological, physical, and biochemical factors (Fig. 2) (45-48, 51, 53, 59). It is estimated that because of an increased effect of these factors in the past 10 to 15 years, the free fatty acid content in the raw milk has doubled world-wide (67), primarily due to physical handling. Hydrolysis induced by physiological factors is usually called "spontaneous" lipolysis and hydrolysis induced by physical factors is called "induced" lipolysis.

Mechanism of the Formation of Free Fat and Free Fatty Acids in Milk

The stability of milk fat globules is based on an energy barrier formed by equidirectional electric surface charges (93). The stability of disperse systems can

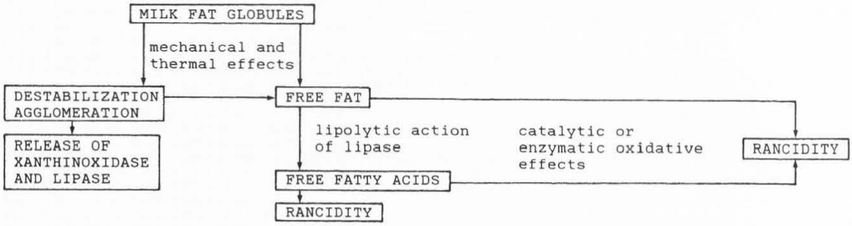

Fig. 1. Mechanism of changes in the milk fat.

be explained by the so-called DLOV theory. [The name DLOV is derived from the first letters of the names of the authors of the theory, i.e., Derjaguin, Landau, Overbeek and Verwey (91)]. Mechanical energy, e.g., as provided by foaming, can suppress the energy barrier. Under the effect of low energy, hydrate envelopes of milk fat globules are ruptured. At a higher energy, the protein and phospholipid layers of the milk fat globule membrane disintegrate and the fat globules form a uniform mass, i.e., free fat. Nordlund and Heikonen (71) discussed a theory on the formation of free fat during cooling of the milk. The theory postulates that due to radial solidification of the milk fat, low-melting, mostly non-solidified triglycerides are present in the core of the milk fat globules during cooling. The non-solidified fat occupies a larger volume than the same amount of solidified fat. Thus, the liquid glyceride part in the centre of the milk fat globules is subjected to a pressure caused by the inner stress of the molecules. Compressibility of liquid fat is low, and shifts in the crystal structures thus occur in the milk fat globules as well as in the solidified fat layers. This may lead to the destruction of the fat globule membranes, i.e., to the formation of free fat. Therefore, milk fat having a higher content of short-chain or unsaturated fatty acids is more sensitive to lipolytic changes.

Milk fat globule membranes rupture during the foaming of milk under the effect of an increased surface tension as the milk fat globules enter the boundary layer between air and milk. Thus, free fat is also formed by this process. The formation of free fat is a prerequisite for the hydrolysis of glycerides. Influenced by inherent or microbial lipases, lipolysis leads to the formation of free fatty acids in the milk (82-85).

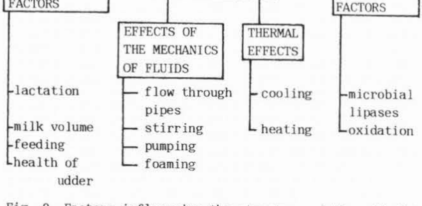

Fig. 2. Factors influencing the structure of the milk fat.

Influence of Physiological Factors on Milk Fat

Physiological factors affecting the milk fat comprise, in particular, feeding, stage of lactation, milk output, health of the udder, and exogenous factors. These factors contribute the most to the lipolysis of milk.

In the case of a reduced milk output, which may be due to the stage of lactation (87) as well as to feeding, the level of free fatty acids in the milk is increased (13, 41, 63). A particularly marked increase in the free fatty acids content occurs when the milk production is reduced to less than 3 kg of milk per milking (41). Feeding plays an important role in the stability of the milk fat. Nonruminant-related feeding causes changes in the protein fractions. This may be reflected by the composition of the milk fat globule membranes, which may be formed only partly or incompletely (20).

An excessive supply of feed energy (34, 66, 90) leads to a soft milk fat, which is subsequently subjected to lipolytic alterations during cooling and under mechanical effects. The lack of feed energy causes the formation of a weak milk fat globule membrane whereas an excessive supply of raw protein in the feed results in an increased incorporation of long-chain, unsaturated fatty acids in the milk fat. These latter fatty acids are formed due to the decomposition of the body fat of the cows (20, 23, 33, 43). In the case of an energy-deficient diet, the long-chain unsaturated fatty acids of the fatty tissues are digested more slowly than the short-chain saturated fatty acids that are used for the energy supply of the cow. A greater amount of long-chain fatty acids thus enters the udder. Formation of oleic acid is catalyzed in the udder by an enzyme called desaturase. Hence, an increased oleic acid content in the milk fat indicates a lack of energy in the feed ration (20). Associated with this effect of feeding is a changed composition of fatty acids and a softer consistency of the milk fat. According to the theory of Nordlund and Heikonen (71), the change in the fatty acid composition results in a stronger lipolytic sensitivity of the milk (3, 49, 54, 59). Generally, it has been observed that barn feeding has a more pronounced effect on the free fatty acid formation than pasteurization of the milk (12, 13). In this respect, the energy and protein contents of the rations are more important than the type of feed or the feeding practice (66).

Some enzymes are part of the milk. Lipoprotein lipase, which participates in the transfer of fat from the blood into the milk and catalyzes lipolytic reactions (12), is one of them. It is likely that greater quantities of this enzyme are secreted along with somatic cells into the milk in a diseased than in a healthy udder. However, Olivecrona et al. (73) assume that an inactive proenzyme existing in the milk is activated by substances existing in the somatic cells. Jellema (40) and Jellema and Schipper (41) reported a correlation between the number of somatic cells and lipolysis in milk.

Lipolytic problems are more frequent in dairy cattle herds producing medium cell counts in the milk (300,000 to 500,000 cells/mL) than in those producing low cell counts (<300,000 cells/mL). The higher counts were found in milk obtained by improper machine milking. Salin et al. (86) reported an additional increase in the free fatty acid values in milk with counts exceeding 500,000 cells/mL. Data obtained in their experiments *in vivo* are listed in Table 1. Effects of subclinical symptoms of mastitis on lipolysis were reported by Velitok (95), Ingr (36) and others.

Several authors also described the effects of exogenous factors such as activators and inhibitors on the activity of lipoprotein lipase. It was found that an addition of bovine blood serum (4, 41, 89), blood fractions containing α-lipoproteins, or blood constituents such as high-density lipoproteins, induced spontaneous lipolysis in milk. Activation of lipolytic processes in the milk by somatic cells may also be a consequence of the effect of activators (15).

Downey and Murphy (19) found an effect of glycomacropeptides on the formation of free fatty acids. Driessen and Stadhouders (21) detected the presence of another lipase in addition to lipoprotein lipase and reported that it is similar to lipase, which can be activated by biliary salts and which was found by Engelrud and Olivecrona (22) in human milk. Thus it may be deduced that the biliary salts also function as activators for some lipolytic reactions (26). Apart from the investigations indicating the presence of activators, it is reasonable to assume that lipolysis may also occur due to inactivation or absence of some specific inhibitors. Deeth and Fitz-Gerald (17) have established the existence of inhibitors of lipolysis in skim milk. These thermostable and dialyzable factors are present in the milk at varying concentrations and may affect the lipolytic sensitivity of various milk samples to some extent. Experimental findings by various authors on the effects of possible activators and inhibitors are reviewed in detail by Olivecrona (72).

Kuzdzal-Savoie et al. (62) suggested that grass feeding may have an inhibitory effect on lipolysis due to the presence of some specific substances present in the grass.

Table 1.

Effect of elevated somatic cell count on the free fatty acid content in milk according to Salin and Anderson (86)

Source* of milk:	Number of somatic cells per mL:	Free fatty acid content (mmol/100 g fat):
A	127,000	0.878
B	1,567,000	1.380
A	200,000	1.374
B	1,582,000	1.904
A	433,000	1.926
B	1,561,000	1.794
A	365,000	0.824
B	1,768,000	1.448

*A = Milk from two healthy udder quarters;
B = milk from two udder quarters of the same cows with induced increase in the cell counts.

Influence of Physical Factors on Milk Fat

During and after milking, milk is subjected to the effects of flow processes. Such processes probably affect the milk fat to some extent.

Laminar flow takes place in smooth pipes at low flow rates. Turbulent flow develops with the increase of the flow rate, due to rapid changes in direction of the flowing liquid, and in pipes having rough surfaces and edges (1, 44).

The nature of the flow is characterized by the Reynold's number. It is low for laminar flow until the so-called critical Reynold's number (R = 2300) is reached. Beyond it, the flow changes from laminar form into the turbulent form with a considerable increase in resistance (78). Back (8) and Back and Reuter (9) found that with a laminar flow (up to a maximum flow velocity of 2.03 m/s), fat globules in milk do not agglomerate. In experiments using a model apparatus, an increase in the free fatty acid concentrations and related defects in sensory attributes developed at flow velocities of 6 m/s in raw milk passed through the pipes for 15 min. Reuter (80) found similar changes in relationship to the friction shear stress τ above 4 kp/m^2. Reuter (79) estimated that flow processes in dairy farming are mostly turbulent. This is caused, e.g., by pipe bends, by flow through constricted pipes (at valves and taps), etc. (10). According to the equation of continuity (68):

$$A_1 x V_1 = A_2 x V_2,$$

where A_1 = sectional areas of the pipe,
A_2 = sectional areas of the constricted pipe,
V_1 = flow velocity in the pipe, and
V_2 = flow velocity in the constricted pipe,

the flow velocity and thus Reynold's number are increased in locations with reduced sectional areas of the tube (68). This may initiate lipolytic processes in the milk. Stirring can also cause changes in the structure of the milk fat and attention should be paid to its effect. When the agitator is off for longer periods of time, the milk is subjected to creaming, although the rising of fat may not exactly follow the Stokes equation (45). To avoid creaming, a continuous or occasional agitation of the milk is necessary. The agitation of milk depends on the extent of mixing and on the mixing turbulence. The extent of changes in the original milk fat globules depends on the energy flow per unit area existing in the turbulently flowing liquid, i.e., on the energy introduced into the liquid by stirring per unit volume and time (77).

In studies of the effects of pumping on milk fat, various effects of individual pumps were reported (45). This means that attention should be paid to the selection of the proper pumps and their appropriate use; displacement pumps should preferably be used.

When milk is transported through long smooth pipelines at flow velocities below 1.5 m/s, the free fat content is affected only to a very small extent. An increase in free fat by only 0.42% was reported (46). High flow velocities through constricted pipes (e.g., at samplers), however, led to an increase by almost 10%.

To maintain acceptable microbiological quality of the milk, efficient cooling is required which would restrict microbial growth in the milk as effectively as possible. In the past decades, considerable changes have been introduced into the way in which milk used to be cooled. Oldfashioned cooling by tap water was replaced by storage cooling (cooling tanks) in the sixties. As modernization of the industrial milk production processes has advanced, high-performance heat exchangers have been used to cool the milk on the farm. However, a rapid cooling also leads to lipolytic changes in the milk fat. Compared to the conditions prevailing several years ago, the microflora in the raw milk has also been changed. Earlier, lactic acid bacteria formed the base of the raw milk flora but now psychrotrophic bacteria predominate. These microorganisms are more lipolytic than lactic acid bacteria (75, 98). Lipases formed by the psychrotrophs can intensify lipolytic processes in milk (30, 75) in accordance with the mechanisms described by Kirst (47) and Nordlund and Heikonen (71).

In addition to flow-conditioned effects which lead to turbulence and to changes in the structure of the milk fat, an excessive absorption of air in the milk, i.e., foaming (39) also stimulates lipolysis. Foaming in milk can be caused by air absorption into the teat cups, leaking pipeline joints, different pipeline diameters, or large pipeline diameters through which only a small volume of milk is transported, and by idling of centrifugal pumps (continuous operation of the milk pump even in case of insufficient milk volume) (96, 97). Using model systems, Worstorff et al. (97) studied the effects of various gases used in the stirring of milk. Considerably higher concentrations of free fatty acids were produced in the milk in the presence of oxygen than in the presence of air or nitrogen. Aule and Worstorff (7) found that the free fat concentration in milk was increased 2.6-fold when air was present in the milk processing equipment; the free fatty acid concentration under the same conditions was increased 4.3-fold. These results were confirmed by Bakke et al. (10), who found a two-fold increase in the concentration of free fatty acids after air had entered the collector of a milking plant. Lipolysis can also take place due to foaming developed during transportation of the milk through elevated pipelines in the milk processing plant (29). In studies of leakage in milk-producing plants, the free fat concentration in milk decreased to 2.89% (which is approximately 20% of the share of total free fat with respect to the total fat content) after the leaks had been eliminated (51).

Influence of Biochemical Factors on Milk Fat

In general, microbial lipases are assumed to have effect on milk fat only if the total count of bacteria exceeds 10^6 cells/mL. However, Mabbitt (69) showed that severe sensory problems may develop in raw milk at total counts lower than 10^6/mL if psychrotrophic bacteria predominate. The most important producers of lipases are, e.g., *Pseudomonas fluorescens*, *P. fragi*, and *P. putrefaciens*, *Alcaligenes* sp., some yeasts and molds (14, 25, 28, 58).

Microbial lipolysis in milk may occur at the stage of primary treatment as well as afterwards. Lipolysis after the primary treatment is caused by contamination of already pasteurized milk during subsequent processing and storage, or by thermo-resistant lipases. Lipolysis in pasteurized milk usually causes more intense off-flavours, especially in products having high fat contents, than lipolysis which takes place during the primary treatment, although in each case, the milk may have the same content of free fatty acids (14). This depends on the specificity of the lipases and on the type of free fatty acids (e.g., short-chain fatty acids) which are thus produced. Furthermore, lipolysis in the finished milk product may also be influenced by environmental conditions, e.g., pH value, salt content, etc. Effective cleaning and disinfection in the processing plant as well as cooling of the milk to the required temperature to avoid the development of high bacterial counts of the raw milk reduce the risk of microbial lipolysis during the primary treatment of raw milk.

The effect of high microbial counts on the free fatty acids content in raw milk stored at 5°C is presented in Table 2.

Table 2.

Changes in the free fatty acid content during the storage of raw milk at 5°C as a function of total cell counts

Storage period (h):	Free fatty acid content (mmol/100 g fat)*	
	at total counts of 12,000/mL	at total counts of 3,500,000/mL
12 – 16	1.2	1.5
72	1.4	2.7

*In this study, the fat was obtained by churning the cream separated by skimming from the raw milk tested. The free fatty acid values were reduced by the passage of the free fatty acids into the skim milk as well as into the buttermilk. Consequently, the ascertained fatty acid content was lower than the value obtained by using the method of Deeth et al. (18).

Oxidative changes in the milk fat are indirectly connected with the lipolytic changes because catalytic-oxidative processes can principally occur with free fat as well as free fatty acids (Fig. 1).

Influence of Lipolytic Changes on the Quality of Milk and Milk Products

Lipolytic changes can affect sensory as well as processing properties of milk and milk products. The quality of the milk and milk products is affected, in particular, by the following factors:

- loss of fat due to "free", destabilized fat adhering to the walls of the containers, pipelines, etc. (76),
- decrease of skimming efficiency (increase in the residual fat content of the skimmed fresh milk) (29, 37, 55),
- losses due to increased fat content in buttermilk (6), and
- flavour of the milk and milk products (35, 38, 58, 61),

Poor quality of milk results in:

- low volume of whipped cream (29, 30, 42, 52),
- reduction of shelf life during storage of the milk products (62), and
- reduction in solubility, wettability, and flow characteristics of the dry milk powder (24, 50).

Apart from the common quality characteristics, however, some technological procedures require a defined minimum amount of free fat to be present in the milk in order to make possible basic processes such as the phase reversal (64). Riedel and Hansen (81) showed that high free fatty acid content in the milk powder used to produce chocolate is very important. As the free fat becomes part of the fat phase of chocolate, the viscosity of the chocolate mass is reduced; this makes it possible to limit the amount of the coconut oil used. In contrast, other industrial applications require an easily wettable milk powder which has a long shelf life and contains the fat in a "concealed" form.

Separability of the milk fat is influenced to a great extent by the size of the fat globules and by the content of free fatty acids. Mechanical effects are partly responsible for the destruction of the fat globules and for the subsequent difficulties encountered when separating them from the milk (37). For example, an increase in the free fatty acids content in raw milk by 0.91 mmol/L during separation leads to an increase in the fat content in the skimmed milk by 0.045% (55).

Table 3.
Threshold values at which single free fatty acids cause rancid flavour in pasteurized milk. According to Connolly et al. (16).

Fatty acid:	Number of C atoms in the molecule:	Concentration in milk	
		mg/kg	mmol/kg
butanoic	4	46.1	0.52
hexanoic	6	30.4	0.26
octanoic	8	22.5	0.16
decanoic	10	28.1	0.16
dodecanoic	12	29.7	0.15
tetradecanoic	14	80.5	0.35
hexadecanoic	16	244.5	0.96
octadecanoic	18	142.1	0.50
cis-9-octadecenoic	18*	221.1	0.78

*One C=C double bond.

Sensory attributes of milk and milk products are affected negatively by the presence of free fatty acids (11). However, low concentrations of free fatty acids contribute to the characteristic flavours of raw milk, cream, butter, and yoghurt (27, 94). Also in some cheese varieties, free fatty acids, which are formed during ripening, produce characteristic flavours (32). On the other hand, Jamotte (38) noted that off-flavours, which usually develop as the result of lipolytic reactions, can be described as impure, old, trickling, oily, pungent, bitter, and rancid. In general, such sensory defects may be noticed at a free fatty acid content exceeding 4 mmol/100 g fat, which corresponds to approximately 1.5 mmol/L of milk. Individual free fatty acids have different effects on the flavour; a low concentration of a particular fatty acid may affect the flavour more severely than a high concentration of another fatty acid. The off-flavours mentioned above are usually caused by short-chain fatty acids, i.e., butyric to lauric acids (62). According to Paulet et al. (74), soapy flavour is caused mainly by decanoic acid (capric acid) and dodecanoic acid (lauric acid). Connolly et al. (16) determined the threshold flavour values of individual fatty acids present in pasteurized milk (Table 3).

Atramentov et al. (5) found that during the separation of fat, about 90% of the free fatty acids passed into the cream and only about 10% passed into the skim milk. Consequently, the quality of butter was influenced by the raw milk to a great extent (38). According to Černá (13), a free fatty acid content of 1.4 to 1.8 mmol/100 g of fat in raw milk can lead to the production of second-grade butter. A similar finding was made by Jamotte (38). To inhibit lipolysis, the latter author recommended to heat the cream before its storage.

Even in milk products having a low fat content, high concentrations of free fatty acids can have detrimental effects on the sensory attributes. Supported by the result of factor analysis, Sonntag (92) showed that the concentration of free fatty acids in raw milk has the most important influence on the quality of pasteurized milk.

Effects of Energy-Restricted Feeding on Milk Fat

Feed rations, which allow farmers to reduce the use of feed concentrates, have been used extensively at present in various countries. In addition, the crude protein content in grass and in grass silage has been increased world-wide. This means that dairy cows are fed partly energy-deficient yet high-protein rations. To examine the consequences of the changes in the feeding practice, the effects of energy-deficient, high-protein rations on milk fat were studied by the author of this review.

The experiments were conducted using one group of cows in each the first and second thirds of lactation (Groups 1 and 2) and a group of 6 cows in the last third of lactation (57, 70) (Group 3). While the cows in Group 1 were in the dry phase, they were fed in accordance

Table 4.
Effect of energy-deficient feeding of cows in the last third of lactation (Group 3) on the fat and fatty acid contents in milk

Feeding period:	Energy content of the ration (% of standard):	Crude protein content of the ration (% of standard):	Fat content in the milk (%):	Free fatty acids content in milk fat	
				(%):	(mmol/100 g)
1 (day 201-230)	140	135	4.3	10.6	2.5
2 (day 231-249)	171	131	5.7	20.9	2.8
			4.8	16.1	2.6
			3.9	21.6	2.7
3 (day 250-267)	40	64	4.1	14.1	2.6
			4.1	12.8	2.4
4 (day 268-281)	62	103	4.0	13.7	2.2
			3.6	16.5	2.9
5 (day 282-295)	152	126	4.7	13.7	2.4

with the feeding standards suggested by the Department of Agriculture of the German Democratic Republic (88). Cows in Group 2 were fed energy-deficient rations at the end of the dry phase. Milk obtained from the 6 cows in the last third of lactation was tested weekly, whereas milk from the other two groups of cows was tested once a month. The free fat content was determined according to the method of Lagoni and Peters (65) and the free fatty acid content was determined by the procedure of Deeth et al. (18). The suitability of these analytical methods for the given purpose was confirmed by our own tests (56). In the examination of cows in the last third of lactation (Table 4), the increase in the fat content in the milk is clearly evident immediately following the administration of energy-deficient rations and may be explained by the degradation of body fat (fat mobilization syndrome). Later, the fat content in the milk is decreased. Its concentration rapidly recovers during re-alimentation (5th feeding period). The onset of energy-deficient feeding in each period induces a temporary increase in the free fat content in the milk but is followed by a decrease and stabilization of the free fat concentration at the lower level. Return to a standard diet (re-alimentation) results in the recovery of the free fat concentration. The free fatty acid content in the milk remains approximately constant with cows in the late stages of lactation.

A marked effect of feeding on the free fatty acid content, however, can be determined at the beginning of lactation. At that time, the fat content of the milk is severely reduced (Table 5). In both groups of cows, the effect of the previous energy-deficient diet on the fat

Table 6.
Passage of free fatty acids from raw milk into cream*

No.:	Free fatty acids in mmol in:			Passage into cream (%):
	1 L of raw milk	1 L of cream	cream from 1 L of raw milk	
1	1.31	8.96	0.640	48.8
2	1.36	8.16	0.646	47.5
3	1.22	6.75	0.541	44.4
4	1.34	8.16	0.637	47.6
5	1.29	7.20	0.540	41.9
6	1.38	8.46	0.648	47.0
7	1.30	8.67	0.637	49.0
8	1.40	8.00	0.600	42.9
9	1.37	5.00	0.668	48.8
10	1.40	7.21	0.834	59.6
11	1.33	4.80	0.737	55.4
12	1.37	7.65	0.815	59.5
13	1.33	8.12	0.824	62.0
14	1.40	7.50	0.740	52.8
15	1.49	11.44	1.066	71.5
16	1.44	13.18	1.263	87.7
17	1.41	10.62	0.947	67.2
18	1.46	10.00	1.041	71.3
19	1.42	10.92	1.012	71.3
20	1.38	11.71	0.938	68.0

*Fat content approximately 50%.

Table 5.
Effect of energy-deficient feeding of cows in the first and second thirds of lactation on the fat and fatty acid contents in the milk

Feeding period: (duration in weeks):	Energy content of the ration (%):	Crude protein content of the ration (%):	Fat content in milk (%):	Contents of	
				free fat (% of total fat):	free fatty acids (mmol/100 g fat):
Group 1					
1 (4 w of energy-deficient feeding)	reduced 30-50%	reduced 30-50%	3.6	20.0	3.0
			3.8	20.4	4.8
2 (8 w of realimentation)	raised 30%	raised 30-50%	2.0	14.6	4.0
			2.0	18.0	5.2
			2.5	18.6	4.6
3 (5 w)	reduced 30%	standard	3.0	14.6	3.6
4 (3 w)	raised 30%	raised 30%	2.8	15.6	6.2
5 (5 w)	standard	raised 30%	3.0	11.4	3.0
6 (4 w)	reduced 30%	standard	4.0	11.0	3.0
Group 2					
1 (2 w*)	reduced >50%	reduced >50%	5.0	13.6	2.7
			3.0	19.2	4.4
2 (8 w energy-deficient feeding)	raised 30%	standard	2.9	17.8	2.6
			1.9	12.8	4.5
			2.8	17.2	4.5
3 (5 w realimentation)	standard	raised 30%	2.3	12.0	6.6
4 (3 w)	raised 30%	raised 30%	2.8	12.4	7.0
5 (4 w)	reduced 30%	standard	3.2	14.0	2.6
6 (5 w)	standard	raised 30%	3.6	11.2	2.3

*From 2 weeks before the beginning of lactation until the 4th week of lactation.

content and the content of free fatty acids is extended even following realimentation (2nd feeding period). This is shown also when the feeding patterns are varied (3rd to 6th periods). Under the condition of the energy-deficient diet beginning as early as in the dry period and by severe underfeeding during the first lactation period, a marked effect of feeding on milk fat is found with the second group of cows. Pooled milk from these cows contained a maximum of 7.0 mmol free fatty acids per 100 g of fat and the maximum content of free fatty acids in the pooled milk obtained from the first group of cows was 6.2 mmol/100 g of fat. The fat contents were reduced to 2.0% and 1.9%, respectively. The effect of feeding appears to be even stronger on the milk from individual cows. In both groups, the increase in the fat content and the reduction of the share of free fat as well as of the content of free fatty acids is particularly obvious in the last two feeding periods, during which the cows were fed in accordance with the feeding standards.

Passage of Free Fatty Acids into Cream and Butter

It was found that in skim milk, the free fatty acid content was higher than expected (5). For this reason, the passage of free fatty acids from raw whole milk into skim milk and cream and from cream into butter was examined. Free fatty acids in milk and cream were determined according to Deeth et al. (18). In butter, the free fatty acid content was titrated after the butter had been dissolved in a mixture of ethanol and diethyl ether (1:1). Approximately 55% of the free fatty acids passed into cream obtained from milk in two milk-collecting districts (Table 6). To check these results, a comparative examination was carried out using raw milk before it entered the separator and then using the corresponding skim milk and cream after separation (Table 7). The results show a good agreement between the amount of the free fatty acids in the raw milk and the totals of the free fatty acids in skim milk and in cream.

The passage of free fatty acids from cream into butter is shown in Table 8. In these examinations, a technology based on the phase reversal without separation of buttermilk (samples 1-10) and a foam-churning process (samples 11-22) have been compared with each other. The results show that when the butter serum is separated, approximately 0.1 mmol free fatty acids, related to 100 g butterfat, passes into this serum. A comparison of the mean difference between the free fatty acid content of the cream and that of the butter in the foam-churning process with the mean difference obtained by the churning process without separating the buttermilk indicates that 0.15 mmol of free fatty acids, related to 100 g of butterfat, passed into the buttermilk. The buttermilk could not be examined for comparison because the method according to Deeth et al. (18) cannot be applied to the determination of free fatty acids in sour products. Lactic acid (and probably also citric acid) is extracted in this method. Therefore, it is necessary to carry out additional tests in order to find the proportions of free fatty acids originating from raw milk and those passing into the skimmed milk.

Table 7.
Comparison of the passage of free fatty acids into skim milk and cream

No.	Concentration of free fatty acids (mmol/L)			Sum of free fatty acids in cream and skim milk
	in raw milk	in cream	in skim milk	
1	1.37	0.605	0.668	1.273
2	1.40	0.528	0.834	1.362
3	1.33	0.590	0.737	1.327
4	1.37	0.568	0.815	1.383
5	1.33	0.516	0.824	1.340
6	1.40	0.566	0.740	1.306

Table 8.
Passage of free fatty acids from cream into butter

No.:	Free fatty acids (mmol/100 g fat)		
	in cream	in butter	difference
1	1.8	1.6	0.2
2	1.7	1.6	0.1
3	1.6	1.6	0.0
4	1.5	1.4	0.1
5	1.7	1.5	0.2
6	1.4	1.2	0.2
7	1.3	1.4	-0.1
8	1.7	1.6	0.1
9	1.2	1.1	0.1
10	1.3	1.3	0.0
11	2.6	2.2	0.4
12	2.1	1.9	0.2
13	2.5	2.3	0.2
14	2.3	2.6	-0.3
15	1.6	1.7	-0.1
16	2.1	1.8	0.3
17	2.1	1.6	0.5
18	1.9	1.5	0.4
19	2.3	1.8	0.5
20	2.0	1.6	0.4
21	2.0	1.9	0.1
22	2.3	1.9	0.4

Conclusions

Although an inquiry by the International Dairy Federation has indicated that lipolysis is not a major problem in most countries (2), the effects of lipolytic changes on the quality of milk products must not be underrated. Special attention should be paid to lipolytic changes in the fat of raw milk caused by physiological factors, in particular by feeding. To reduce lipolytic processes in milk, special attention should be paid to:

- ruminant-related feeding practices for cows,
- restriction of other physiological factors,
- minimization of mechanical and thermal effects,
- prevention of foaming in the milk, and
- minimization of the growth of psychrotropic bacteria in milk.

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Discussion with Reviewers

D. E. Carpenter: The author refers to protection by the milk fat globule membranes of the fat from lipolytic and oxidative processes. Is there any evidence that hydrolysis of the membrane protein by natural milk proteases or psychrotrophic milk proteases gives rise to higher levels of free fatty acids?

Author: The effect of hydrolysis of the milk fat globule membrane proteins by microbial and milk proteases was studied by several authors (28, 99, 100). Disruption of the fat globule membrane by proteases exposes the fat globules to the action of lipase, particularly at high counts of psychrotrophic bacteria. Thus, the increase in the free fatty acid concentration is the result of lipolytic action on fat globules destabilized by proteases. To prevent this from happening, it is important to keep the psychrotrophic bacteria counts in milk as low as possible.

S. Saito: Homogenization does not produce free fat but is the most effective treatment to induce lipolysis. Will the author please give his opinion on the effect of homogenization on the microstructure of fat globules?

Author: Homogenization results in the disintegration of the original (large) fat globules in milk and in the production of a great number of smaller fat globules. Consequently, also the fat globule membranes are disintegrated and lipase is released. As the total surface of the fat globules is increased 5 to 6 fold (101), the newly formed fat surface is rapidly coated with surface-active material such as the fragments of the fat globule membranes, casein, and undenatured whey proteins from the milk serum. Liberated lipase participates in lipolysis. Lipolysis may be induced by homogenization only in raw milk but not in pasteurized or UHT-treated milk.

Z. Saito: The effect of energy-deficient feeding on the fat content in milk and the proportion of fatty acids (Table 4) seems to be small. Is it statistically significant?

Author: Statistical tests were carried out by analysis of variance (ANOVA) [to study the interactions between the lactation stages and feeding] and by a multiple comparison of the mean values using a Newman-Keuls Test (102).

The increase in the milk fat content at the beginning of the energy-deficient feeding was found to be statistically significant ($P < 0.05$). This effect is called 'the fat-mobilization syndrome' (103). In the subsequent period, the fat content in the milk decreased; this decrease was also significant. However, the increases in the free fat content at the beginning of each feeding period were statistically not significant.

Additional References

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