DSC of Milk Fats from Various Animals with High Levels of Medium-Chain, Unsaturated and Polyunsaturated Fatty Acids

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ABSTRACT

Mammals of different species provide milk lipids with a wide variety of fatty acid composition yet with common stereospecific features. This allows the investigation of crystallographic properties of milk lipids that cannot be achieved by interesterified lipids due to the random stereospecific distribution that is obtained. The milk fats of elephant and white rhinoceros contain high amounts of 8:0, 10:0 and 12:0 which form triglyceride species that melt between 8 and 22 °C. The crystallographic behaviour of the milk lipids from blesbok and blue wildebeest differ from the other ruminant lipids, and that of horse and vervet monkey differ from the other non-ruminant lipids. It seems that a low content of 18:0 and 18:1, and a high content of saturated short- to medium-length fatty acids prevent the formation of the high and low temperature melting isotherms, between 35 and 42 °C, and between –45 and –10 °C, respectively, which are normally observed for milk fats.

KEY WORDS

Triglyceride, differential scanning calorimetry, milk, medium-chain fatty acid, polyunsaturated fatty acid.

1. Introduction

The fatty acid composition of milk fats varies widely among mammals. A phylogenetic relationship has been noted for the preference of which lipid synthesis mechanisms are employed.[1] The milk fats of ruminants are characterized by the presence of up to 15 % short-chain fatty acids (4:0 and 6:0), appreciable amounts of medium chain length fatty acids but relatively low levels of polyunsaturated ones. Some non-ruminant herbivores, such as the horse and rhinoceros, have extraordinarily high contents of medium-chain fatty acids (8:0–12:0), while in elephant (Proboscidae) milk the contents may reach amounts of up to 90 %,[2] the composition of which may change drastically over lactation time[3]. Synthesis of medium-chain fatty acids is also a property of the primates and specific phylogenetic relationships are found.[4,5]

Triacylglycerides in general do not only differ in fatty acid composition, but also in their stereospecific arrangement on the three sn-positions of the glycerol.[6] The distribution in animal fats is also different from that of vegetable fats. Due to the chemical methods for the determination of this arrangement being complex, milk fats of only a few animals have been studied. While the overall fatty acid composition amongst mammalian milk fats may differ, the stereospecific arrangement shows common features. The 14:0 and 16:0 acids are concentrated in position sn-2, and 18:0 in position sn-1. Unsaturated fatty acids are mainly found in positions sn-1 and sn-3. Of the short-chain fatty acids in ruminant milk, 4:0 and 6:0 are found entirely at sn-3, while 8:0 is also found mainly at this position. As the chain length increases, the acids are to be found in positions sn-2 and sn-1. While the stereospecific distribution sets some limits to the amount of different possible molecular triglyceride species that can be formed, a large number is still possible. In cow's milk 81 different molecular species have been identified[7] and in goat's milk 137.[8] The number of molecules is actually higher, as isomers have not been taken into account. In human milk only 30 triglyceride species have been identified,[9,10] however, the analytical methods used to obtain this low number were different from those used for cow's and goat's milk. Nevertheless, a high degree of similarity is observed for the triacylglyceride molecular species found in the milk fat of all three these mammals, however, the amounts differ between the ruminant species and human which can be related to the fatty acid composition.

Simple fats with single type or limited types of fatty acids regularly form β-type crystals, the most stable form, or they may be transformed to this from the less stable β′- and least stable α-type crystals.[11,12] In complex fats, such as milk fats, the combination of different triglyceride molecule species leads to complicated crystallization patterns. Polymorphism during crystallization is observed, i.e. more than one crystal type is formed due to different patterns of molecular packaging in the crystal.

The crystallization and melting of fat from cow's milk has been, and still is, studied in great detail.[11,13,14] Due to the large number of triglyceride molecule species present, the crystallization is very complex, resulting in the formation of compound crystals. It is not possible to relate specific molecules to the formation of these crystals, however, three major crystal types have been identified in milk fats. These are α2L crystals which consist of certain triglycerides that contain 18:0, and $\alpha 3L_2$ crystals of 18:1 containing fats, and a mixture of α- and β′ crystals.[15] Upon melting, these crystals are transformed to more stable crystal types, specifically β′2L crystals of 18:0 containing molecules and

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β′3L structure of 18:1 containing fats[16].

With nutritional intake, milk fat composition may be altered, which may affect the crystallization properties.[17–19] Such alteration in cow's milk is only limited, due to the effect of the ruminant bacteria controlling the fatty acid types that are eventually absorbed. To investigate the effect of more extensive changes in fatty acid on milk fat crystallization, than is possible with dietary changes, other species as well as non-ruminant species may be studied. Apart from the commercially exploited mammals, we were only able to locate work on crystallization of camel (*Camelus dromedarius*) milk fat.[20] It was found that the camel milk fat contained higher amounts of high melting triacylglycerides and lower amounts of lipids that melt in the medium range. It was ascribed to a low content of short and medium-chain fatty acids and higher levels of 16:0.

The aim of the current work was to compare the melting and crystallization behaviour of milk fats consisting of high levels of medium-chain, unsaturated and polyunsaturated fatty acids, as may be obtained from milk fats from dairy and non-dairy animals of different taxa, and relate it to fatty acid composition by differential scanning calorimetry (DSC).

2. Materials and Methods

The origin and drawing of milk of the animals under study have been described in earlier studies. Milk was obtained from African elephant (*Loxodonta africana africana*),[2,3] white rhinoceros (*Ceratotherium simum*),[21] vervet monkey (*Chlorocebus pygerythrus*),[5] cheetah (*Acinonyx jubatus*),[22] serval (*Felis serval*),[23] sable antelope (*Hippotragus niger*), goat (*Capra hircus*) and sheep (*Ovis aries*),[24] cow (*Bos taurus*),[25] blesbok (*Damaliscus dorcas phillipsi*) and blue wildebeest (*Connochaetes taurinus taurinus*).[26] Milk from pig (*Sus scrofa*) and horse (*Equus caballo*) was obtained from farms in the Bloemfontein area, human milk from a volunteer donor at the National Hospital in Bloemfontein, bottle nose dolphin (*Tursiops truncates*) from the Zoo-Aquarium de Madrid, pudu (*Pudu pudu*) and okapi (*Okapi okapi*) from the Bristol Zoo Gardens, and scimitar oryx from the National Zoological Gardens in Pretoria. Milk was collected from eland and oryx during culling operations for meat production respectively on a game farm 50 km west of Bloemhof, North West Province, and Vaalpan Ballistic Test Range, Copperton, South Africa. Drawing, handling and storage of milk from culled animals were described by Osthoff *et al.*[25]

Extraction of total fat from milk samples was performed quantitatively according to Folch *et al.*[27] using chloroform and methanol in a ratio of 2:1. Fatty acids were transesterified to form methyl esters (FAME) using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol.[28] The FAME were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness). The column temperature was 40–230 °C (hold 2 min; ramp 4 °C min^{-1}; hold 10 min). The FAME in hexane (1 μl) was injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen was used as the carrier gas at 45 psi and nitrogen was the makeup gas. Chromatograms were recorded using Varian Star Chromatography Software. Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples with those of standards of all 37 fatty acids obtained from Supelco (Supelco 37 Component Fame Mix 47885-U).

Differential scanning calorimetry was performed on a Mettler Toledo DSC 822e/700 utilizing sample sizes of approximately 8 mg in a 40 microlitre aluminium crucible that was hermitically sealed. Experiments in triplicate were performed under a nitrogen atmosphere utilizing a nitrogen flow rate of 3.5 cm^3 min^{-1}. The crystallization history was destroyed at 80 °C for 1 min, followed by cooling to –50 °C at a cooling rate of 10 °C min^{-1}. Thereafter, the temperature was cycled three times from –50 °C to 85 °C and back with an isothermal break of 1 minute between each temperature apex. Finally the temperature was returned to 25 °C. DSC thermograms of the first cooling and second heating cycle are presented. Tempering of fat was accomplished by destroying the crystallization history followed by one cooling and heating cycle, after which the fats were kept at 22 °C or 4 °C for one day or two weeks. Only one heating and cooling rate was chosen, that is used fairly universally.[29–31]

3. Results

Although more than one milk sample was available for most of the animals other than the African elephant, only one from each species is presented here for the comparative study between fatty acid composition and melting and setting properties. This was done because drastic differences in melting and setting properties are caused only when the fats differ substantially in fatty acid composition, as was observed for the elephant milk fats (Table 1 and Fig. 1). This is not the case with small differences in fatty acid composition, as for ruminant milks (Table 2 and Figs 2 and 3), and was also reported in a recent study of adipose fat of Nile crocodiles.[31] The aim was to study the melting and setting properties of milk fats of different fatty acid composition, rather than an interspecies comparison. The animal names are therefore merely used as means of identification rather than describing phylogenetic relationships between species. Comparisons of enthalpy were not attempted. Comparisons were based on the positions of peaks at respective temperatures. The thermograms of replicates differed very little, not meriting reporting and discussion.

3.1. Fatty Acid Composition

The fatty acid compositions of milk fats from six African elephants at different stages of lactation (A–F) and one white rhinoceros (G) are listed in Table 1 according to decreasing amounts of 10:0. They are distinguished from other milk fats by the high content of medium-chain fatty acids, more specific 8:0, 10:0 and 12:0, which are responsible for levels of total saturated fatty acids of above 90 %. It has been shown previously that the 10:0 content of elephant milk increases during the progression of lactation,[3] which is probably also the case in the white rhinoceros[21].

The fatty acid compositions of milk fats from all the other species under study are shown in Table 2. The fatty acid composition of the milk fats of the other mammals studied differ from the above by having much less 8:0, 10:0 and 12:0, more 14:0 and 18:0, a 16:0 content above 18 % and a 18:1 content above 15 % and high amounts of short to medium chain length fatty acids, which is in agreement with the literature.[1] The only two exceptions are the milk fats from blesbok and blue wildebeest which have the long-chain fatty acids replaced by short to medium-chain acids.[26]

Based on the fatty acid composition, these fats can be grouped into two larger groups; the ruminants and non-ruminants. The ruminant milk fats are distinguished by the presence of short fatty acids, higher amounts of 14:0 and 18:0, lower amounts of 18:2 and 18:3 and very little long-chain polyunsaturated fatty acids. Within this group, the blesbok and blue wildebeest milk fats are distinguished by containing more than 12, 6 and 18 % of, respectively, 10:0, 12:0 and 14:0, and less than 7 and 11 % of 18:0 and 18:1.

Within the non-ruminants, greater differences are observed.

Table 1 Fatty acid composition of milk fats from African elephant (*Loxodonta africana africana*) (A–F) and white rhinoceros (*Ceratotherium simum*) (G).

FAME (% of total fatty acids)		A	B	C	D	E	F	G
Caprylic	8:0	9.1	12.7	8.3	5	4	3.2	3.1
Capric	10:0	62.1	63.6	54.8	48	45.6	36.5	26.6
Hendecanoic	11:0	0.9	1.3	0.9	0.9	0.9	1.3	ND
Lauric	12:0	18.9	16.0	18.7	23.6	27.8	23.7	17.2
Tridecoic	13:0	ND	ND	ND	ND	0.24	0.3	ND
Myristic	14:0	1.3	0.9	1.9	2.6	3.5	4.5	10
Pentadecylic	15:0	ND	ND	0.2	ND	0.2	0.2	0.4
Palmitic	16:0	2.9	2.3	6.0	6.4	6.2	11.8	17.1
Palmitoleic	16:1c9	ND	ND	0.3	ND	0.3	1.3	1.2
Margaric	17:0	ND	ND	0.4	ND	0.4	0.4	0.5
Heptadecenoic	17:1c10	0.6	ND	0.1	0.6	ND	0.3	ND
Stearic acid	18:0	1.5	0.4	1.4	1.6	1.3	1.4	9.2
Oleic	18:1c9	2.7	2.4	6.0	10.2	7.3	12.4	8.9
Vaccenic	18:1c7	ND	ND	ND	ND	ND	0.9	ND
Linoleic	18:2c9,12 (n-6)	0.4	0.2	0.8	0.6	1	1.2	3.9
α-Linolenic	18:3c9,12,15 (n-3)	ND	0.2	0.8	0.8	1.3	0.7	2.6
Eicosenoic	20:1c11	ND	ND	ND	ND	ND	0.2	ND
Eicosadienoic	20:2c11,14 (n-6)	ND	ND	ND	ND	0.1	0.1	ND
Eicosatrienoic	20:3c11,14,17 (n-3)	ND	ND	ND	ND	ND	0.1	ND
Eicosatrienoic	20:3c8,11,14 (n-6)	ND	ND	0.1	ND	0.2	ND	ND
Arachidonic	20:4c5,8,11,14 (n-6)	ND	ND	ND	ND	ND	0.1	ND
Nervonic	24:1c15	ND	ND	ND	ND	ND	ND	0.8
Total saturated fatty acids	96.7	97.6	92.0	87.8	89.7	83.6	83.3	
Total monounsaturated fatty acids	3.3	2.4	6.3	10.8	7.6	14.2	10.9	
Total polyunsaturated fatty acids	0.4	0.4	1.7	1.4	2.7	2.2	6.5	
Total omega-3 fatty acids	ND	0.2	0.8	0.8	1.3	0.8	2.6	
Total omega-6 fatty acids	0.4	0.2	0.8	0.6	1.3	1.4	3.9	

ND = not detected

The vervet monkey milk fat is the only one with high amounts of 8 and 10:0, that of the horse with high amounts of 10:0, 12:0, 14:0, while that of the bottle nosed dolphin contains high amounts of 16:1, 20:1 and polyunsaturated isomers of 20 and 22 carbon length.

3.2. Differential Scanning Calorimetry

The thermograms of the milk fats from African elephants (A–F) and white rhinoceros (G) are shown in Fig. 1. They display very sharp temperature ranges for both setting and melting. This may be ascribed to the limited number of fatty acid types in the composition, and consequently limited numbers of triglyceride molecular species. None of the fats displayed transformation to other crystal forms during tempering at either 22 °C or 4 °C for up to two weeks.

The setting isotherms of the other milk fats under study are shown in Fig. 2. When the setting isotherms are compared, the milk fats can broadly be grouped into a group with high temperature isotherms between 22 and 15 °C followed by a setting isotherm from 15 to as low as –40 °C. These properties are displayed by the fats of the ruminants. Only the setting isotherms of milk fats from lechwe, blesbok and blue wildebeest do not display the high temperature setting isotherm.

The second group of setting isotherms, of non-ruminant milk fats, display a small high temperature peak between 12 and 4 °C followed by a large peak between 4 and –40 °C or even lower. Only the milk fat from vervet monkey does not display the peak at 12 to 4 °C; instead setting is initiated at 2 °C with a large peak between –10 and –34 °C and a peak maximum at –14 °C.

The melting isotherms of the milk fats under study other than from African elephant and white rhinoceros are shown in Fig. 3. Grouping the melting isotherms is complex. However, if isotherms after tempering are inspected, those of the ruminant milk fats are very similar and differ completely from that of the non-ruminants. The ruminant milk fats display a high temperature melting isotherm between 30 and 42 °C and a large melting isotherm between –10 and 26 °C. One or more peaks may be displayed in the latter, the most prominent at approximately 18 °C (Fig. 3). The peak of high temperature melting isotherm between 30 and 42 °C of the lechwe milk fat is very small. The isotherms of the milk fats from the blesbok and blue wildebeest do not display the high temperature melting peak between 30 and 42 °C after tempering, but resemble that of the horse milk fat, with one single broad peak between –10 and approximately 30 °C.

The melting isotherms of milk fats from all the non-ruminants, except vervet monkey and horse, display a small peak of a high melting temperature isotherm between 32 and 42 °C, similar to that of the non-ruminant fats, only after tempering for either one day or two weeks at 4 °C. They also display low temperature melting isotherms from as low as –45 °C, best visible for the milk fats of bottle nosed dolphin, serval and pig.

4. Discussion

The elephant milk fats A and B may be distinguished from fats C–G in containing above 60 % 10:0, approximately 10 % 8:0, less than 20 % of 12:0 and 14:0 combined, and also low amounts of 18:1, 2.7 % and 2.4 %, respectively. Fats A and B also display the lowest onset of setting temperature of –22 °C and peak at –25 °C. This is in accordance with these fats containing the highest number of short-chain fatty acids. The melting thermograms of these two fats show a very small exothermic peak at approximately –13 °C, and an endothermic peak at approximately 2 °C, visible as a shoulder to the larger peak (best visible for fat B), with

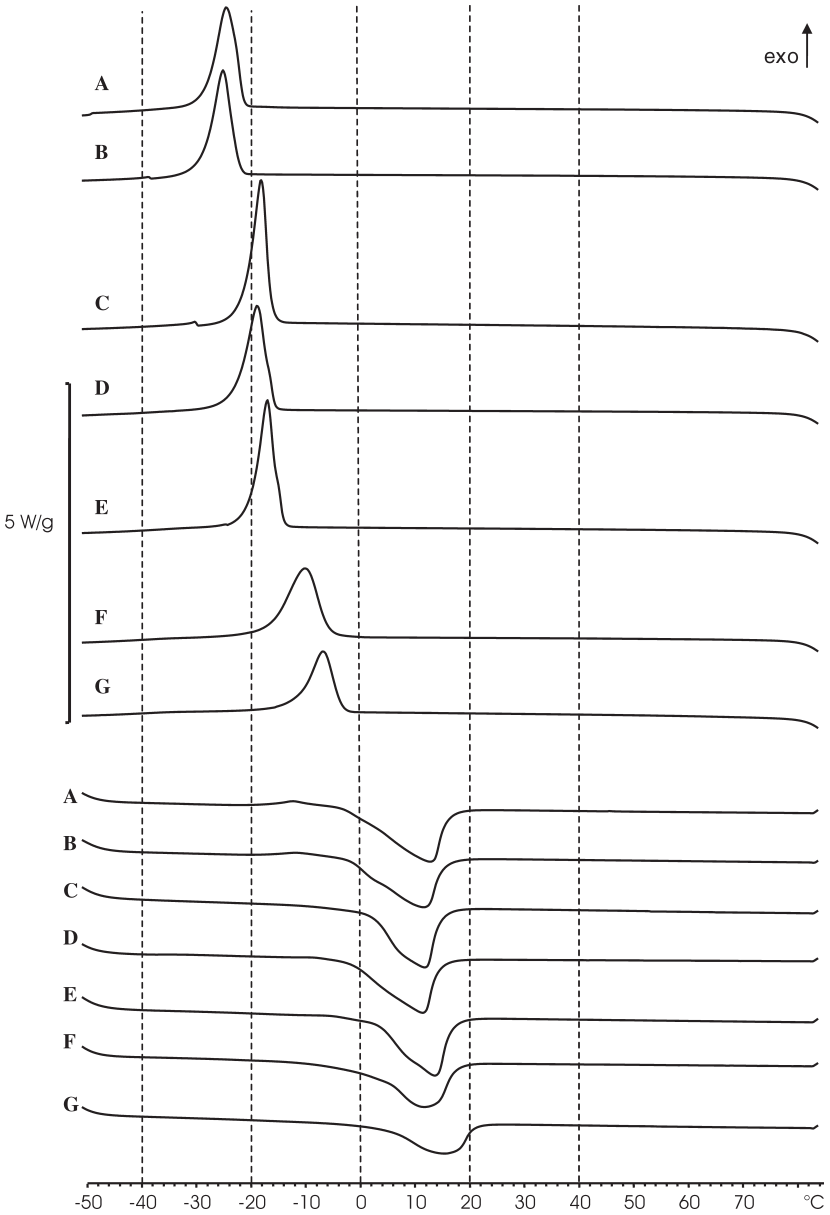

Figure 1 Differential scanning calorimetry profiles of milk fats from African elephant (*Loxodonta africana africana*) (A–F) and white rhinoceros (*Ceratotherium simum*) (G). Profiles at top are of cooling to –50 °C and bottom of melting to 85 °C at a rate of 10 °C min^{-1}.

a peak maximum at 11–13 °C. According to Hagemann[12] an exothermic peak would indicate a polymorphic transition in crystal structure. Since this exothermic peak is very small, and specific data on crystal structure, as may be observed with X-ray diffraction spectrometry, is not available, further discussion is not possible. The closest match with a specific triacylglyceride that displays these temperatures would be that of 18:1-10-10 (3-oleoyl didecanoglyceride), with a melting point of 4 °C for β-type crystals.[12] Based on the stereospecific arrangement of fatty acids in milk fats found by Christie[6] as well as mathematical probability, a small number of 18:1-10-10 molecules may exist. The fact that the large endothermic peak is divided in a shoulder at approximately 2 °C and a larger peak at 11–13 °C may indicate that two separate groups of compound crystals are present.

The elephant milk fats C, D and E are distinct from the others. With approximately 50 % 10:0 (respectively 54.8, 48.0 and 45.6 %) the fatty acids responsible for the greatest differences to fats A and B, and F and G are respectively 18.7, 23.6 and 27.8 % 12:0 and 6.0, 10.2 and 7.3 % 18:1. The thermograms of both fats show higher setting point peaks of –17 °C. The melting peak may be divided into a peak at 12 °C which contains a smaller shoulder peak at approximately 8 °C, best visible for fat C, which is an indication that two separate groups of compound crystals may be present. Despite the differences of up to 10 % of the abovementioned major fatty acids, the thermograms of fats C, D and E do not differ much. The difference in thermograms between fats A and B, and C, D and E are therefore caused by different triacylglyceride molecular species due to the lower 10:0 content, which is more than 10 % lower in the latter two, as well as the 8:0 content, which is almost halved, while the 12:0, 14:0 and 16:0 collectively have increased. This is in agreement with the results of Hagemann[12] that the melting points of compound triacylglycerides increase with an increase in fatty acid length.

The fatty acid composition of the elephant milk fat F and the white rhinoceros G contain half to a third of 10:0, and less 8:0 than fats A and B and much less than C–E, with a consequent increase in the other fatty acid content, especially 14:0 and 16:0, and 18:0, and a large increase of 18:2 in fat G. A more diverse group of triacylglycerides is present resulting in the broader

Table 2 Fatty acid composition of milk fats from dairy and non-dairy animals.

FAME (% of total fatty acids)		Okapi	Pudu	Eland	Cow	Oryx	Scimitar oryx	Sheep	Lechwe	Goat	Sable antelope	Blesbok	Blue wildebeest	Horse	Serval	Cheetah	Pig	Human	Bottlenosed dolphin	Vervet
Butyric	4:0	0.3	1.3	0.8	0.9	0.8	0.8	0.4	1.3	0.4	0.5	1.0	0.5	ND	ND	ND	ND	ND	ND	ND
Caproic	6:0	0.7	0.7	0.6	1.4	1.4	0.8	0.7	1.0	0.8	1.2	1.6	1.9	ND	ND	ND	ND	ND	ND	ND
Caprylic	8:0	1.1	0.5	0.2	1.2	1.5	0.9	0.7	1.0	1.2	2.0	3.1	5.0	1.1	ND	ND	ND	ND	ND	5.8
Capric	10:0	4.9	1.0	0.6	3.3	6.0	3.4	3.3	1.5	4.1	8.7	12.2	21.0	3.4	ND	ND	ND	0.6	ND	9.1
Lauric	12:0	0.7	1.6	0.9	4.1	2.9	2.0	2.1	1.8	1.7	3.1	6.0	10.6	3.5	ND	0.1	ND	3.3	ND	2.5
Tridecoic	13:0	0.1	ND	ND	ND	ND	ND	ND	ND	ND	0.1	0.2	ND	ND	ND	ND	ND	ND	ND	ND
Myristic	14:0	8.7	8.8	5.7	13.8	15.2	10.8	8.1	4.8	5.3	12.2	18.4	23.0	5.1	0.6	3.0	0.7	4.2	6.6	1.4
Myristoleic	14:1c9	0.2	0.2	0.4	0.7	0.3	0.2	0.4	0.1	ND	0.2	0.5	0.5	0.1	ND	0.2	ND	ND	0.2	ND
Pentadecylic	15:0	1.6	1.0	2.2	1.4	1.1	0.6	1.1	0.5	0.4	1.3	1.0	1.0	0.5	0.2	0.3	ND	ND	0.5	ND
Palmitic	16:0	28.0	40.6	22.1	35.0	42.1	32.7	27.1	22.2	23.2	23.3	31.3	20.8	28.4	23.9	21.1	22.1	23.4	19.0	21.7
Palmitoleic	16:1c9	1.8	1.3	2.0	2.5	1.8	1.8	1.4	1.8	1.7	1.6	1.4	0.5	4.5	2.1	5.9	2.9	3.3	11.8	2.7
Margaric	17:0	0.9	1.0	1.8	1.2	1.1	0.8	1.2	1.8	1.2	1.5	0.9	1.3	0.5	0.4	0.4	0.4	ND	0.4	ND
Heptadecenoic	17:1c10	0.2	0.2	0.8	0.3	ND	0.2	0.4	0.4	0.9	0.2	0.2	ND	0.6	0.2	0.5	0.3	ND	0.3	ND
Stearic acid	18:0	18.7	11.6	17.7	10.3	7.5	10.3	14.9	12.6	15.9	20.3	6.7	4.9	4.7	7.2	5.0	7.4	6.9	5.2	6.3
Elaidic	18:1t9	3.4	0.6	0.6	1.1	0.1	1.1	1.9	0.4	2.5	0.3	0.9	0.5	ND	ND	ND	0.1	ND	0.1	ND
Oleic	18:1c9	23.2	21.0	35.0	19.2	15.6	27.1	31.2	44.4	36.9	19.1	10.6	6.6	29.3	34.7	33.0	44.8	35.2	20.3	19.1
Vaccenic	18:1c7	ND	ND	ND	ND	0.3	ND	ND	ND	0.3	ND	ND	ND	0.8	ND	ND	1.5	0.9	ND	1.0
Linolelaidic	18:2t9,12 (n-6)	0.2	ND	0.2	ND	ND	0.2	0.3	0.2	ND	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Linoleic	18:2c9,12 (n-6)	4.5	7.1	4.4	1.4	0.9	4.0	2.9	2.7	3.3	1.9	1.5	1.2	10.8	27.5	16.3	17.4	19.1	2.3	28.2
Conjugated linoleic acid	18:2c9t11(n-6)	ND	0.2	0.4	0.4	0.3	0.8	0.7	0.7	0.3	ND	0.4	ND	0.1	ND	ND	ND	ND	ND	ND
α-Linolenic	18:3c9,12,15 (n-3)	0.8	0.7	2.2	0.5	0.5	0.5	1.0	0.4	0.3	1.0	0.9	ND	5.8	1.3	11.0	0.5	1.1	2.3	0.9
α-Linolenic	18:3c6,9,12 (n-6)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	0.3	ND	ND	ND
Nonoadecanoic	19:0	0.4	0.1	0.3	0.4	ND	0.2	ND	0.2	ND	0.3	0.2	ND	ND	ND	0.1	ND	ND	0.3	ND
Arachidic	20:0	0.3	0.2	0.4	0.6	0.5	0.4	0.4	0.3	ND	0.9	0.9	0.6	0.1	0.2	ND	ND	0.1	0.7	ND
Eicosenoic	20:1c11	ND	ND	0.2	0.3	ND	0.1	ND	ND	ND	ND	ND	ND	0.3	0.2	0.4	0.5	0.1	10.5	ND
Eicosadienoic	20:2c11,14 (n-6)	ND	ND	ND	ND	ND	0.1	ND	ND	ND	0.2	ND	ND	0.1	0.5	0.3	0.5	0.2	0.6	0.3
Eicosatrienoic	20:3c11,14,17 (n-3)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.2	ND	0.5	ND	0.3
Eicosatrienoic	20:3c8,11,14 (n-6)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	0.6	ND	ND	0.2	ND
Arachidonic	20:4c5,8,11,14 (n-6)	ND	0.4	0.2	ND	ND	ND	ND	0.2	ND	ND	ND	ND	ND	2.5	1.0	0.8	0.6	0.4	0.3
Eicosopentaenoic	20:5c5,8,11,14,17 (n-3)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	0.03	5.0	0.1
Heneicosanoic	21:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND	0.02	ND	0.1
Behenic	22:0	ND	ND	0.2	ND	0.2	0.1	ND	ND	ND	0.1	0.2	0.3	0.3	ND	ND	ND	0.1	ND	ND
Docosapentaenoic	22:5c7,10,13,16,19 (n-3)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	1.9	0.1
Docosahexanoic	22:6c4,7,10,13,16,19 (n-3)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.9	0.1	ND	0.1	8.3	0.1
Erucic	22:1c13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	1.1	0.1
Docosadienoic	22:2c13,16 (n-6)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	1.3	0.2
Tricosanoic	23:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	0.01
Lignoceric	24:0	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	0.02
Nervonic	24:1c15	ND	ND	ND	ND	ND	0.2	ND	ND	ND	ND	ND	ND	ND	0.2	0.5	ND	0.1	0.7	0.02
Total saturated fatty acids		66.4	68.4	56.1	73.6	79.2	63.2	60.0	49.0	50.1	72.9	82.8	90.9	47.6	32.3	30.0	30.6	38.6	32.7	44.4
Total monounsaturated fatty acids		28.8	23.3	38.4	24.1	18.1	30.7	35.3	47.1	42.0	21.4	13.6	8.0	35.0	37.5	39.8	50.1	39.7	44.2	22.9
Total polyunsaturated fatty acids		5.5	8.4	7.4	2.3	2.7	6.1	4.7	3.9	7.9	5.7	3.6	1.2	17.4	30.2	30.2	19.4	21.7	23.1	32.7
Total omega-3 fatty acids		0.8	0.7	2.2	0.5	0.5	0.5	1.0	0.4	0.3	1.0	0.9	0	5.8	21.2	11.3	0.5	1.6	17.7	1.2
Total omega-6 fatty acids		4.7	7.7	5.2	1.8	2.2	5.6	3.7	3.5	7.6	4.7	2.7	1.2	12.4	29.0	18.9	18.9	20.1	5.4	33.9

ND = not detected.

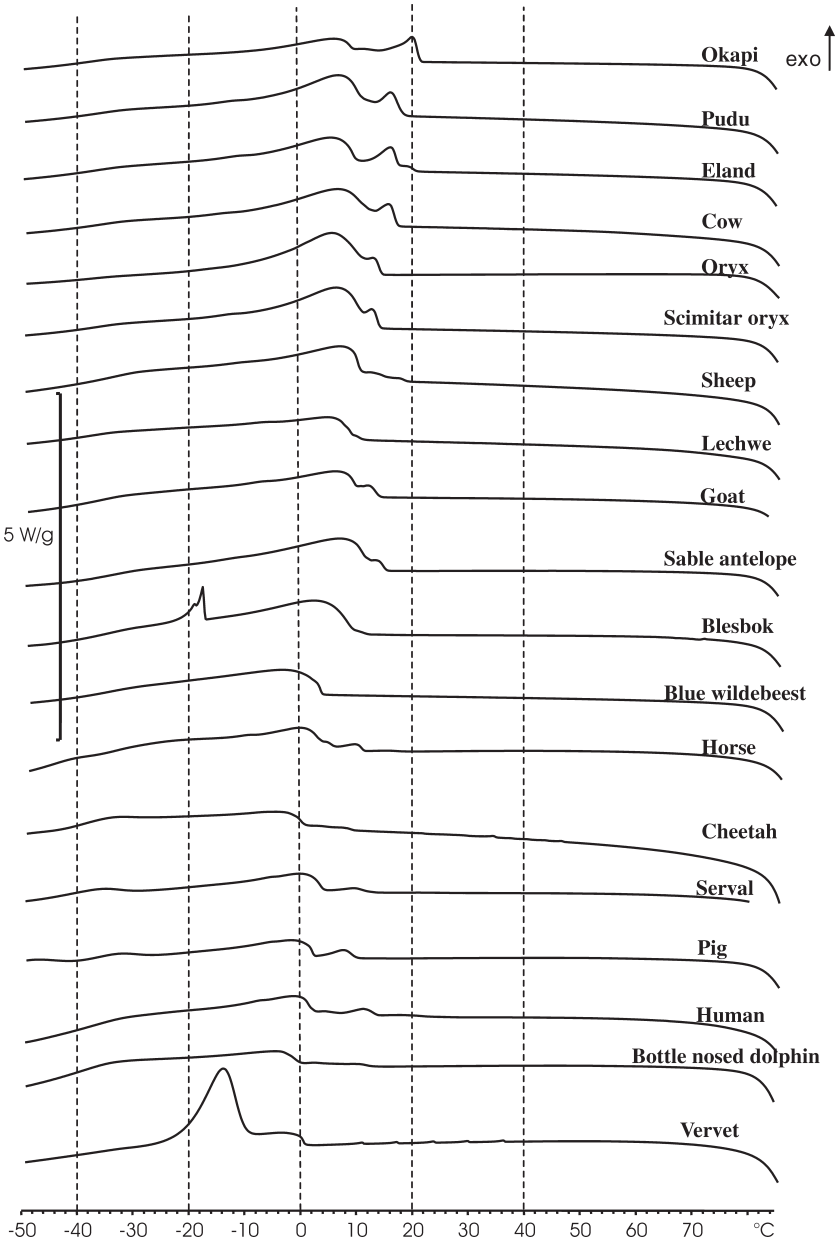

Figure 2 Differential scanning calorimetry profiles of milk fats from dairy and non-dairy mammals during cooling from 85 °C to –50 °C at a cooling rate of 10 °C min^{-1}.

melting isotherm with an onset around –10 °C, and less defined peaks between 10 and 14 °C. It may also be noted that the fatty acid composition between fats C, D and E differ much more than between A and B, yet the melting isotherms are very similar. It therefore seems as if a large degree of tolerance in molecular lipid structure, determined by fatty acid composition, is allowed before a crystal lattice with different melting and setting properties is established. This indicates that there is no gradual change from a crystal lattice with melting properties of A and B to that of C, D and E, and then to that of F and finally G, but that certain threshold levels of fatty acid content have to be exceeded in order to form a crystal network with a suitable environment that can accommodate different molecular species.

Triglyceride molecule species containing high amounts of 10:0 together with fatty acids between 8 and 16 carbon in length are also found in other milk fats such as cow and goat, respectively at 9.42 and 5.13 %[7,8]. If these molecules, or at least some of them, would form the same compound crystal structure when the ruminant fats are crystallized, as observed for milk fats A–G, their melting isotherm would be contained in the low temperature end of the middle melting point fraction that melts between 8 and 22 °C.[16]

The fatty acid composition of the milk fats of the other mammals studied differ from the above, which effects different setting and melting behaviour. The ruminant milk fats display high temperature setting isotherms between 22 and 15 °C followed by a setting isotherm from 15 to as low as –40 °C (Fig. 2). For the crystallization of cow's milk fat described by Lopez *et al.*,[15] a setting isotherm between 22 and 16 °C may be ascribed to the crystallization of the 18:0 containing fraction as α2L1 crystals. Setting between 16 and 10 °C was described as crystallization of 18:0 containing fraction as α2L2 crystals as well as 18:1 containing fraction as α3L2. With the lack of X-ray diffraction spectroscopic data, such claims of specific crystals cannot be made for the individual milk fats under study. However, taking into account the work of Fontecha *et al.*,[8] Fraga *et al.*,[7] Morera Pons *et al.*[10] and Winter *et al.*[9] that same types of triacylglyceride molecular species occur in milk of different species, it may be

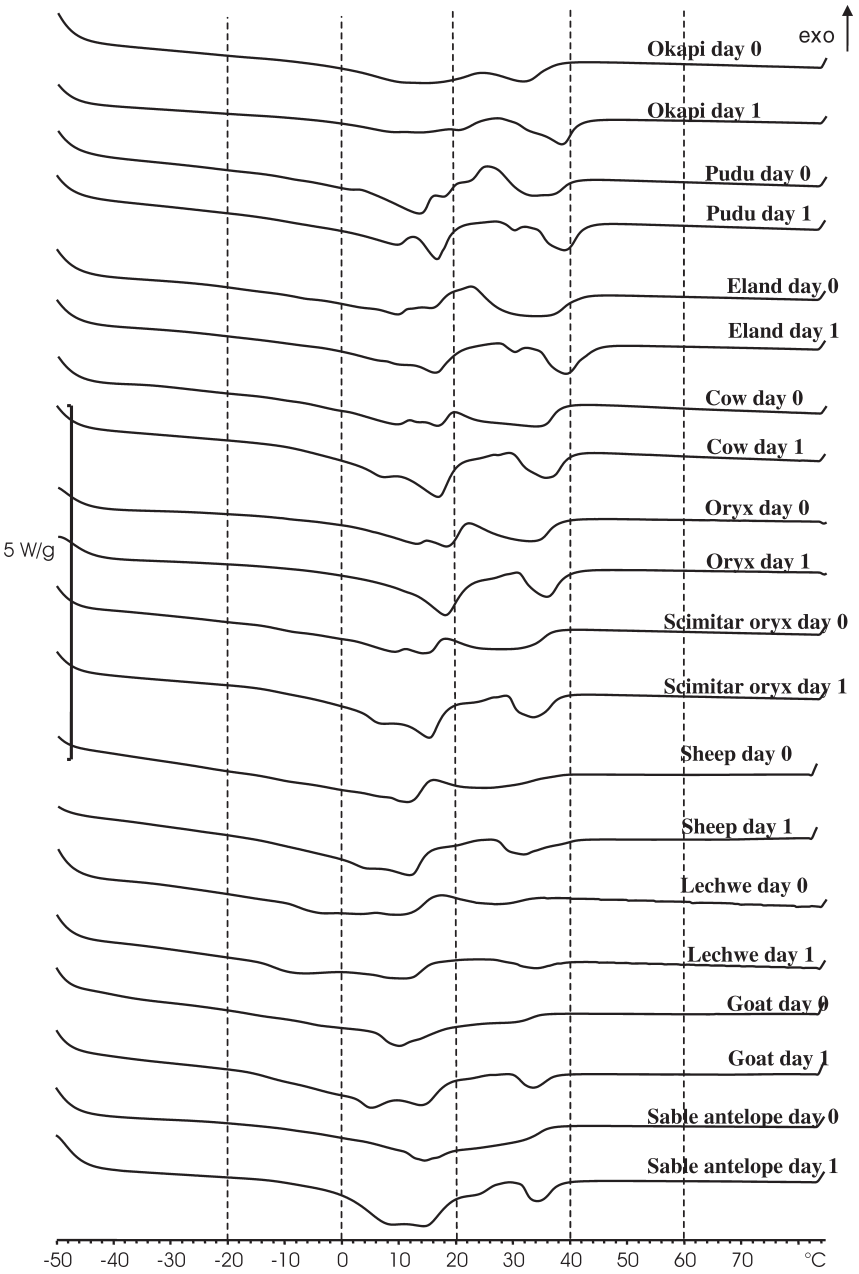

Figure 3 Differential scanning calorimetry profiles of milk fats from dairy and non-dairy mammals during heating from –50 °C to 85 °C at a heating rate of 10 °C min^{-1}. (*Continued overleaf.*)

assumed that the 18:0 containing molecules set at the highest temperature and 18:1 containing molecules at the medium temperature, irrespective of the specific crystal type formed. Only the setting isotherms of milk fats from lechwe, blesbok and blue wildebeest do not display the high temperature setting isotherm.

The non-ruminant milk fats, display a small high temperature setting isotherm peak between 12 and 4 °C followed by a large peak between 4 and –40 °C or even lower. Only the milk fat from vervet monkey does not display the peak at 12 to 4 °C; instead setting is initiated at 2 °C with a large peak between –10 and –34 °C and a peak maximum at –14 °C.

The ruminant milk fats display a high temperature melting isotherm between 30 and 42 °C and a large melting isotherm between –10 and 26 °C. One or more peaks may be displayed in the latter, the most prominent at approximately 18 °C (Fig. 3). For the melting of cow's milk fat described by Lopez and Olivon[16], the shift to a melting peak between 30 and 42 °C is due to the formation of a more stable β′-type crystal of the 18:0 containing fat fraction, more specific a β′2L structure from α crystals with a melting range of 25 to 30 °C. The β′2L-type crystals of the 18:0 containing fat fraction are the crystals that display the highest melting temperature of any milk fat. Shifts to a peak between 10 and 20 °C are due to the formation of the β′3L structure of 18:1 containing fats. Again, with the lack of X-ray diffraction spectroscopic data, such claims of specific crystals cannot be made for the individual milk fats under study. However, taking into account the work of Fontecha *et al.*,[8] Fraga *et al.*,[7] Morera Pons *et al.*[10] and Winter *et al.*[9] that same types of triacylglyceride molecular species occur in milk of different species, it may be assumed that the 18:0 containing molecules melt at the highest temperature and 18:1 containing molecules at the medium temperature, irrespective of the specific crystal type involved. Although the melting isotherms in the region –10 to 26 °C differ between the ruminant milk fats, it cannot be merely explained by the fatty acid composition alone. Data from fat fractionation and X-ray diffraction spectroscopy would be needed.

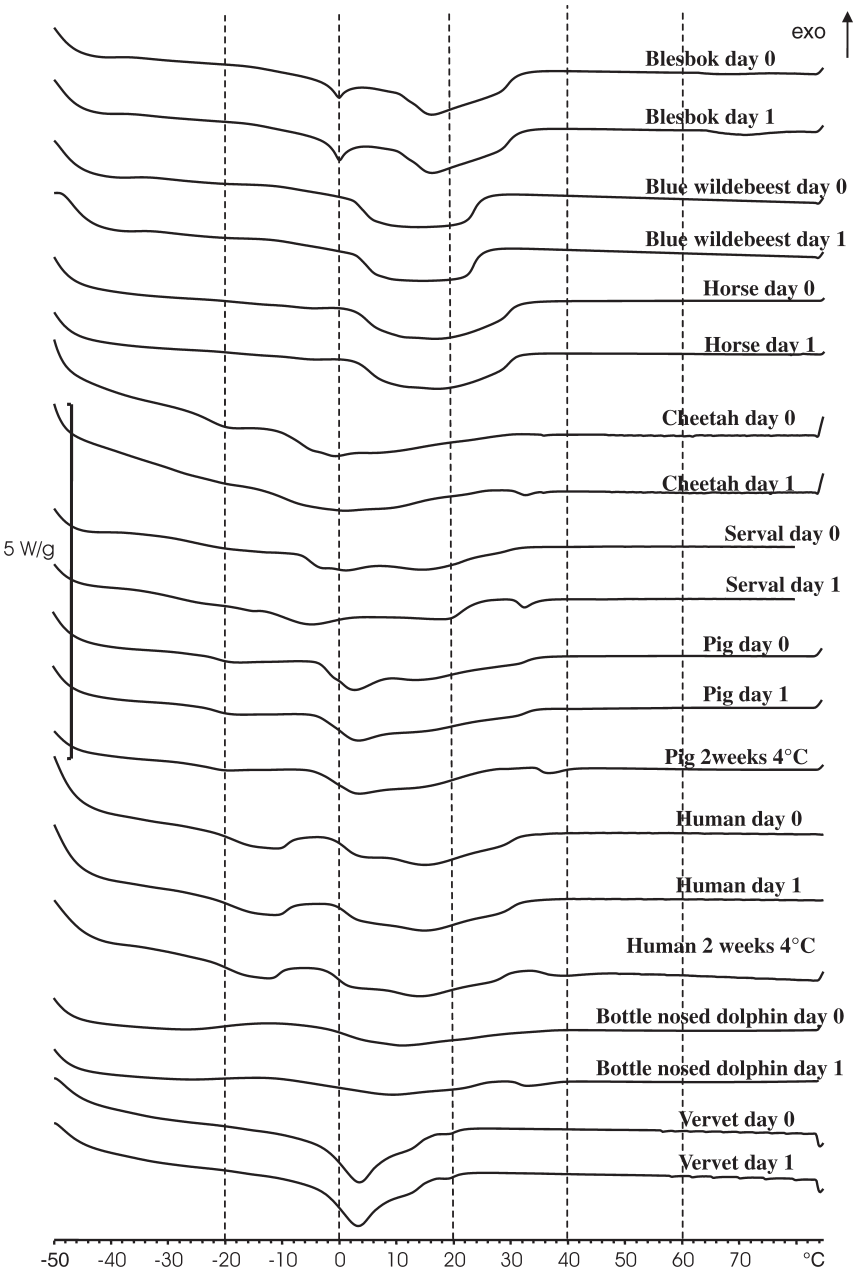

Figure 3 (*continued*)

The melting isotherms of the non-ruminants, except vervet monkey and horse, display a small peak of a high melting temperature isotherm between 32 and 42 °C, similar to that of the non-ruminant fats, only after tempering for either one day or two weeks at 4 °C. They also display low temperature melting isotherms from as low as –45 °C, best visible for the milk fats of bottle nosed dolphin, serval and pig. This low temperature melting is due to triacylglyceride molecule species containing long-chain polyunsaturated fatty acids as reported for fats of vegetable,[32,33] pork,[34] fish[35] and crocodile.[31] Compared to the other non-ruminants, the single melting isotherm of vervet milk fat is sharp, between –10 and 22 °C. Two shoulder peaks are observed at the higher temperature end, which might be due to the presence of different crystal structures. The horse milk fat melting isotherm is a single broad peak between 2 and 32 °C with a barely detectable low temperature melting peak between –10 and 0 °C.

The setting and melting behaviour at specific low, medium and high temperatures of most of the milk fats can be described and related to triacylglyceride composition of respectively long-chain polyunsaturated; short- to medium-chain and mono-unsaturated; and long-chain saturated fatty acids[12] as well as comparison with thermographic studies of cow's milk.[11,13–16] The milk fats from blesbok, blue wildebeest, horse and vervet monkey, however, deserve further discussion.

Firstly, the milk fats of blesbok and blue wildebeest contain the lowest amounts of 18:0 compared to the ruminant milk fats, but they are also very low when compared with the non-ruminant ones, which might immediately explain the absence of the high setting isotherm between 22 and 15 °C and high melting isotherm peak between 30 and 42 °C. Their content of unsaturated as well as polyunsaturated fatty acids is of the lowest, which might explain the absence of the low temperature melting isotherm between approximately –45 and –15 °C. Based on the information provided by Christie,[6] Fontecha *et al.*,[8] Fraga *et al.*,[7] Morera Pons *et al.*[10] and Winter *et al.*[9] on triacylglyceride structure and molecular species, and the presence of 18:0 as well as polyunsaturated fatty acids, it is highly unlikely that the molecu-

lar species with setting and melting behaviour in these stipulated temperature ranges would not be present in the fats under study, although their amounts might be low. The only explanation left is that the crystals of low and high melting temperature properties are not formed during crystallization and tempering, although the triacylglyceride molecules are present, but that these are rather drawn into the compound crystal structures of the lipids of the medium temperature melting range, i.e. the short to medium length as well as the 18:1 containing groups. The cause of this would be that a favourable environment is being created by the molecular species that contain the high amounts of low to medium-chain fatty acids (8:0 to 14:0). The content of 82.8 and 90.9 % saturated fatty acids in the milk fats of blesbok and blue wildebeest, respectively, second only to that of the elephant and rhinoceros discussed above, and of the lowest amounts of unsaturated as well as polyunsaturated fatty acids would imply that very little adaptation of the molecules is needed to form a crystal structure, and that the crystal structure is so stable that it would not be re-arranged during tempering.[36] The blesbok milk fat, in particular, also displays a sharp setting peak between –10 and –20 °C and a sharp melting peak between –10 and 10 °C which might be due to crystal structures similar to the fats of elephant and rhinoceros, which are formed by the high levels of 8:0, 10:0, and 12:0. The separate peak between 10 and 30 °C would probably represent the melting range of the triacylglyceride molecular species that contain the 18:1 or the long-chain saturated fatty acids. Why the crystallization behaviour of the blue wildebeest and blesbok milkfats are not exactly the same, specifically the lower setting temperature and lower final melting temperature of the blue wildebeest milk fat, might be due to certain threshold levels of fatty acid composition, brought about by the higher content of 10:0, 12:0 and 14:0, and lower content of 16:0, 18:0 and 18:1.

The milk fat from lechwe also deserves discussion at this point, as it does not display the high temperature setting peak between 22 and 15 °C, but in turn displays the high temperature melting peak at 30 and 42 °C after tempering, although very small, similar to the other ruminant milk fats. This milk fat contains the highest amount of monounsaturated fatty acids at 47.1 %, of which 18:1 at 44.4 % forms the greater part, while 16:0 and 18:0 are found in similar amounts as in other ruminant milk fats. It is therefore possible that the molecular species containing the 18:1 are creating an environment that favours the incorporation of the high melting 18:0 containing species into a compound crystal during setting. However, the latter are then able to re-crystallize during tempering.

Within the group of non-ruminants, the milk fats of horse and vervet monkey contain the highest amounts of saturated acids (above 44 %) and the amounts of 8:0, 10:0 and 12:0 are comparable to that found in ruminant milks. The horse milk fat contains the lowest amounts of 18:0, which might immediately explain the absence of the high melting isotherm peak between 30 and 42 °C. At 17.4 % the horse milk fat also contains the lowest amounts of polyunsaturated fatty acids, which might explain the absence of the low melting isotherm peak. However, the content of the latter is not much lower than that of the pig milk fat, and much higher than that of the 2.3 to 8.4 % of the ruminant milk fats that display such a melting peak. It also contains no polyunsaturated fatty acids longer than 20 carbon atoms in length. The combination seems to result in the formation of fat crystals in the middle temperature melting range between 5 and 30 °C that consist of triacylglycerides of saturated as well as monounsaturated fatty acids. Although it is in the same range as observed for the milk fats from blesbok and blue wildebeest, the crystal structures might not be the same, and may indicate a different threshold level of fatty acid composition that is reached to form certain crystals.

The vervet monkey milk fat has commonalities with the horse milk fat regarding the high amounts of saturated acids (above 44 %), contains very low amounts of polyunsaturated fatty acids longer than 20 carbon atoms in length, and contains high amounts of 8:0, 10:0 and 12:0, the latter three being almost comparable with the amounts found in the blesbok milk. Compared to the other non-ruminant milk fats, the polyunsaturated fatty acid composition is almost completely restricted to the 28.2 % 18:2, the highest in all the milks. It also contains a low amount of 18:0, which might immediately explain the absence of the high melting isotherm peak between 30 and 42 °C, although the amount is not lower than that found in the other non-ruminant milk fats. The combination seems to result in lipid crystals of the middle temperature melting range between –10 and 22 °C that consist of triacylglycerides of saturated as well as monounsaturated fatty acids. The setting and melting behaviour of this fat has much in common with that of milk fats E–G of elephant and white rhinoceros regarding the sharp setting peak between –10 and –20 °C and sharp melting peak between –10 and 22 °C, and might be ascribed to the triacylglyceride molecular species that contain the 8:0 and 10:0.

The formation or disappearance of high melting temperature crystals has been described for cow's milk fat that has been transesterified with vegetable fat.[30,37] However, in these instances the fatty acid compositions and their stereospecific distribution of triacylglyceride species are being changed indiscriminately, resulting in triacylglyceride species of random stereospecific distribution. In the current study random structure can be ruled out because of the stereospecific arrangement of milk fats showing common features,[6] i.e. 4:0 and 6:0 are found entirely, and 8:0 preferably, at sn-3, while 14:0 and 16:0 acids are concentrated in position sn-2. The 18:0 is found mainly in position sn-1 and unsaturated fatty acids mainly in positions sn-1 and sn-3.

To conclude, it may be mentioned that fats in general consist of a mixture of triacylglyceride molecular species which normally do not crystallize as segregate crystals, but rather as compound crystals with polymorphic properties. The milk fats studied here are of a variety of fatty acid composition, varying from a high content of medium-chain fatty acids to a high content of long-chain polyunsaturated fatty acids, with a mixed content in between. Specific melting isotherm peaks are observed by DSC, which may be replaced by others, as the fatty acid compositions of the triglycerides change. Restructuring from less stable crystal polymorphs is observed for some lipids. High setting temperature peaks above 15 °C and high melting peaks above 30 °C are associated with lipid crystals containing 18:0, while melting peaks below –10 °C are associated with lipid crystals containing polyunsaturated fatty acids. The middle temperature melting range between –10 and 30 °C is associated with lipid crystals containing monounsaturated, mainly 18:1, and short- to medium-chain saturated fatty acids. From the milk fats of elephant and rhinoceros, it can be learnt that the melting isotherm of fats containing high amounts of 8:0, 10:0 and 12:0 lies between –10 and 20 °C. When the content of specific fatty acids in some lipids reach certain threshold amounts, new crystals with different melting isotherms are observed. It appears as if the triacylglyceride molecular species of short to medium-chain fatty acids are able to create stable crystal structures during setting that are not transformed to other polymorphs, and that they create a suitable environment to incorporate low and high temperature melting lipids.

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