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Fatty acid analysis of dairy products after in vitro digestion by using offline SPE and GC-MS.

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M.S. in Chemistry

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Abstract

Dairy products are an essential source of nutrition in Nordic diets. Lipids are a substantial part of bovine milk and a vital energy source, as well as providing physiological components in enzymes and immune systems, hormones and vitamins. Lipids are arranged mostly into triacylglycerols (TAG), containing three fatty acids (FA) in ester bonds to a glycerol moiety. TAGs hydrolyse into monoacylglycerols (MAG) and free fatty acids (FFA) by digestive enzymes before uptake in the stomach and small intestine. The arrangement of FAs on the glycerol in TAG affect their physical/chemical properties, absorption and health effects.[1] The Norwegian Center for Research-based Innovation (CFI) funds the "Foods of Norway" research project, aimed at developing technologies within agriculture and aquaculture. *LipidInflammaGenes* is a part of this project and aims at investigating the structure and composition of triacylglycerols in dairy lipids and their effect on digestibility and inflammation. This thesis will identify and quantify FAs in 2-MAG of digested dairy products.

A static *in vitro* digestive model (INFOGEST) containing an array of digestive enzymes is employed in digesting four independent dairy products; gouda cheese (Norvegia), cream cheese (kremgo), whole cream (kremfløte) and sour cream (seterømme). The lipids are extracted from the digestion matrix using Folch's extraction. The extracted lipids are separated into polar lipids (PL), FFA and neutral lipids (NL) using automated offline solid phase extraction (SPE). The NL fraction is further split into TAG, diacylglycerol (DAG), and MAG by the same method. Transesterification then derivates each fraction into its corresponding fatty acid methyl ester (FAME). FAMEs are analysed using GC-MS. The Glycerol content for each sample is analysed using a Megazyme® glycerol assay kit.

The stereospecificity of digestive enzymes ensures that fatty acids attached in the sn-2 position on the TAG are not hydrolysed; the MAG fraction will reveal the FA composition in this position and is the focus of this investigation.

This investigation has identified 47 unique fatty acids, of which 36 have been quantified. Undigested dairy products concur well with previous reports on milk lipids and show 70 % saturated fatty acids (SFA), 30 % mono-unsaturated fatty acids (MUFA) and 1 – 2 % poly-unsaturated fatty acid (PUFA). In addition, FA profiles are almost identical for each dairy product with C16:0 (33 – 36 %), C18:1c9 (23 – 26 %), C18:0 (16 – 17 %), C14:0 (9 – 10 %) and C12:0 (2

– 3 %). MAG analysis shows 77 % SFA, 21 % MUFA and 1 % PUFA. Compared to the undigested, MAG has a relative increase in C16:0 and C14:0 (12 % and 8 % larger, respectively) with a simultaneous decrease in C18:1c9 and C18:0 (8 % and 10 % smaller, respectively). The greatest difference was in C18:0 concentration, which had halved. The MAG analysis showed no short-chain fatty acids (SCFA) presence. These results concur well with previous reports on TAG structure in bovine milk, which suggests that SCFAs are attached in the sn-3 position, C18:0 and C18:1c9 are preferentially attached in the sn-1 or sn-3 position, and long-chain fatty acids are attached in the sn-2 position. This concurrence with TAG structures in undigested whole milk suggests that the effect of fermentation, processing and digestion is minimal to the structure of TAG. Glycerol analysis shows free glycerol in digested samples, indicating isomerization of 2-MAG into 1(3)-MAG, which digestive enzymes hydrolysed into free glycerol. Digested whole milk shows a substantial presence of free glycerol (10 %).

The FA analyses have good precision with minor standard deviations (<5 %). However, the analyses report a too high amount of FAs, especially for FFA fractions. This results from the internal standard (IS) concentration being lower than estimated, likely from evaporation or deposition from the solution. It is also known from previous FAME analysis that SCFAs are under-quantified because they are more volatile and polar, thus evaporating or extracting out of solution. These problems can be rectified by controlling the concentration of the IS before quantification and adding shorter IS (e.g. C5:0). Several factors influence variations in FA composition (season, genetics, feed, stage of lactation), which are difficult to control and complicates analysis. The *In vitro* digestion model does not remove products from solution, as it would *In Vivo*, and does not give an accurate representation of the reaction kinetics, as less product will be formed.

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Introduction

Health is the cornerstone for living a fulfilling life and is highly affected by lifestyle and diet. Western societies consume an excess of saturated fats, which are associated with adverse health effects and have been shown to contribute to cardiovascular diseases and type 2 diabetes. Over 62 % of saturated fats come from meat and dairy products. The fatty acid composition and the structure of triacylglycerol, the main component of dietary fats, has been shown to significantly effect properties, absorption and health effects in human beings.[1, 2] *LipidInflammaGenes* [3] is a research project investigating the composition and structure of triacylglycerols in Norwegian animal products and their effect on low-grade inflammation and digestibility in humans and animals. The fatty acid composition and triacyl structure differ between meat, dairy, and animals. During digestion, fatty acids attached to the TAG are released and absorbed with MAG. Figure 1 shows a general reaction pathway of enzymatic hydrolysis of TAG.

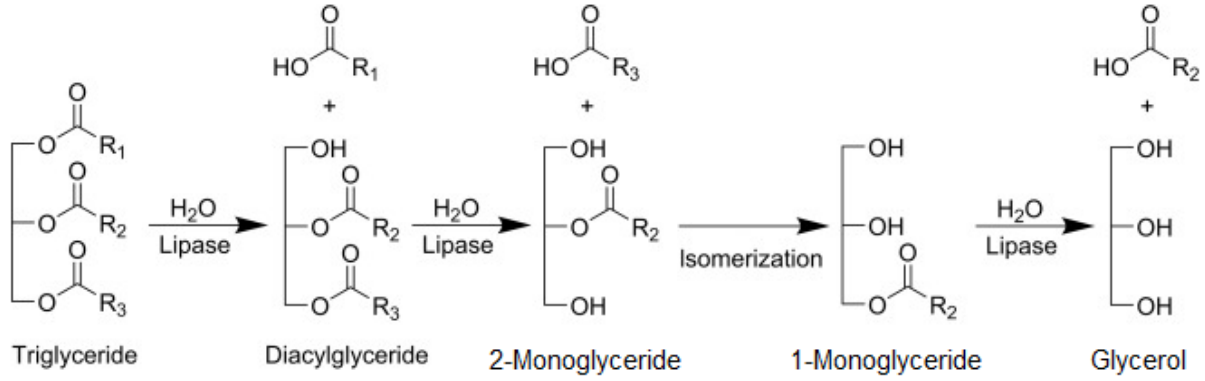

Figure 1: Enzymatic hydrolysis of triacylglycerols into di- and monoacyl glycerols, free fatty acids and free glycerol.[4]

The absorbed fatty acids are synthesized to TAG and stored in adipose tissue. The metabolic fate of 2-MAG is still unclear and is little investigated. Spontaneous isomerization of 2-MAG into 1(3)-MAG facilitates complete hydrolysis of 1(3)-MAG, producing free glycerol. This thesis aims to elucidate fatty acid composition in 2-MAG for digested dairy products. [5-7]

Aim

This thesis aims to produce a complete lipid analysis of digested dairy products, namely: gouda cheese (Norvegia), cream cheese (Kremgo), whole cream (Kremfløte) and sour cream (Seterømme). Samples of purchased dairy products will undergo static *in vitro* digestion, and

extracted lipids will be separated into polar lipids, free fatty acids and neutral lipids via SPE. Neutral lipids will be further separated into MAG, DAG and TAG via same method. These fractions will be derivatized into FAMEs and analysed using GC-MS. LOD and LOQ will be measured by calibration curve method for FAME standards.

The main aim is to quantify FAs in MAG fraction after digestion. This will subsequently elucidate the FAs attached in the sn-2 position on the TAG molecule, thanks to the stereospecific nature of the digestion enzymes employed. It will be necessary to analyse undigested dairy products to consider the effect of the digestion model on FA composition. Comparison between the undigested product and MAG will be helpful for evaluation of the FA composition in the TAG molecule. Complete FA profile of the different dairy products is necessary for evaluating any effect of processing or digestion model on the FA composition. Glycerol analysis of digested samples will demonstrate if the digestion model completely hydrolyses TAG into glycerol and FFA. Complete hydrolysis will suggests spontaneous isomerization of 2-MAG.

Theory

Fatty acid chemistry

Fats are ubiquitous macromolecules and a staple in human diets worldwide. It is the most energy-dense macronutrient, with as much as 37 kJ (9 kcal) per gram of fat.[6] Western society depends on as much as 40 % of caloric intake from dietary fats.[8] Fats serve many other bodily functions than simply as an energy source, and certain fats have a clear health benefit over others.

Quantitatively, fats consist mainly of triacylglycerols (TAGs), phospholipids, and minor fractions of glycolipids, sterols, and fat-soluble vitamins. TAG molecules consist of 3 fatty acids (FA) connected via an ester linkage to a glycerol backbone, designated position sn1 – sn3 (see figure 2). The FA constituents may vary, creating unique enantiomers and often very complex profiles. Suppose the number of fatty acids present are denoted n. In that case, there can be as many as n^3 possible enantiomers for TAG molecules. Fatty acids are long hydrocarbon chains with a carboxylic acid at the alpha end (α) and a methyl group at the omega (ω). Fatty acids typically vary in length from C2 to C26 long carbon chains, which can either be saturated or unsaturated with as many as six or more double bonds. Chains can also be branched or have unique constituents such as heteroatoms or alkyl rings. For simple fatty acids, shorthand notation, annotating the number of carbon atoms, the number of double bonds and their placement, may be employed, i.e. for oleic acid "cis-9-C18:1" or "C18:1c-9" or just 18:1. [8, 9] This thesis will primarily use the second annotation.

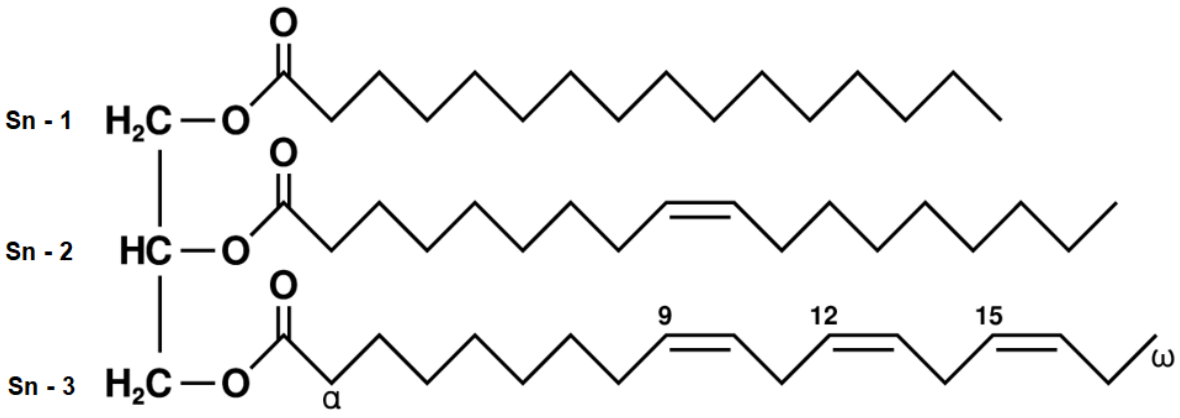

Figure 2: Chemical structure of a triacylglycerol molecule.[10]

Fatty acids may be classified according to the number of double bonds. Fatty acids without double bonds will have the maximum number of hydrogens around each carbon, thus called "saturated fatty acids" (SFA). Fatty acids containing a single, double-bond will be classified as mono-unsaturated (MUFA); any more than one double bond will be classified as poly-unsaturated (PUFA). The position of the double bond will be labelled as the distance from the ω end (annotated n- or ω-); typically, this will be n-3 (ω-3), n-6 (ω-6) or n-9 (ω-9).

Milk

All mammalian species produce and secrete milk to feed suckling neonates, who often depend entirely on their mothers as the sole food source. Besides purely nutritional, milk serves many other essential physiological needs, such as enzymes, enzymatic inhibitors, immunoglobulins, hormones, vitamins and anti-bacterial agents.[11] Milk composition will be unique for each species to fit their nutritional and physiological needs. Milk composition will also be markedly different between individuals and can be affected by breed, diet, health, season, geography, age and more.[5] Of more than 4000 species that produce milk, only about 180 have been analysed, of which bovine has been extensively studied. Lipids are a substantial constituent of milk, serving mainly as an energy source, and fat content will often appropriate the energy requirements of the species. Arctic and marine animals will have an exceptionally high concentration, with hooded seals having as much as 60% fat content. [12] Bovine milk has a long tradition in Nordic diets and is an essential source of macro- and micronutrients. In addition, milk fermentation practices are employed to extend shelf-life and avoid spoilage by inoculating milk with safe bacteria or moulds. As a result, fermented products such as cheeses will have the same nutritional value as the original milk but with less bulk from water and safer storage from unwanted pathogens. Rao et al. has shown some changes in the lipid profile of whole milk after fermentation. There was a significant increase in saturated FAs and oleic acid (C18:1) with a simultaneous decrease in linoleic and linolenic FA (C18:2 and C18:3) and complete disappearance of monoglycerides after fermentation of whole milk with select bacteria. [13]

Milk fat composition

Lipid profiling of bovine milk has been reported extensively, providing a suitable scaffold for further inquiry. [11, 14, 15] Table 1 shows a typical composition of bovine milk, the subject of this study. Bovine milk has about 3 – 5 % lipid content which exists as emulsified globules coated with a membrane from the secreting mammary cells, 2 – 4 µm wide. This milk lipid globule membrane

(MLGM) stabilises the emulsion and prevents the globules from coalescing. The large surface area facilitates lipolysis by digestive enzymes and absorption of the digestive products. All commercial milk is pasteurised and homogenised. Although pasteurisation has little effect on the lipid composition, homogenisation reduces the diameter of the lipid globules, increasing their number at least 100-fold and altering the MLGM structure and composition. The globules are recoated almost entirely with caseins. These smaller particles should stabilise the emulsion better, but other effects, e.g. digestion, have not been studied. [16]

Most (97 – 98 %) lipid content in milk, primarily found in these globules, will be triacylglycerols (TAG) (see table 2). Small amounts of mono- and diacylglycerols (MAG, DAG) are left over from incomplete synthesis and partial hydrolysis of TAG. Polar lipids, including phospholipids, ceramides, cerebrosides and gangliosides, represent less than 1% lipid fraction and are primarily derived from membranous materials and are in increased proportions in skimmed milk and buttermilk. Cholesterol represents approx. 0.3 % w/w of total lipids in milk and other sterol compounds, such as hormones, appear in trace amounts. Milk also contributes significantly to the dietary requirement of some fat-soluble vitamins in western countries. [12] Table 1 and 2 lists the components present in bovine milk.

Table 1. General composition of bovine milk (wt %) [17, 18]

Component	Amount
Protein	3.2
Casein	2.6
Fat	3.9
Lactose	4.6
Total solids	12.7
Ash	7
Energy	276 kJ/100 g

Milk fat is the most complex of natural fats, with bovine milk estimated to contain upwards of 400 different FAs. The milk fat is derived from the feed or the rumen's microbial activity. [19] Amount and composition will vary due to various factors such as genetics, age, health, feed, lactation and other environmental factors. [7, 20, 21]

Table 2. Composition of lipid fractions in bovine milk. [22]

Component	Content (wt %)
Triacylglycerols	97-98
Diacylglycerols	0.3-0.6
Monoacylglycerols	0.02-0.04
Free fatty acids	0.1-0.5
Cholesterol	0.2-0.4
Phospholipids	0.2-1.0
Carotene	Trace
Lactones, aldehydes, ketones	Trace
Vitamins A, D and E	Trace
Tocopherol	Trace

As seen in table 3, bovine milk contains primarily long-chain fatty acids (LCFA), defined as FAs, which are fourteen to twenty carbon atoms (C14 – C20). There is also a good deal of short-chain fatty acids (SCFA), which are defined as shorter than six carbon atoms (<C6:0).[23] FAs with carbon length <C9 are liquid at room temperature. SCFA in milk is derived from bacterial fermentation of plant cellulose in the rumen, serves critical physiological functions in the rumen and is used in *de novo* FA synthesis in the mammary gland. SCFA in milk and serum are associated with the nutritional value of dairy products and can be used as biological markers for predicting the animal's overall health.[24] LCFAs have been associated with a negative energy balance when the cow mobilises lipids from its fat storage, mainly C16:0, C18:0 and C18:1c9. [25] Studies have shown that LCFA is in higher concentrations early in lactation due to increased energy demands after calving. Later in the lactation period, the mobilisation of lipids from fat storage decreases. In contrast, *de novo* synthesis of C4:0 to C16:0 FAs increases.[26, 27] These differences in the lactation period on the concentration of SCFAs and LCFAs are likely to influence the lipid composition within the TAG molecule. High amounts of SCFAs will produce low- and medium-molecular weight (LMW, MMW) TAG species, while high concentrations of LCFAs will produce more high-molecular-weight (HMW) TAG. As of yet, no studies have investigated the relationship between the lactation period and TAG composition in bovine milk. [28-30]

Table 3. FA composition of bovine milk in wt %. Adapted from Pachebo-Pappenheim et al. (2022)[30], Jensen (2002)[16] and Lindmark-Månsson et al. (2003)[31].

Fatty acid	Carbon number	Abundance (wt %)
Butyric acid	C4:0	2-5
Caproic acid	C6:0	1-5
Caprylic acid	C8:0	1-3
Capric acid	C10:0	2-4
Lauric acid	C12:0	2-5
Myristic acid	C14:0	8-14
Pentadecanoic acid	C15:0	1-2
Palmitic acid	C16:0	22-35
Palmitoleic acid	C16:1c9	1-3
Margaric acid	C17:0	0.5-1.5
Stearic acid	C18:0	9-14
Oleic acid	C18:1c9	20-30
Linoleic acid	C18:2c9,12	1-3
Linolenic acid	C18:3c9,12,15	0.5-2

The composition of TAG is usually described in terms of the types and amount of FAs present. The structure of TAG, however, includes the organisation of FAs within the TAG molecule, among TAG molecules and individual molecular species of TAG. The structure of TAG influences the lipolytic activity of enzymes and the absorption of its products. The structure also influences the physical properties of milk lipids, such as melting points, crystallisation, rheological properties, and even flavour properties of dairy products. [16, 32-35] Composition and concentration of FAs at the sn-2 position on the TAG are important for infant formula development. High concentrations of C16:0 were associated with increased FFA and calcium absorption in infants. [36, 37]

Although the amount may vary, the fatty acid composition and triacyl structure are unique and not readily altered by ordinary factors mentioned previously. Bovine milk contains at least 400 unique FAs and can theoretically be 400^3 or 64 $x10^6$ possible combinations for TAG. Although FAs are not esterified on the glycerol at random but are preferentially attached at the three positions on the

glycerol, the actual number of TAGs is much less. Still, there are estimated to be several thousand combinations of TAG in bovine milk, most in trace amounts. This nonrandom distribution of FAs in the TAG molecule is due to the specific properties of acyltransferases in ruminants. The sn-glycerol-3-phosphate pathway synthesizes most of the TAG. Short-chain FAs are esterified in the sn-3 position by 1,2-diacyl-sn-glycerol acyltransferase, and long-chain acyl coenzyme A preferentially attaches FAs in the sn-1 position and then sn-2 position. Esterification of SCFA is much more rapid than long-chain. Reports have shown that SCFA is exclusively attached in the sn-3 position, and LCFA is equally attached in the sn-1 and sn-2 positions. Medium-chain fatty acids are esterified in all positions but decrease in the sn-3 position as chain length increases. Stearic acid (C18:0) is highest in the sn-1 position, while oleic acid (C18:1) is acylated equally in all positions. Fatty acid composition in each position is given in table 4.[19, 38-40]

Table 4. Milk fatty acid composition, in mol %. Means calculated from bimonthly samples over one year. Adapted from Parodi (1979)[15], Gresti et al. (1993)[40] and Jensen (1991)[18].

Fatty acid	**Carbon number**	**Overall**	**Positional distribution**		
			Sn-1	Sn-2	Sn-3
Butyric	C4:0	10.3	1.6	0.3	98.1
Caproic	C6:0	4.3	3.1	3.9	93.0
Caprylic	C8:0	2.0	10.3	55.2	34.5
Capric	C10:0	3.3	15.2	56.6	28.2
Lauric	C12:0	3.2	23.7	62.9	13.4
Myristic	C14:0	9.4	27.3	65.6	7.1
Palmitic	C16:0	19.9	44.1	45.4	10.5
Hexadecenoic	C16:1c9	2.6	35.4	45.3	17.3
Stearic	C18:0	11.7	54.0	16.2	29.8
Oleic	C18:1c9	21.7	37.3	21.2	41.5
Linoleic	C18:2c9,12	2.5	21.4	48.6	30.0

The gross composition of milk fats is 70% saturated FAs and 30% unsaturated FAs, of which 1-2% are PUFAs. This is subject to seasonal variations as it has been shown that for Sweedish cows, the SFA content is lowest in the summer when the cows are grazing outside and highest in the

winter during indoor feeding. The opposite is true for unsaturated FAs, which are highest in the summer. [39, 41] Quantitatively by weight, the most abundant FAs are C16:0 (25-30 %), C14:0 (11 %) and C18:0 (12%). Of the SFAs, approximately 10 % are SCFAs, of which C4:0 and C6:0 is the most abundant (3-4 % and 2-3%, respectively). Due to lower molecular weight, these SCFAs will have a higher concentration when expressed as molar percentages (approximately 10 and 5 % for C4:0 and C6:0, respectively). [38] For MUFA, oleic acid (C18:1) accounts for 24 %. Linoleic acid (C18:2) and α-linolenic acid (C18:3) are the most abundant PUFAs and account for 1.6 and 0.7 % by weight of the total FAs. The ratio between ω-6 and ω-3 is reported to be 5.7 for conventional milk and 2.3 for organic milk. Bovine milk can thus be an important source of ω-3 in the human diet. In France, animal products account for 40% of ω-3 intake. [16, 31, 41-43]

Digestion

Lipid digestion is complex, involving an array of lipolytic enzymes. These enzymes, predominantly in the stomach and small intestine[2], catalyse the hydrolysis of various dietary lipids, including acyl glycerides, phospholipids, galactolipids, cholesterol and vitamin esters. The enzymes include gastric lipase, colipase-dependent pancreatic lipase, pancreatic lipase-related protein 2 (PLRP2), bile salt-stimulated lipase (BSSL), and pancreatic phospholipase A2. The presence of lingual lipase in human digestion is still controversial.[44-46] Under normal circumstances, the digestion of triacyl glycerol will have upwards of 95% effectiveness. Excretion of undigested fats and fatty stool (>5%) indicates underlying malabsorptive conditions.[8] Digestion begins as soon as the food enters the mouth and is mechanically minced, increasing surface area and dispersing the food components, resulting in the formation of a bolus. After swallowing, the food is transferred to the stomach, where TAG will undergo partial hydrolysation into DAG and FFA by gastric and lingual lipases. When the bolus passes into the small intestine, fats will disperse into micelles with aid from bile salts excreted from the gall bladder. Pancreatic lipases will hydrolyse TAG on the surface of the micelles into MAG and FFA before absorption into the intestinal wall. After absorption, MAG and FFA can be rearranged into DAG, TAG, and even phospholipids. Intestinal cells secrete these synthesised lipids, and lipoproteins carry them to the recipient organs and tissues in the blood and lymphatic system. TAG is hydrolysed, absorbed into adipose tissue, synthesised and stored in adipose cells. The TAG is available on-demand by hormone-mediated enzymatic hydrolysis into FFA and glycerol. [47, 48]

Human gastric lipase (HGL) is produced in the stomach mucosa and initiates the gastrointestinal lipolysis of dietary fats. HGL can potentially hydrolyse all ester bonds on the TAG yet is severely inhibited by the presence of FFA, and thus MAGs are rarely observed in gastric contents. HGL also hydrolyses polyethene glycol (PEG) mono- and di-esters but has no activity on cholesterol esters or phospholipids. [49] HGL has demonstrated, both *in vitro* and *in vivo*, preferential hydrolysis of FAs in the sn-3 position, selectively releasing short- and medium-chain fatty acids located here. The C4:0-C10:0 FAs are absorbed through the stomach lining, into the portal vein, and transported to the liver, where they are oxidised. As the molecular weight of the FAs released increases, fewer FAs are absorbed due to higher hydrophobicity and melting point. Approximately 25-30 % of TAGs are digested in the stomach. [16, 50, 51]

Human pancreatic lipase (HPL) is the major lipase involved in the hydrolysis of TAG. In the small intestine, it acts in the presence of bile where specific protein cofactor colipase, also produced by the pancreas, anchors the enzyme on the oil-water interface and counteracting the inhibitory effect of the bile salts. HPL also acts in DAG but with a lower rate of hydrolysation relative to TAG. HPL is 1,3-regioselective, producing 2-MAG and FFA from TAG. [52, 53] HPL is inactive on 2-MAG, and LCFAs attached in sn-1/3 position, while weakly active on medium-chain 1(3)-MAG. HPL also has no activity on cholesterol esters, galactolipids or phospholipids. [54, 55] Pancreatic lipase has a greater affinity for ester bonds in the sn-1 rather than sn-3 position. [56] Some types of pancreatic lipase can synthesise MAG from glycerol and FFA via esterification reactions. [57] All sn-1/3 bonds are hydrolysed in digestion, and 22% of sn-2 bonds are hydrolysed. [58] Despite acyl migration to the sn-1/3 position, there is still a 75% conservation of FAs in the sn-2 position. [8] This conservation is because of pancreatic lipase regiospecificity for the sn-1/3 position and chain length of sn-2 FAs. LCFA contain more than 12 carbon atoms and undergo different absorption pathways than the sn-1/3 medium and short-chain FAs. [53] LCFAs are absorbed by a protein-mediated process, while 2-MAG is absorbed by passive diffusion. [59] FA transport proteins have different affinities based on chain length. [60, 61]

Bile salt-stimulated lipase (BSSL) is a non-specific esterase in pancreatic juice and breastmilk and has been shown to hydrolyse various substrates *in vitro* like cholesterol ester, TAG, MAG, vitamin (A, E) esters and phospholipids. [62-65] BSSL can also hydrolyse carotenoid esters (lutein and capsanthin diesters, esters of β-cryptoxanthin)[65], galactolipids (mono-galactosyl diglycerides)[66]

and PEG mono- and di-esters. [54, 55] BSSL is not regioselective and can hydrolyse all three ester bonds on the TAG molecule, whatever their position on the glycerol. [67] BSSLs' ability to hydrolyse 2-MAG seems only relevant during the lactation period of newborn infants. [68] HGL and HPL will hydrolyse most of the milk TAG into MAG and FFA. Addition of BSSL hydrolyses the MAG. The concerted action of HGL, HPL and BSSL will hydrolyse milk TAG completely into free glycerol and FFA as end-products.

Isomerisation of DAG and MAG

Historically, acyl migration of 2-MAG poses a problem for the characterisation, isolation and synthesis of acylglycerols, hindering research done in lipid metabolism, especially *in vivo*. It has been shown that both 2-MAG, 1,3- and 1,2-DAG isomerise, changing the stereospecific position of the attached acyl chain on the glycerol backbone.[69, 70] Figure 3 and 4 shows the isomers of MAG and DAG respectively. [71]

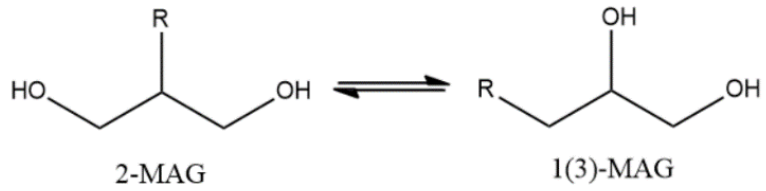

Figure 3: Monoacylglycerol isomers 2-MAG and 1(3)-MAG, where R is attached acyl chain.

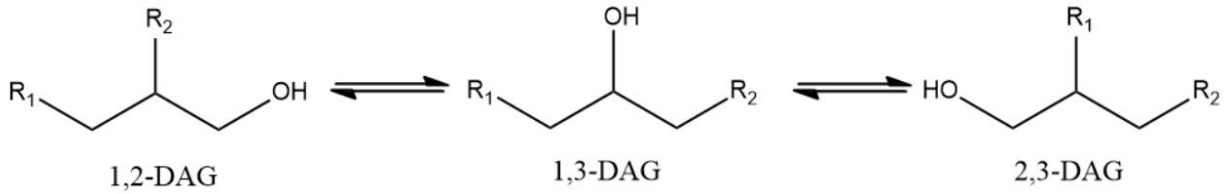

Figure 4: Diacylglycerol isomers 1,2-DAG, 1,3-DAG and 2,3-DAG, where R is attached acyl chain.

The isomerization of MAG was first suggested by Fischer et al. in 1924.[72] Fureby et al. (1996)[69] suggested a reaction mechanism for acyl isomerisation where the sterically stable hydroxyl group in the sn – 1 position reacts with the ester group in the sn-2 position and forms a cyclic intermediate. See figure 5.

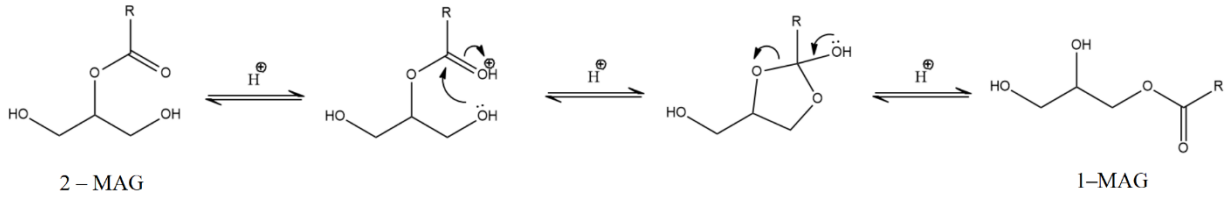

Figure 5: Acyl isomerisation mechanism of MAG from sn-2 to sn-1 position as proposed by Fureby et al (1996).[69]

Figure 5 shows that the carbonyl in the ester is firstly protonated by an acid. A nearby hydroxyl group performs a nucleophilic attach on the carbonyl carbon. A five membered ring intermediate is formed. The hydroxyl group performs a nucleophilic attack on the same carbon, opening the ring and reforming the carbonyl group. The acyl group is now attached in the sn-1 position on the glycerol.

2-MAG and 1-MAG are reported to be in equilibrium at a 1:9 ratio, suggesting that spontaneous isomerisation of 2-MAG occurs to form the more stable 1-MAG. The ester group attached in the sn-2 position will interact more with the neighbouring hydroxyl groups in the sn-1 and sn-3 positions, and equilibrium will favour the thermodynamically more stable 1-MAG. Primary alcohols also act as a stronger nucleophile than secondary alcohols, further supporting this positional preference. [70, 73, 74] Acyl migration is a universal phenomenon during alcoholysis and esterification, where partial acylglycerols are the product. For the synthesis of bioactive 2-MAG, isomerisation may cause problems but can be desirable in enzymatic transesterification of TAG with alcohol for obtaining high yields of biodiesel. [75-77] It is, therefore, desirable to be able to control, promote or inhibit isomerisation based on the desired synthetic product. Various factors such as temperature, type of solvent, pH, acyl chain length and enzyme support materials will affect migration profoundly. [78]

Health Index – index of atherogenicity and index of thrombogenicity

The IA evaluates the atherogenic potential of FA in food. The old health index of PUFA/SFA is too general and does not consider specific FA concerning cardiovascular health (CVH). Ulbricht and Southgate developed IA and IT in 1991, based on the available evidence at the time, and concluded that the results were in accordance. The IA considers the relation between the sum of SFA and UFA. The main SFAs (C12:0, C14:0 and C16:0), except C18:0, are generally

proatherogenic. [79, 80] The UFAs are considered beneficial, as they can reduce cholesterol and other harmful lipids and inhibit the build-up of plaque in arteries, i.e. antiatherogenic. [80, 81] Thus, foods with a lower IA have a greater potential for reducing blood LDL and cholesterol concentrations. [82] An important caveat to the IA is that although it is a more reasonable index for evaluating the atherogenicity, it is still not a perfect formula, as suggested by Ulbricht and Southgate: C18:0 should be included in the denominator when sufficient evidence is presented for its antiatherogenic properties. Neither should all UFAs be weighted equally. Moreover, there is still conflicting research on the effect of trans-FAs, which is subsequently omitted. [83]

$$IA = \frac{[C12{:}0 + (4 \times C14{:}0) + C16{:}0]}{\Sigma UFA} \quad (1)$$

Equation 1 – Index of Atherogenicity (IA)

The IT evaluates the thrombogenicity of FAs and the potential for forming clots in blood vessels. It assesses the contribution and relationship of pro-thrombogenic FAs (C12:0, C14:0 and C16:0) with anti-thrombogenic FAs (MUFAs, especially ω-3 and ω-6). [83] Consumption of food with low IT is considered beneficial and promotes CVH. As with IA, IT lacks accuracy, and the formula should be updated as the research surrounding FA thrombogenicity increases.

$$IT = \frac{\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}}{\left[(0.5 \times \Sigma\mathrm{MUFA}) + (0.5 \times \Sigma\omega - 6\ \mathrm{PUFA}) + (3 \times \Sigma\omega - 3\ \mathrm{PUFA}) + \left(\frac{\omega - 3}{\omega - 6}\right)\right]} \quad (2)$$

Equation 2 – Index of Thrombogenicity (IT)

IA and IT are useful for assessing the CVH effects according to the FA composition. Food with lower IA and IT have healthier FAs, better nutritional quality, and promotes CVH. There are, as of yet, no recommended values for IA or IT. As research on FAs increases, modifications on IA and IT will be more appropriate.

Chromatography

Chromatography is a collective term for separation techniques where the analyte is separated from a mixture by being pushed through a stationary phase by a mobile phase (gas or liquid). The

analytes are distributed between the mobile and stationary phases according to the distribution equilibria K_d. See equation 3.

$$K_d = C_s/C_m \quad (3)$$

Equation 3 – Distribution equilibrium of analyte between stationary (C_s) and mobile (C_m) phase, where C is the analyte concentration. [84, 85]

When the analyte is in the mobile phase, it will move with a velocity equal to the mobile phase; when it is in the stationary phase, it will stand still. Both places reach a new equilibrium when the analyte moves with the mobile phase from A to B. These moves are imagined as independent segments (plates) with independent equilibria. Each segment moves, a new equilibrium is reached, and the process repeats. Analytes with high K_d will move slower because more of the analyte is dispersed in the stationary phase. Thus, analytes of different K_d will be separated in a sample mixture.

Band broadening will affect the efficiency of the column. The signal in the chromatogram will be a gaussian peak, where the analyte is distributed according to its velocity. The poor separation will affect the width of the peak and can cause phenomena called fronting or tailing, in which the front or back of the peak is protracted, and the opposite end is shortened. Narrow bands of compound fully separated are ideal. Column efficiency will depend on the theoretical plate count (N) and its height (H) and will be proportional to the length of the column. See equation 4. Both H and N can be determined experimentally by measuring the retention time of the analyte.

$$N = L/H \quad (4)$$

Equation 4 – Theoretical plate count

The van Deemter equation can calculate the efficiency of columns.

$$H = A + \frac{B}{u} + C_s u + C_m u \quad (5)$$

Equation 5 – *van Deemter* equation

Equation 5 describes the *van Deemter* equation where H is the plate height in cm, u is the linear velocity of the mobile phase in cm/s, A describes eddy diffusion, B describes longitudinal

diffusion, $C_s u$ and $C_m u$ describe the mass transfer between stationary and mobile phase respectively. [84, 85]

The most crucial thing in chromatography is to get the highest resolution in the minimum time. Good resolution ensures that the compounds are sufficiently separated to where the peak area can be measured accurately. Resolution is thus defined as the difference in retention time for two peaks divided by the average peak width at the base (W) or the peak width at half maximum height (FWHM).

$$R_s = \frac{t_B - t_A}{(W_A + W_B) * \frac{1}{2}} \quad (6)$$

Equation 6 – Peak resolution

Equation 6 shows that the resolution, R, depends on the difference in retention time (t) and peak width for the separated compounds A and B. [85]

Gas chromatography

Gas chromatography (GC) is a separation technique where the mobile phase is gaseous, and the sample is volatile and thermostable. GC is the most common analytical technique widely used to test the purity of a sample or separate a mixture into its different components. GC is always carried out in a column, either packed or capillary. Packed columns can handle larger samples, while capillary can separate more complex mixtures. Capillary columns are open tubular columns in which the stationary phase adheres to the column walls in different ways. The stationary phase can be either solid or liquid and needs to be non-absorbent and chemically inert materials, often silicone based. Packed columns are made from stainless steel or glass, while capillary columns are made from quartz or fused silica. The sample is injected into the column at the inlet using an autosampler. The injection is usually either split, splitless or on-column injection. Split and splitless injection heats the sample in a chamber, and the carrier gas pushes the sample in its entirety (splitless mode) or a small portion (split mode) into the column. On-column inlet places the sample into the column entirely without heat so that the sample is condensed into a narrow sone, where it is subsequently heated, releasing the sample into the gas phase as boiling point is reached.

The sample is carried in the column by an inert carrier gas. The choice of carrier gas is essential, as the plate height efficiency (see equation 5) is related to the flow rate of the carrier gas, as described by a van Deemter curve. Nitrogen (N_2) is cheap but has an optimum efficiency at a low flow rate, so the analysis will take longer. Helium is widely used but is very expensive, while Hydrogen (H_2) is linked to explosion hazards. After the separation in the column, the analyte is detected in the detector, of which flame ionisation (FID) or mass spectrometry (MS) is the most common. In FID, the ions are detected by the analyte being combusted in a hydrogen flame, making carbon cations and electrons, which induce a current between two electrodes placed in the chamber. The current is proportional to the concentration of the sample and translates to a signal in the chromatogram. FID is cheap, easy to use and has low detection limits. It only works for organic compounds and does not qualitatively identify samples like a mass spectrometer can. [84-86]

Mass spectrometry

In mass spectrometry, sample molecules are ionised in the gas phase, and the components are separated according to their mass-to-charge ratio, *m/z*. The detector measures the number of ions, and a computer interprets the signal creating mass spectra. There is a plethora of different ionisation sources and mass filters. This project employs electron ionisation (EI) and quadrupole mass filters.

Electron ionisation is the most widely used ionisation method. It is an ionisation method where high-energy electrons (70 eV) interact with a solid or gaseous analyte, producing ions. When an electron has a higher kinetic energy than the ionization energy of the sample molecule, an electron from the molecule is removed upon collision, converting the sample molecule to a positive ion. In EI, the high-energy electron produces energetically unstable molecular ions, readily dissociating into fragment ions by homolytic or heterolytic cleavage of molecular bonds. Fragment ions produced are often unique to a specific molecule and can be used in structural identification of unknown molecules. The ion source is made of metal, where the cathode is a rhenium or tungsten filament inserted through a slit in the source block. When the filament is heated, with current running through it, it will produce ions according to a process known as thermionic emission. The anode is placed outside the ion source opposite the cathode. The potential difference between the filament (cathode) and the trap electrode decides the electron energy of the electrons produced, usually 70 eV. Magnets on the outside direct the flow of electrons in a helix inside the ion source, increasing the path length. The sample is injected through the sample hole, whereupon interaction

with the electron beam, is ionised. A repeller opposite the exit pushes the generated ions out from the ion source. The ion source is heated to 300°C to prevent sample condensation, and vacuum pumps ensure less background interference with pressures as low as 10^{-6} torr. A schematic drawing of a generic EI ions source is shown in figure 6.

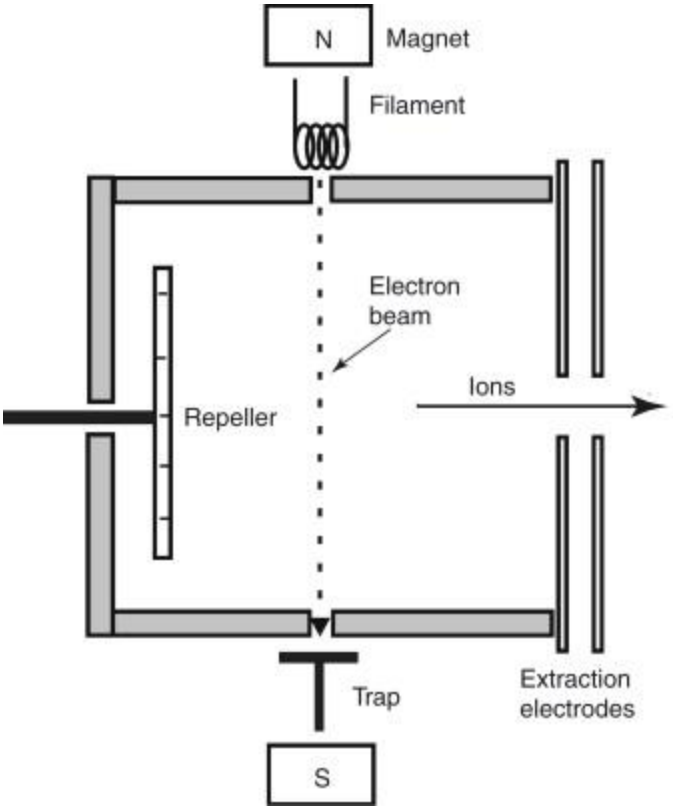

Figure 6: Schematic drawing of an EI ion source. N and S denote the direction of the magnetic field, going from north (N) to south (S).[87]

Electron ionisation only works for samples that are volatile and thermally stable. The extensive fragmentation often removes the molecular ions, requiring supplementary softer ionisation techniques for identification. EI is also only valid for organic compounds with a molecular weight of less than 600 mu. EI is, however, a simple and sensitive ionisation technique. Fragmentation patterns serve as compounds' fingerprints, and unique mass spectra can be easily identified by searching in a library database.

A quadrupole mass filter is a mass analyser, the component responsible for selecting ions entering the detector based on their mass-to-charge ratio. A quadrupole consists of four parallel rods surrounding a central axis. Each opposing rod is connected to either direct current (DC) or radio

frequency (RF). The ions entering the electric field between the rods will be separated by the stability of the trajectory along the z-axis. The positive ions will be drawn towards the negative rod, and the oscillating potential difference causes the ion to change direction, ultimately containing in along the central axis. Ions' stability is proportional to the potential applied to the rods. Only ions of a certain *m/z* will reach the detector for a given voltage, and ions with unstable trajectories will collide with the rods. A typical quadrupole mass filter is shown in figure 7.

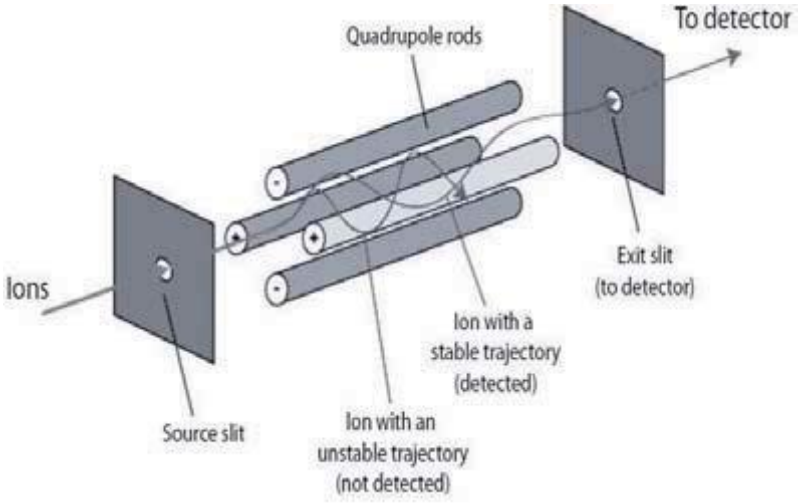

Figure 7: Schematic drawing of a quadrupole mass filter.[88]

Although quadrupole mass filter has a lower resolution and mass range than both sector instruments and orbitrap, it is cheaper, smaller, easy to use and has a high throughput.

There are several types of detectors used in mass spectrometers. The most common is an electron multiplier, where the selected ion collides with a metal plate, which induces secondary emission of electrons. The electrons continue to collide in the walls of the dynode, resulting in a cascading effect which exponentially increases the number of electrons that are emitted until a metal anode ultimately collects them—the amount of emitted electrons is proportional to the m/z ratio of the entering ion. The computer interprets the potential collected in the anode, generating mass spectra.[89-91]

Method

Chemicals and standards

Chloroform, methanol, n-heptane (HiPerSolv® chromanorm™ quality), diethyl ether, sodium chloride (AnalaR® Normapur™ quality), acetic acid and 2-propanol (Rectapure ® quality) was supplied by VWR (VWR part of Avantor, Radnor, PA, United States). In addition, metallic sodium and boron trifluoride-methanol solution (14 %) was supplied from Merck KGaA (Darmstadt, Germany).

A total of six different internal standards (IS) were used: triundecanoin (C11:0 TAG), trinondecanoin (C19:0 TAG), diundecanoin (C11:0 DAG), monoundecanoin (C11:0 MAG), 1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine (C19:0 Pl) and nonadecanoic acid (C11 FFA), supplied by Larodan AB (Solna, Sweeden). A 37-component fatty acid methyl ester (FAME) mix was used (Food Industry FAME MIX, Restek Corporation, Bellefonte, PA, USA) for the identification of the FAMEs. In addition, several individual standards were employed; heptadecanoic acid methyl ester (C7:0), nonadecanoic acid methyl ester (C9:0) supplied by Fluka, now Merck KGaA, (Darmstadt, Germany), methyl nonadecanoate (C19:0), methyl 11-methyldodecanoate (11me-C12:0), methyl nonadecanoate (C19:0), methyl 11-methyldodecanoate (11me-C12:0), methyl 10-methyldodecanoate (10me-C12:0), methyl 12-methyltridecanoate (12me-C13:0 (14C) iso), methyl 13-methyltetradecanoate (13me-C14:0 (15C) iso), methyl 14-methylpentadecanoate (14me-C15:0 (16C) iso), methyl 14-methylhexadecanoate (14me-C16:0 (17C) anteiso), methyl 7(Z)-hexadecenoate (C16:1cis-7), methyl 13(Z)-octadecenoate (C18:1cis-13), methyl 7(Z),10(Z),13(Z),16(Z),19(Z)-docosapentaenoate (C22:5c7,10,13,16,19), trans-9-hexadecanoic acid ME (C16:1cis-9), cis-10-nonadecanoate ME (C19:1cis-10), cis-9-,trans-12-octadienoic acid ME (C18:2cis-9 trans-12), methyl hexacosanoate (C26:0), methyl 3,7,11,15-tetramethylhexadecanoate (3,7,11,15me-C16:0) supplied by Larodan AB (Solna, Sweden)

General remarks

The analytical method consists of six distinct phases. First, the samples were prepared, mixed, and diluted to have an equal fat percentage (3.5 %). Then, the samples were digested according to the INFOGEST 2.0 procedure[92] followed by Folch's extraction[93] of the lipids. Next, the extracted lipids were separated using SPE fractioning into NL, FFA and PL. The NL fraction was further separated into TAG, DAG and MAG by the same SPE procedure. Then, in a methanol solution,

the fractions were esterified into FAMEs by sodium methanolate or BF3. Lastly, the FAMEs were analysed using GC-MS.

Sample preparation

Eirin Stork and Lene Ruud performed sample preparation. Four dairy products were selected for analysis: gouda cheese (Norvegia), cream cheese (Kremgo), whole cream (Kremfløte) and sour cream (seterømme). These were purchased on 20th June 2022; a sample (10 – 20 g) was taken from each and stored in a freezer (-20 °C). Three replicates of each product were created where a sample was weighed approx. 1g and diluted with appropriate amounts of distilled water so that all samples had a normalised and equal fat percentage of 3.5 %. A blank sample was made with pure MQ water. In total, 13 samples. Samples were minced, crushed and mixed in the water, simulating mechanical oral chewing before digestion.

Digestion of the dairy samples

The digestion protocol follows as described in the INFOGEST 2.0 procedure.[94] The dairy samples were prepared and checked for pH the day before digestion. The enzymes simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were readymade and stored in the freezer (-20 °C). These were weighed, diluted, and heated to 37 °C in an incubator before adding to the samples.

Oral digestion (2min) - 0.8mL SSF was added to the sample test tube with 5 µL 0.3 M $CaCl_2$ and 0.195 mL MQ water (amylase was substituted with water because there is no presence of fibre in the samples), a total volume of 2 mL.

Gastric digestion (120min) - To the sample reaction mixture, 1.60 mL SFG (37 °C), 0.1 mL of rabbit gastric extract (RGE) solution (diluted in water and chilled in ice), 0.10 mL MQ water (pepsin was substituted with water since the RGE activity is sufficiently high) and 1.0 µL 0.3M $CaCl_2$ solution were added. The pH was adjusted to pH 3.0 with 1 M HCl solution. MQ water was added for a total sample volume of 4 mL. The samples were incubated at 37°C and shaken at 180rpm horizontally for 2 hours.

Intestinal digestion (120min) - To the reaction mixture, 1.7 mL SIF (37°C), 0.5 mL bile solution, 1.0 mL pancreatin and 8.0 µL $CaCl_2$ were added. Next, the pH was adjusted to 7.0 by adding

NaOH. Finally, MQ water was added for a total sample volume of 8 mL. The samples were incubated at 37°C for 2 hours.

Extraction of lipids

Lipids were extracted by a modified Folch's method[93], as reported previously.[95] The lipids were extracted by adding 20 mL chloroform:methanol (2:1 v/v) and the digested sample to 50 mL tubes (Greiner). Internal standards were added to the digested samples with a Hamilton syringe: C11:0 FFA - 400 µL (5 mg/mL); C11:0 MAG - 200 µL (5 mg/mL); C11:0 DAG – 20 µL (5 mg/mL); C11:0 TAG – 100 µL (5 mg/mL); C19:0 TAG (for evaluation of SPE separation of TAG and DAG) - 100 µL (5 mg/mL); C19:0 PL - 100 µL (1 mg/mL).

The mixture was shaken for 20 minutes at 250 rpm on an orbital shaker (PSU-10i, Biosan, Riga, Latvia). Phase separation was induced by the addition of 4.0 mL 0.9 % NaCl in MilliQ water. The flasks were gently shaken after this addition and then left to settle for 20 minutes. The organic phases (approx. 66 mL) were transferred to vacuum evaporation tubes (Buchi Labortechnic AG, Flawil, Switzerland). The polar phase of each replica was re-extracted twice with 66 mL chloroform. Next, the organic phases of each replica were combined before evaporation at 40 °C with a vacuum evaporator (Q-101, Buchi Labortechnic AG, Flawil, Switzerland). The samples were evaporated to dryness, redissolved in 1.0 mL chloroform and transferred to microtubes (MCT-150-C, Axygen®, a Corning brand, Corning, NY, USA) for centrifugation. The samples were centrifuged at 14800 rpm for 5 min (sigma 1-14 microcentrifuge, Sigma Laborzentrifugen GmbH, Osterode, Germany). The supernatant was transferred to new microtubes and evaporated to dryness. Afterwards, the samples were redissolved in 1.0 mL chloroform, transferred to GC-MS vials, and stored cold (-24 °C) before fractionation using solid phase extraction.

SPE fractioning of NL, FFA and PL

The lipid extracts were fractioned using an automatic SPE robot (Gilson, GX-274 ASPEC, Middleton, WI, USA) and 500 mg aminopropyl SPE columns (SigmaA art. nr 52637-U) with a flowrate of 1.0 mL min^{-1} according to the procedure by Devle et al.[96] Each sample, its respective column and collecting tube were placed in the chamber. The columns were conditioned with 7.5 mL hexane. 500 µL of sample is applied to the column. The neutral lipids were eluated with 5 mL chloroform. The free fatty acids were eluated with 5 mL diethyl ether:acetic acid (98:2), and the

phospholipids were eluted with 5 mL methanol. The fractions were collected in reaction test tubes, and the solvent was evaporated at 30°C under N_2(g).

SPE fractioning of NL into MAG, DAG and TAG

The lipid extracts were fractioned using an automatic SPE robot (Gilson, GX-274 ASPEC, Middleton, WI, USA) and 500 mg aminopropyl SPE columns (SigmaA art. nr 52637-U) with a flowrate of 1.0 mL min^{-1} according to the procedure by Haraldsen & Hausberg[97] The neutral lipid were redissolved in 1 mL hexane:chloroform:methanol 90:6:4 and transferred to sample vials. The NL samples were reloaded into the SPE robot, and new columns and sampling tubes were installed. The columns were conditioned with 7.5 mL heptane. Then, 150 µL of the sample was applied to the column. TAG was eluated with 800 µL heptane:diethyl-ether (93:7). DAG was eluated with 3200 µL heptane:diethyl-ether (93:7). MAG was eluated with 3 mL chloroform:methanol (2:1). The fractions are collected in reaction test tubes, and the solvent is evaporated at 30 °C under N_2(g).

Formation of fatty acid methyl esters (FAMEs).

FFA fraction – The dry samples were redissolved in 1 mL BF_3 in methanol (14 %) and incubated in an 80 °C water bath for five min. 2 mL n-heptane was added, vortexed and centrifuged (2000 rpm 5min) before the organic phase is transferred into GC-vial using a Pasteur pipette and stored in a -20 °C freezer.

NL fractions – The lipids were redissolved in 2 mL n-Heptane, vortexed, and sonicated. A 1.5 mL sodium methanolate solution (5 mg/mL) was added to the test tube. The samples were capped and placed horizontally on an orbital shaker at 30 min/350 rpm. The samples were centrifuged at 5 min/3000 rpm; the organic phase was transferred to a GC vial and stored at -20 °C in the freezer.

PL fractions – The dry samples were redissolved in 1mL BF_3 in methanol (14%) and incubated in a 100 °C water bath for 90 min. Next, 2 mL n-heptane was added, vortexed and centrifuged (2000rpm 5min) before the organic phase was transferred into GC-vial using a Pasteur pipette and stored in a -20 °C freezer.

FAME analysis by GC-MS

FAME analysis used a GC-MS (GC: Thermo Fisher Scientific, TRACE™ 1310, Waltham, MA, USA; MS: Thermo Fisher Scientific, ISQ™ QD, Waltham, MA, USA). GC column was Restek, Rtx®-2330 Columns (fused silica biscyanopropyl cyanopropylphenyl polysiloxane stationary phase), with 0.25mm diameter, 0.2mm film thickness and 60m long. An AI/AS 1310 Series Autosampler (Thermo Fisher Scientific, Waltham, MA, USA) injects the sample (1.0 µl at a split ratio of 1:10) into an injection chamber set to 250°C. Helium (99.99990%, from Yara, Rjukan, Norway) is the carrier gas, with a constant flow of 1.0ml/min.

The MS used electron ionisation (70 eV electrons) as the ionisation method and single quadrupole as a mass filter in mass range *m/z* 60-600, full-scan acquisition mode and scan time of 0.2 s. The ion source and transfer line had a constant temperature of 250°C.

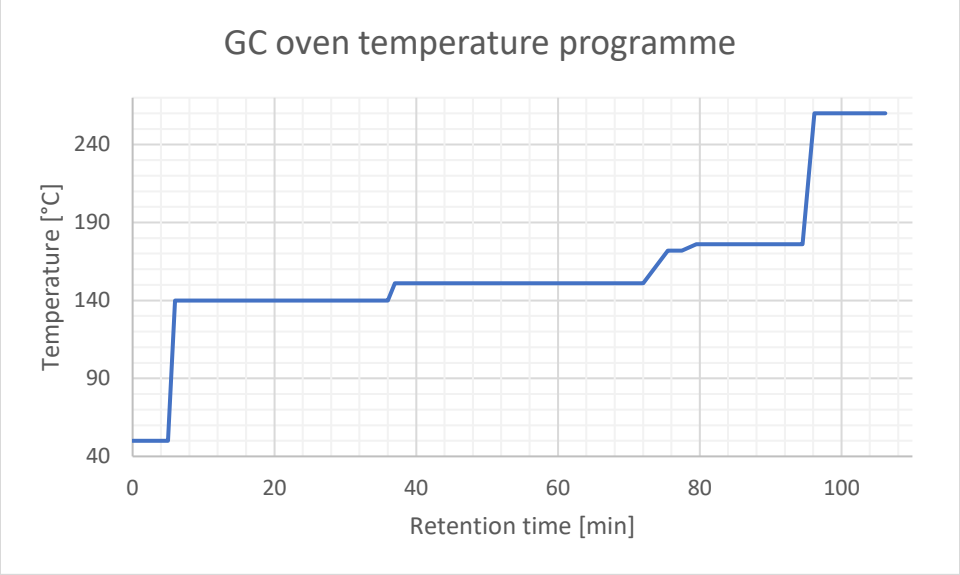

Figure 8: GC oven temperature program

Table 5. GC oven temperature program

NO	RETENTION TIME [MIN]	RATE [°C/MIN]	TARGET VALUE [°C]	HOLD TIME [MIN]
1	0	run		
2	5	0	50	5
3	35.9	100	140	30
4	72	10	151	35
5	77.5	6	172	2
6	94.5	2	176	15
7	106.18	50	260	10

Samples were diluted in heptane to a 10 µL/mL concentration before transfer to GC-MS vials for analysis. To identify and quantify the complete FA profile for each product, all FAME standards, divided into three different sample mixtures, are injected before and after each sequence. Each sample is injected in order of Blank, three sample parallels and a short program with pure n-Heptane for each dairy product. In total, 25 injections. Each fraction of the different products was divided into a new sequence. RRF for C11:0 and C19:0 standards were obtained from previous work on FAMEs performed on the same instrument. [98-100]

Data handling

The software used for obtaining and handling raw data was Chromeleon v7.2.8 (Thermo Fisher Scientific, Waltham, MA, USA)). Compound peaks in the chromatogram are identified by comparing retention time to the standard FAME analyses and a NIST library search of the mass spectra (NIST 08, Gaithersburg, MD, USA). Compounds without standard, non-FAME compounds, or compounds with match factor (SI) <700 are disregarded. The peak area is then integrated, labelled and exported to an excel document.

Calculations of FAME concentration were made according to equation 3.

$$[FAME] = \frac{A_{FAME} * [IS]}{A_{IS} * RRF} \quad (7)$$

Equation 7 – FAME concentration from GC-MS analysis

Equation 7 shows that the concentration of the individual FAME is proportional to the signal in the chromatogram compared to the internal standard signal (A_{FAME} and $A_{IS,}$ respectively). Therefore, the signal response of the individual FAMEs will be unique and thus must be adjusted relative to the internal standard (RRF or relative response factor).

Calculation of mean concentration and standard deviation was performed on each triplicate. In addition, simple one-way ANOVA and Tukey tests using Rstudio were performed for each FAME present in all products to evaluate differences between products. The presentation of raw data is collected in supporting information.

Determination of LOD and LOQ

LOD and LOQ were calculated by creating a linear equation based on known concentrations of FAME standards and their respective signal in the GC-MS. A triplicate sequence of eight known concentrations in increasing dilution (150, 100, 50, 20, 10, 5, 2, 1 µg/mL) with n-heptane was prepared and subsequently analysed in the GC-MS in mass range *m/z* 60-600, full-scan acquisition mode and scan time of 0.2 s. The chromatogram determined the signal/noise ratio from the integrated peak for each FAME and exported it into excel, where the different concentrations are graphically represented. From the linear equation, the concentration required for an S/N of 10 and 3 for LOQ and LOD can be deduced.

Glycerol analysis

Glycerol analysis was performed using the glycerol assay kit (Megazyme®, K-GCROL). Digested and undigested samples were prepared before SPE. The detection limit for this method is 0.34 µg/mL. The smallest differentiating absorbance is 0.010 absorbance units. The linear range is between 0.8 and 35 µg of glycerol in the sample. During sample preparation, the extracted FAMES were washed using a brine solution. Because glycerol is highly water soluble, brine washing would potentially wash out most of the free glycerol suspended in the solution. Regardless, 1 mL of the FAME solutions, dissolved in chloroform, was washed twice with 1 mL distilled water (total of 2 mL). Then the water phase was removed and used in the analysis according to the Megazyme® glycerol assay kit. The analysis was performed on a Hitachi U-1100 spectrophotometer (340 nm) in plastic cuvettes.

Later digestions were done on whole milk. The digestion was stopped by adding 10mL methanol. The methanol was removed by evaporation under N_2(g), and the samples were diluted with MQ

water to a total volume of 20 mL. A 100 µL aliquots sample was taken and analysed for glycerol using the glycerol assay kit (Megazyme®, K-GCROL).

Presentation and organization of data

In this investigation, only FAs are analysed. Quantified FAs are all above LOQ, have an equal retention time of external FAME standard, and have a mass spectra match factor (SI) in the NIST library of more than 800. Identified FAs are above LOD and have a SI match factor of more than 700. The concentration for each FA is given in µg/g or mg/g sample, where the sample is 1g of dairy product normalised to a fat percentage of 3.5 % by dilution with water. This amount is synonymous with mg/mL or µg/mL, as the lipids were extracted into 1mL n-heptane before analysis. The concentrations were calculated based on the known concentration of IS, the peak area of the sample and IS, and the relative response factor (see eq. 7). This was done for ease of analysis in the GC-MS, data handling, and interpretation of data.

Due to the enormous nature of the data collected, only a subset of the most abundant FAs is presented graphically. For complete tables and corresponding graphical representations, consult the supporting information. The fat percentage is normalized to 3.5 % for all products because the relative trends in the FA profile are more straightforward to interpret than having absolute values for each product. The FA analyses are arranged graphically according to concentration and not according to FA carbon chain length. Organizing the results according to decreasing relative abundance facilitates easier visualization of trends, similarities and differences. It also makes comparing the profiles and amounts between and within the dairy products and their respective fractions easier. This report intends to investigate the TAG composition, specifically the FAs attached in the sn-2 position. Due to the stereospecificity of the digestive enzymes employed, the MAG fraction will be comprised exclusively of FAs attached in the sn-2 position on the TAG molecule. Therefore, the results and discussion will focus mainly on the MAG analysis.

Results and discussion

LOD/LOQ

LOD and LOQ were determined for 35 FAME standards using calibration curve method with a signal-to-noise ratio (S/N) on the y-axis and concentration (µg/mL) on the x-axis. The results are compiled in table 6.

Table 6. LOD and LOQ (µg/mL) for FAME standards.

FAME	LOD (µg/mL)	LOQ (µg/mL)	R^2
C4:0	1.130	3.360	0.999806
C6:0	0.172	0.536	0.995753
C8:0	0.183	0.388	0.996131
C10:0	0.058	0.160	0.996045
C11:0	0.116	0.205	0.992697
C12:0	0.146	0.299	0.995492
C13:0	0.115	0.235	0.993027
C14:0	0.192	0.335	0.996584
C14:1-c9	0.211	0.370	0.988128
C15:0	0.182	0.361	0.995371
C15:1-c10	0.173	0.395	0.998422
C16:0	0.269	0.528	0.999063
C16:1-c9	0.195	0.537	0.997935
C17:0	0.294	0.641	0.994866
C17:1-c10	0.272	0.511	0.986093
C18:0	0.595	0.839	0.996582
C18:1-t9	0.272	0.596	0.998841
C18:1-c9	0.349	0.674	0.998065
C18:2-t9,12 n-6	0.282	0.706	0.999918
C19:0	0.294	0.909	0.997487
C18:2-c9,12 n-6	0.292	0.715	0.996823
C18:3-c9,12,15 n-3	0.678	1.940	0.978313
C20:0	1.320	2.450	0.810633
C20:1-c11	0.824	2.060	0.994658
C21:0	0.233	0.594	0.989475
C20:2-c11,14 n-6	0.235	0.633	0.996858
C20:3-c11,14,17 n-3	0.264	0.681	0.98509
C20:3-c5,8,11,14 n-6	0.263	0.812	0.970808
C22:0	0.370	0.675	0.995991
C22:1-c13	0.299	0.914	0.991997
C20:5-c5,8,11,14,17 n-3	0.304	0.809	0.999375
C23:0	0.355	0.971	0.989701
C22:2-c13,16 n-6	0.782	2.030	0.977477
C24:0	0.576	0.712	0.994186

C20:6-c4,7,10,13,16,19	0.218	0.361	0.997432

From table 6, LOD ranges from 0.06 – 1.1 µg/mL and LOQ from 0.2 – 3.4 µg/mL, with R^2 never lower than 0.8. The highest LOD/LOQ values are for C4:0 and C20:0 likely due to increased noise in these areas in the chromatogram. Other FA analyses using the same instrument are Flytkjær (2021)[101] who reports LOD for FAMEs to be 4 – 25 µg/mL, and Østbø (2021)[102] reports 3 – 14 µg/mL, with C20:0 also having the highest concentration. This analysis has thus a much lower LOD and LOQ than previous reports.

Digested dairy products - MAG analysis

From the MAG fraction of the different dairy products, 27 different FAs were identified, of which 23 are quantified. Of the quantified FAs, 17 were SFA, 5 were MUFA, and C18:2-cis-9,12 was the only PUFA. No ω-3 FA was detected in the MAG fraction for any dairy product; thus, the ω-6/ω-3 ratio is omitted. Table 7 summarizes FAs for the MAG fraction in mg per 1g sample of each dairy product at 3.5 % fat in decreasing amount.

Table 7. Summary of MAG analysis of digested dairy products (mg/g per 1g sample at 3.5 % fat ± SD, n = 3) in decreasing amount.

Fatty acid	Gouda cheese	Cream cheese	Whole cream	Sour cream
C16:0	2.9 ± 0.36	3.7 ± 0.029	3.4 ± 0.2	4.3 ± 0.24
C18:1-c9	1.1 ± 0.16	1.4 ± 0.018	1.3 ± 0.068	1.6 ± 0.1
C14:0	1.0 ± 0.13	1.2 ± 0.0064	1.3 ± 0.074	1.6 ± 0.067
C18:0	0.43 ± 0.029	0.67 ± 0.016	0.62 ± 0.037	0.74 ± 0.053
C12:0	0.16 ± 0.022	0.1 ± 0.007	0.18 ± 0.0094	0.22 ± 0.0068
C16:1-c9	0.13 ± 0.009	0.15 ± 0.01	0.17 ± 0.0097	0.21 ± 0.0061
C14:1-c9	0.09 ± 0.01	0.09 ± 0.003	0.1 ± 0.006	0.12 ± 0.007
C15:0	0.07 ± 0.008	0.08 ± 0.005	0.09 ± 0.008	0.11 ± 0.002
C18:2-c9,12	0.07 ± 0.01	0.08 ± 0.007	0.08 ± 0.006	0.1 ± 0.01
C10:0	0.03 ± 0.008	0.01 ± 0.002	0.03 ± 0.003	0.03 ± 0.001
anteiso-C17:0	0.03 ± 0.008	0.03 ± 0.002	0.04 ± 0.002	0.04 ± 0.003
iso-C16:0	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.001	0.02 ± 0.0007
C16:1-c7	0.02 ± 0.001	0.02 ± 0.0007	0.02 ± 0.002	0.02 ± 0.002
iso-C15:0	0.02 ± 0.0009	0.02 ± 0.001	0.02 ± 0.002	0.03 ± 0.001
iso-C14:0	0.009 ± 0.0004	0.009 ± 0.0006	0.01 ± 0.0008	0.01 ± 0.0009
iso-C13:0	0.007 ± 0.0005	0.007 ± 0.0004	0.009 ± 0.0007	0.01 ± 0.0003

C17:0	n.d.	n.d.	0.04 ± 0.005	0.04 ± 0.0008
C13:0	n.d.	0.006 ± 0.001	0.007 ± 0.0008	0.009 ± 0.0004
C6:0	n.d.	n.d.	0.006 ± 0.0002	n.d.
C9:0	n.d.	n.d.	0.004 ± 0.0002	n.d.
C16:0-3,7,11,15-met	n.d.	0.01 ± 0.0006	0.004 ± 0.001	0.006 ± 0.001
C8:0	n.d.	n.d.	0.002 ± 0.0002	n.d.
C18:1-t9	n.d.	n.d.	n.d.	0.05 ± 0.002
∑SFA	4.8 ± 0.57	5.8 ± 0.073	5.7 ± 0.34	7.05 ± 0.37
∑MUFA	1.3 ± 0.18	1.6 ± 0.031	1.6 ± 0.085	1.96 ± 0.12
∑PUFA	0.067 ± 0.013	0.079 ± 0.0072	0.081 ± 0.0056	0.1 ± 0.012
∑ω-3	n.d.	n.d.	n.d.	n.d.
∑ω-6	0.067 ± 0.013	0.079 ± 0.0072	0.081 ± 0.0056	0.1 ± 0.012
∑FA	6.2 ± 0.76	7.6 ± 0.11	7.4 ± 0.44	9.1 ± 0.5
IA	5.1	5	5	5.3
IT	6.6	2.3	6.1	6.5

From table 7, the total FA content is 6.2 – 9.1 mg/g, depending on the dairy product. MAG fraction has 77% SFA, 21 % MUFA and 1% PUFA, for all dairy products. Considering that each dairy product is produced from bovine milk, it is expected that there should not be much deviation in FA composition between the different products, like we see here. Whole cream is unique in that it is the only dairy product which quantified C6:0, C8:0 and C9:0 in the MAG fraction. Whole cream is different from the other products in that it is the least processed, has the shortest shelf life and has a lower viscosity than the other products. Having a lower viscosity means that there should be larger volume of low molecular weight TAG, meaning that we can see presence of short- and medium- chain FAs in the sn-2 position. Amount of unsaturated FAs in the sample will also affect the rheological properties of the product[103], yet from table 7 there are no real differences between the dairy products. The results show a slightly higher SFA and lower MUFA than what is expected. For undigested bovine milk, SFA and MUFA is typically 70 % and 30 %, respectively. Østbø[102] has analysed MAG fraction for these same dairy products, and reports SFA (73 – 83 %), MUFA (16 – 26 %) and PUFA (1.2 – 1.7 %). So, he report a slightly higher amount of SFAs than here, but also a larger difference between the dairy products. There should also be lower SFA during the summer, due to the cows grazing outside, and thus exposed to different feed and climate. The dairy products were purchased in June, but there is little information on when the milk was harvested. Whole cream has the shortest shelf life and does not undergo as much processing, so

this is likely the freshest dairy product. Domestic cattle are reproductively active year-round[104], which makes complete knowledge of the stage of lactation a challenge. Considering what we know from previous reports on the position of FAs in the TAG molecule (see tables 3 and 4) [18, 40, 105], the most abundant SFA in milk, C16:0, is preferentially attached in the sn-2 position, and the most abundant MUFA in milk, C18:1, is preferentially attached in the sn-1 position; it, therefore, coheres well that the MAG fraction will have proportionally less MUFA. This point is illustrated better in Figures 9 and 10.

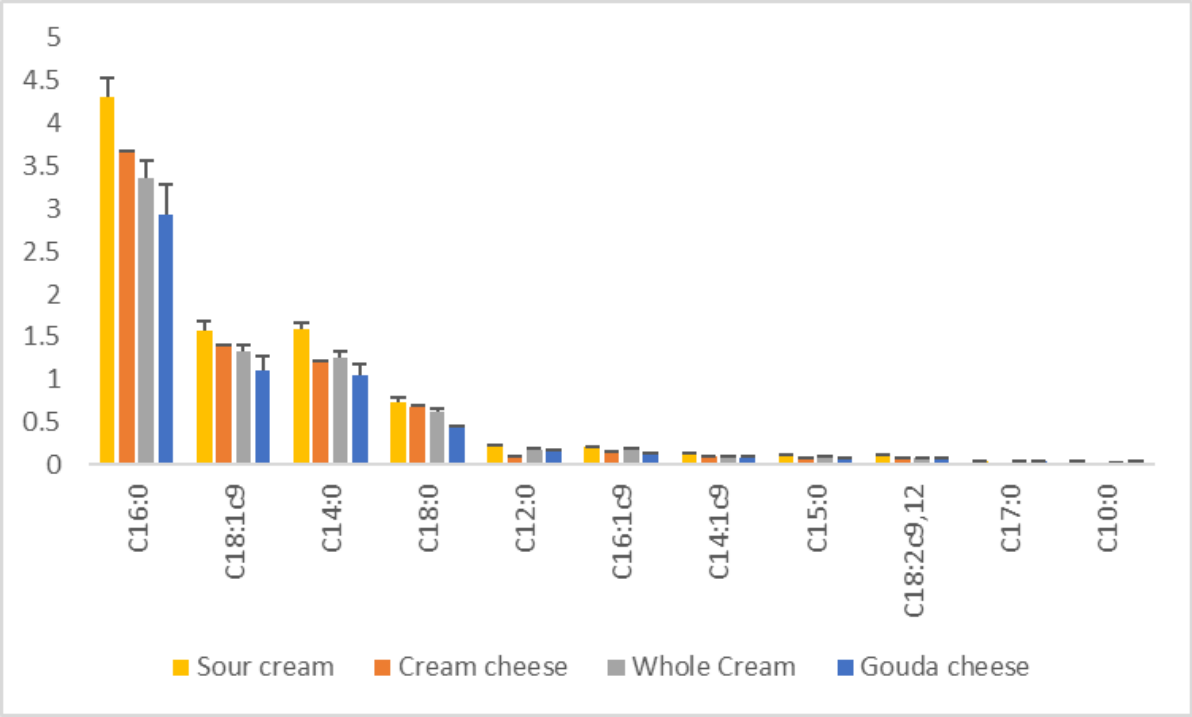

Figure 9: MAG fraction's FA composition (mg/g) for different dairy products.

Figure 9 shows that C16:0 is the most abundant FA in MAG, regardless of dairy product, followed by C18:1c9, C14:0, C18:0 and C12:0, which make more than 90% of the total MAG fraction. There was no presence of short-chain FAs in the MAG, and only minuscule amounts of C10:0, the shortest FA quantified, further suggesting that shorter-chain fatty acids are preferentially attached in the sn-3 position. Figure 9 also shows a distinct difference between the dairy products, with sour cream having the highest amount and gouda cheese the lowest for most of the FAs. Table 7 also corroborates that sour cream has the highest ∑FA and gouda cheese the lowest. Differences in digestion due to the matrix effect may cause these differences. Figure 10 illustrates further the relative FA composition for each dairy product.

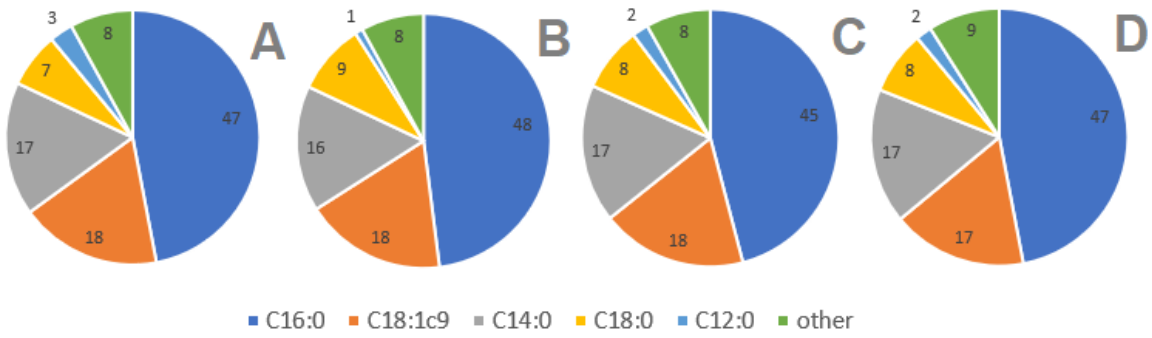

Figure 10: Relative FA composition (%) of MAG fraction for A – gouda cheese, B – cream cheese, C – whole cream and D – sour cream.

From figure 10, we can see that relatively, there is little difference in FA composition between each dairy product, suggesting a very high degree of conservation of milk's FA profile when processing and digestion. From the theory, we know that TAG hydrolyses into 2-MAG. We should then expect to see mostly C16:0, C18:1c9 and C14:0. From figure 10, almost half of the FAs attached in the sn-2 position are C16:0 (45 – 48 %). C18:1c9 and C14:0 are almost equal relative abundance, with 18 % and 17 %, respectively. Combining tables 3 and 4 from the theory and calculating the wt % for FAs in the sn-2 position, the relative FA composition of milk looks like C16:0 (37 %), C18:1c9 (14 %), C14:0 (21 %), C18:0 (5 %) and C12:0 (7 %). From these, it can be seen that the results have a higher amount of C16:0, C18:1c9, and C18:0 and less C14:0 and C12:0 compared to bovine milk. Previous FA analysis on the same dairy products by Østbø, 2021[102], reports similar relative FA composition as this investigation.

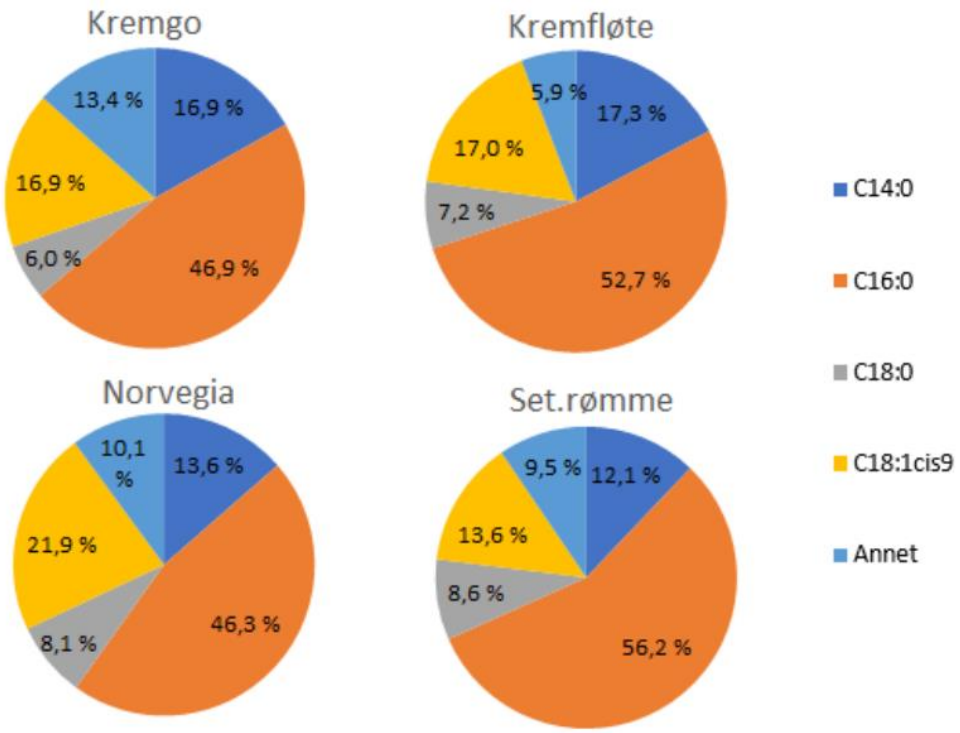

Figure 11: Relative FA composition (%) of MAG fraction for dairy products. The figure is taken from the master's thesis by Østbø, 2021[102].

Østbø's results in figure 11 show a larger difference between each product than found in this investigation (figure 10). Østbø's results report a higher abundance of C16:0 in whole cream and sour cream (53 and 56 %, respectively), the highest amount of C18:1c9 for gouda cheese (22 %), and a lower amount of C14:0 for gouda cheese and sour cream (14 and 12 % respectively). This investigation uses the same analytical method as developed by Østbø. Thus, these differences are not systemic to the method used and likely reflect fundamental sample differences.

Comparison of MAG with undigested dairy products

For a more in-depth understanding of the FA composition in the MAG fraction, it is pertinent to compare it to undigested products. The FA composition of undigested product will show the FAs in the sn-1 and sn-3 position, as they will be missing from the MAG analysis. Undigested FA composition is documented in table 8 and figure 12.

Table 8. Summary of FAs for undigested dairy products (mg/g per 1g sample at 3.5 % fat ± SD, n = 3) in decreasing amount.

Fatty acid	Gouda cheese	Cream cheese	Whole cream	Sour cream
C16:0	7.5 ± 0.7	8.1 ± 0.21	7.1 ± 0.36	8.3 ± 0.21
C18:1-c9	5.7 ± 0.43	5.4 ± 0.026	5.5 ± 0.3	5.6 ± 0.3
C18:0	3.6 ± 0.37	3.6 ± 0.066	3.4 ± 0.25	3.7 ± 0.087
C14:0	2 ± 0.17	2.3 ± 0.081	2.1 ± 0.11	2.5 ± 0.11
C12:0	0.52 ± 0.043	0.6 ± 0.027	0.57 ± 0.034	0.66 ± 0.036
C10:0	0.38 ± 0.031	0.42 ± 0.021	0.42 ± 0.032	0.48 ± 0.026
C16:1-c9	0.41 ± 0.031	0.37 ± 0.0076	0.41 ± 0.019	0.43 ± 0.009
C14:1-c9	0.27 ± 0.02	0.3 ± 0.0099	0.28 ± 0.016	0.33 ± 0.016
C18:2-c9,12	0.34 ± 0.025	0.3 ± 0.0064	0.32 ± 0.0056	0.33 ± 0.01
C15:0	0.22 ± 0.018	0.24 ± 0.0077	0.22 ± 0.012	0.27 ± 0.0097
C6:0	0.19 ± 0.018	0.19 ± 0.016	0.19 ± 0.021	0.23 ± 0.0083
C8:0	0.14 ± 0.016	0.14 ± 0.0073	0.15 ± 0.013	0.18 ± 0.012
C4:0	0.14 ± 0.017	0.13 ± 0.012	0.13 ± 0.024	0.16 ± 0.005
C17:0	0.15 ± 0.0099	0.13 ± 0.012	0.13 ± 0.0065	0.16 ± 0.034
anteiso-C17:0	0.089 ± 0.0067	0.082 ± 0.0012	0.085 ± 0.0018	0.095 ± 0.01
C18:1-t9	0.083 ± 0.0054	0.082 ± 0.0006	0.08 ± 0.0053	0.088 ± 0.004
C18:3-c9,12,15	0.068 ± 0.0027	0.063 ± 0.0059	0.085 ± 0.0026	0.08 ± 0.0016
iso-C15:0	0.047 ± 0.0033	0.051 ± 0.0019	0.049 ± 0.0031	0.058 ± 0.0025
iso-C16:0	0.05 ± 0.0039	0.051 ± 0.0022	0.047 ± 0.0017	0.05 ± 0.003
C16:1-c7	0.035 ± 0.0037	0.031 ± 0.0011	0.033 ± 0.0005	0.034 ± 0.00042
C20:4-c5,8,11,14	0.019 ± 0.0025	0.02 ± 0.00091	0.018 ± 0.0015	0.02 ± 0.0029
iso-C14:0	0.018 ± 0.0013	0.018 ± 0.00072	0.018 ± 0.0005	0.02 ± 0.001
iso-C13:0	0.014 ± 0.0008	0.015 ± 0.0007	0.015 ± 0.0005	0.019 ± 0.0011
C13:0	0.013 ± 0.000021	0.015 ± 0.0018	0.014 ± 0.0003	0.017 ± 0.0005
C20:3-c8,11,14	0.011 ± 0.002	0.014 ± 0.0015	0.01 ± 0.0005	0.011 ± 0.0023
C22:5-c7,10,13,16,19	0.0078 ± 0.0008	0.0079 ± 0.0004	0.0098 ± 0.0011	0.011 ± 0.0004
C19:1-c10	0.0088 ± 0.0013	0.0086 ± 0.00047	0.0084 ± 0.0005	0.0087 ± 0.0003
C14:1-c11	0.0055 ± 0.0004	0.0077 ± 0.0004	0.0066 ± 0.0003	0.0077 ± 0.0005
anteiso-C13:0	0.0047 ± 0.0004	0.0046 ± 0.00006	0.0048 ± 0.0003	0.0051 ± 0.0002
C24:0	0.0048 ± 0.0006	0.0057 ± 0.0017	0.0053 ± 0.0014	0.0048 ± 0.0005
C26:0	0.0036 ± 0.0005	0.0044 ± 0.00009	0.0035 ± 0.0004	0.0041 ± 0.0003
∑SFA	33 ± 1.1	29 ± 1.7	32 ± 0.95	30 ± 2.8

∑MUFA	13 ± 0.64	13 ± 0.68	12 ± 0.093	13 ± 0.98
∑PUFA	0.84 ± 0.028	0.86 ± 0.02	0.77 ± 0.028	0.9 ± 0.067
∑ω-3	0.16 ± 0.003	0.2 ± 0.008	0.16 ± 0.013	0.16 ± 0.008
∑ω-6	0.68 ± 0.025	0.7 ± 0.015	0.67 ± 0.018	0.75 ± 0.06
∑FA	47 ± 1.8	43 ± 2.4	46 ± 1.1	44 ± 3.9
ω-6/ω-3	4.2	3.5	4.2	4.7
IA	2.8	2.4	2.7	2.3
IT	3.9	3.3	1.8	5.1

Table 8 shows the amount of FAs in undigested dairy products. Forty-one unique FAs were identified, of which thirty-one were quantified. The analyses show that the FA content is similar for all dairy products. There is 68 – 70 % SFA, 26 – 30 % MUFA, 1.6 – 2 % PUFA and 0.3 – 0.4 % ω-3 FA, which is a 7 % smaller degree of saturation compared to the MAG fraction, further corroborating the hypothesis that there are more SFA attached in sn-2 than the rest of the TAG molecule. This composition also fits better with the reported composition of undigested whole milk (70 % SFA, 30 % MUFA and 1 – 2 % PUFA). Table 8 shows presence of more unsaturated FAs and especially poly-unsaturated (C18:3, C20:4, C20:3, C22:5) which are absent in the digested MAG in table 7. Table 8 also shows a higher presence of shorter chain FAs (C4:0, C6:0, C8:0 and C10:0). In table 7, much of the saturated FAs and branched FAs remain. This indicates that the saturated medium-long chain FAs are preferentially attached in the sn-2 position, and thus remain in the MAG analysis. We can also say that much of the PUFA in table 8 is likely preferentially attached in either sn-1 or sn-3 position as they are absent in table 7. These esterification preferences are in accordance with other studies in bovine milk. [11, 15, 30, 106] Pacheco-pappenheim et al. (2022) reports a slightly different profile although in low concentrations. They have identified C17:1-*cis*9 and C20:0 as preferentially attached in the sn-2 position, and C16:1-*trans*9, C18:1-*trans*9 and C18:1-*cis*11 preferentially attached in sn-1(3) position. These variations are likely due to natural variations in the milk and dairy products.

The ω-6/ω-3 ratio is 3.5 – 4.7, lowest for whole cream (3.5) and highest for gouda cheese (4.7). Conventional milk is reportedly 5.7, organic milk 2.3, and grass-fed milk 0.95[107]. The total amount of FAs is 43 – 47 mg/g, depending on the dairy product. This is an 8 % difference between whole cream, the lowest and sour cream, the highest. Østbø[102] has reported a FA analysis of these dairy products in relative amounts (%), but a FA analysis of these products in absolute amounts has not been done. Bovine whole milk has been studied extensively [11, 14, 15], and FA analyses put the total

FA content at 33 mg/g milk.[5, 25, 108, 109] Thus, the ∑FA undigested dairy products herein is much higher than expected. Comparing table 7 and 8, undigested ∑FA is 5 – 7 times higher than the ∑FA for digested MAG fraction. Figure 12 will illustrate the differences in the relative FA composition for the undigested dairy products compared to the MAG fraction.

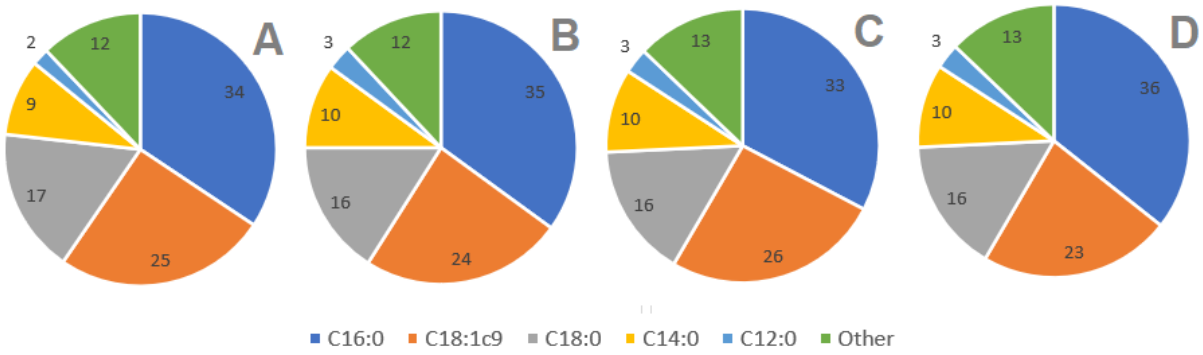

Figure 12: Relative FA composition (%) of undigested A – gouda cheese, B – cream cheese, C – whole cream and D – sour cream.

Figure 12 shows that the FA composition between undigested dairy products is similar. Similar to MAG, >90 % is comprised of C16:0 (33 – 36 %), C18:1c9 (23 – 26 %), C18:0 (16 – 17 %), C14:0 (9 – 10 %) and C12:0 (2 – 3 %). From table 8 we can see the presence of common FAs in bovine milk, su This distribution is the same profile reported for undigested bovine milk, suggesting a minimal effect on the FA composition during fermentation and digestion. [11, 14, 15] The relative abundance of each FA is markedly different from the MAG fraction. The MAG fraction has a 12 % higher content of C16:0 and 6 – 8 % higher C14:0 concentration than the undigested. This fits well with long-chain SFAs preferentially attached in the sn-2 position on the TAG. The MAG has an 8 – 10 % lower C18:0 concentration, or half the relative amount as undigested. It is known that C18:0 will be attached equally to the sn-1 and sn-2 positions. When the FAs in the sn-1 and sn-3 position is hydrolysed, the remaining amount of C18:0 will be halved. This is a good indicator that there is only 2-MAG in the sample. There is a decrease in the amount of C18:1c9 (6 – 8 %) as this is preferentially attached in either the sn-1 or sn-3 position. C12:0 is almost the same concentration for both MAG and undigested. "Other" for undigested is slightly higher than MAG due to the presence of short- and medium-chain fatty acids, preferentially attached in the sn-3 position, which are absent in the MAG fraction.

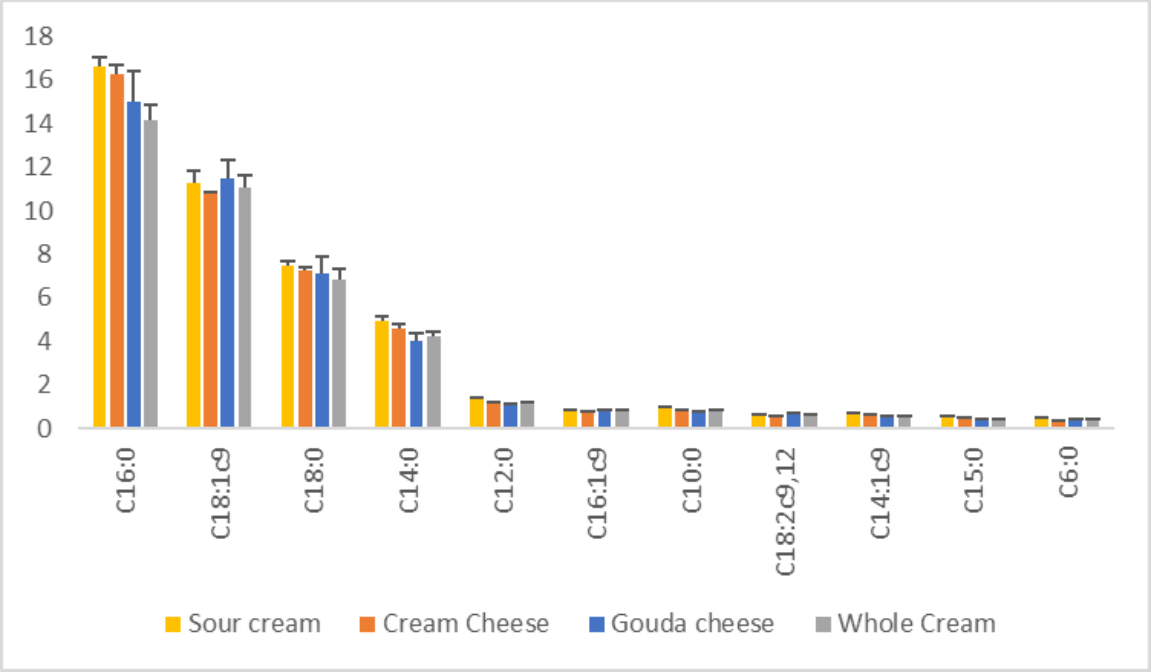

Figure 13: undigested dairy products' FA composition (mg/g ± SD, n = 3) in decreasing amount.

In figure 13, the composition of the most abundant FAs for each dairy product is graphically represented. There is a difference in the amount of C16:0 between the dairy products, with sour cream having the most (17 mg/g) and whole cream the least (14 mg/g), a 17 % difference. The other FAs show no difference in amount, with differences <5 %. Table 8 documents the presence of C4:0, C6:0 and C8:0 for all samples, absent from the MAG fraction (table 7). This further shows that the short-chain fatty acids are preferentially attached in the sn-3 position. Previous FA analysis using the same method reported that SCFAs are under-quantified as they are more polar and have lower boiling points than the longer FAs; they will more readily be extracted into the water phase or evaporate from the sample. This problem could be mitigated using a more appropriate internal standard; for example, C5:0.[102] Figure 9 shows a higher abundance of C14:1 and C17:0 in the MAG fraction than undigested, supporting that the long-chain FAs are preferentially attached in the sn-2 position on the TAG.

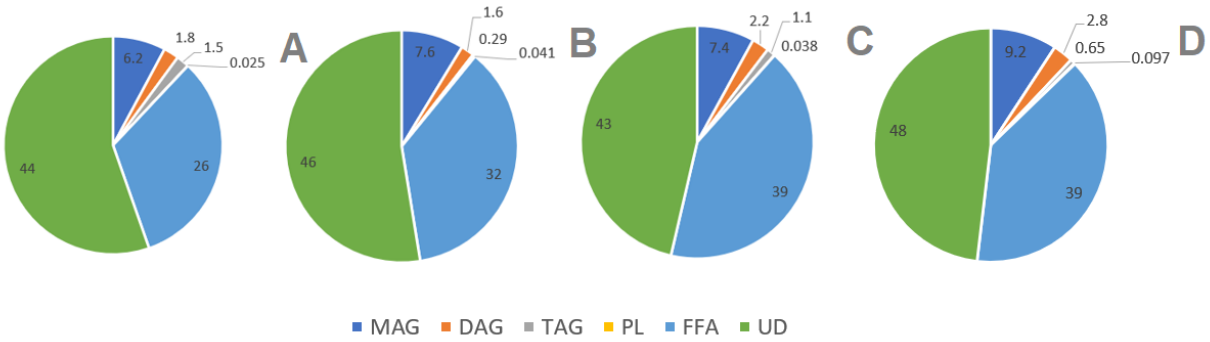

Figure 14: Total amount, ∑FA (mg/g) of each fraction for undigested and digested A – Gouda cheese, B – Cream cheese, C – Whole cream and D – Sour cream.

Figure 14 shows the absolute amount of each lipid fraction for the different dairy products. The undigested FAs make the largest fraction, with 46 – 55 % of the total. The FFA fraction is 32 – 46 %, and the MAG is 8 – 9 %. This is expected as the bulk of the product will be hydrolysed into FFA during digestion, showing that the digestion was successful. If the concentrations are calculated in mol %, there should theoretically be a 2:1 ratio between FFA and MAG, as each molecule of TAG releases two molecules of FAs to produce one molecule of MAG. This, however, is not the case practically, as 2-MAG will spontaneously isomerise into 1(3)-MAG and be further hydrolysed. It is suggested that the rate of isomerisation in water is 5.12 – 5.86 %/hr at 20 °C.[110] The digestion lasted for at least 4 hrs at 37 °C, meaning as much as 23.4 % of the 2-MAG produced is isomerised. The isomerisation will increase yield in FFA and free glycerol, as the 1(3)-MAG can be hydrolysed by the digestive enzymes. From figure 13, the FFA fraction is 4 – 5 times larger than the MAG, suggesting a high degree of hydrolysation of 2-MAG. Because both DAG and MAG isomerise at similar rates, a discussion of the relative composition of DAG and FFA fractions will be difficult, as the position of the FA on the glycerol cannot be discussed with any certainty, unlike the MAG and undigested fractions can. After digestion stops, 2-MAG will continue to isomerise; however, 1(3)-MAG will not be hydrolysed, so the MAG fraction will be a mixture of 2-MAG and 1(3)-MAG. The digestive enzymes ensure that all the FAs in the MAG fraction originate from the sn-2 position on the TAG molecule, regardless of isomerisation.

There is a minuscule presence of TAG and DAG from remaining undigested lipids. The DAG and TAG fractions will be the lipids that are not fully digested. In an *in vitro* model, like in this study, the ratio of undigested/digested FAs will be higher than *in vivo* because the digestion equilibrium

will be pushed towards undigested. It has been documented that gastric lipase is rate sensitive to the concentration of FFAs in solution. As the concentration of MAG and FFA increases, the rate of formation of products will decrease due to the remaining TAG molecules being sterically hindered from reaching the active site on the enzymes. *In vivo*, the MAG and FFA will be absorbed as they are produced, pushing the equilibrium towards products and resulting in greater hydrolysis of TAG.

Other fractions

Although not the focus of this thesis, it is worth taking a more comprehensive approach to discuss observable trends in the different dairy products and their respective lipid fractions. Figures 15 and 16 can help better represent the trends between fractions within the same dairy product.

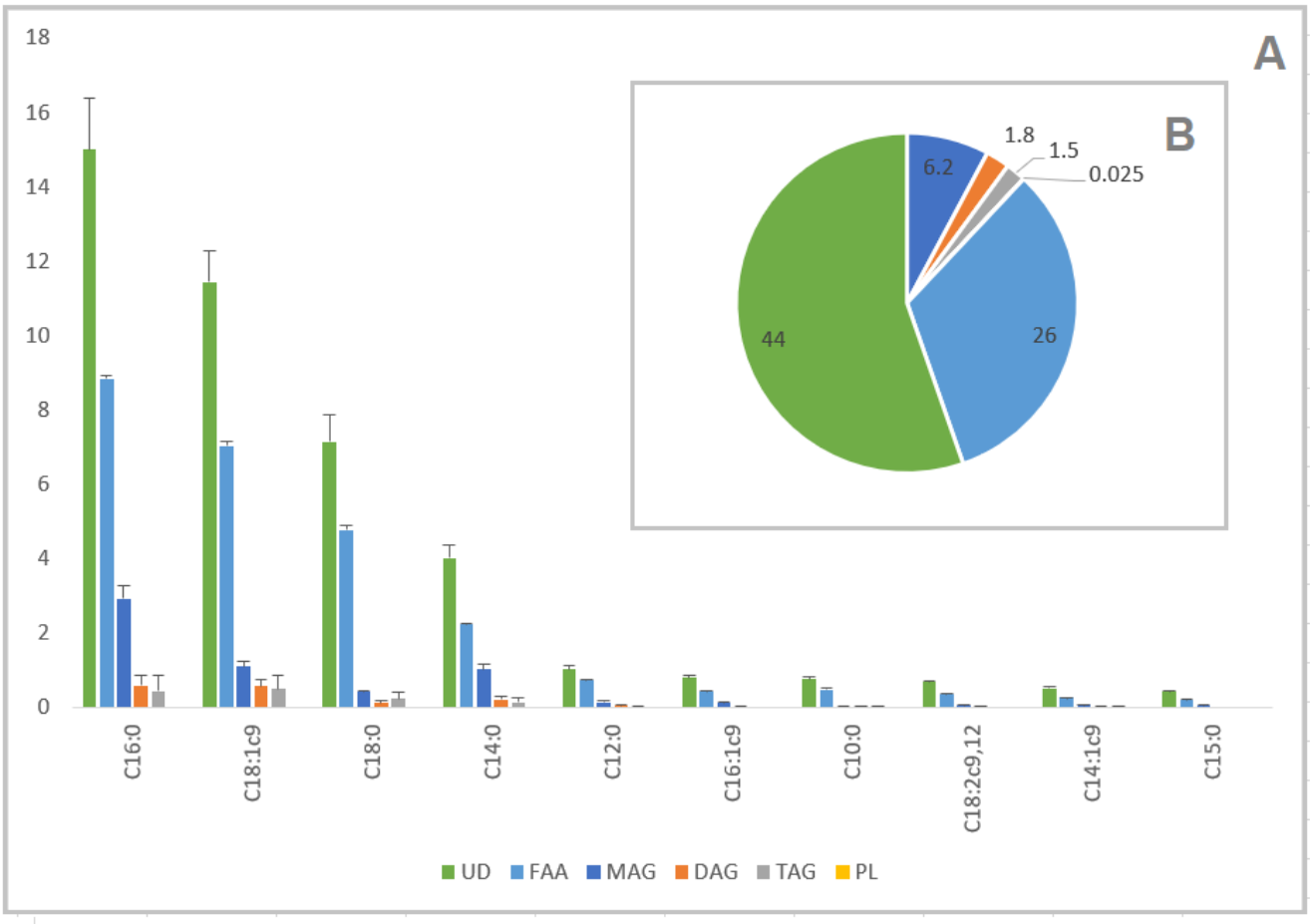

Figure 15: FA composition (mg/g ± SD, n = 3) for gouda cheese. A - The subset of most abundant FAs for each fraction is arranged in order of decreasing amount. B – Total amount of FAs for each fraction.

From figure 15, the undigested fraction is the largest and the only fraction identifying short-chain FAs (C4:0, C6:0). The digested samples have the highest amount of FFAs, followed by MAG, DAG, TAG, and minuscule amounts of PL. The FA profile of all lipid fractions is similar, with >90 % consisting of C16:0, C18:1, C18:0, C14:0 and C12:0. Compared to gouda cheese, whole cream is the most dissimilar. A graphical representation of whole cream is given in figure 16.

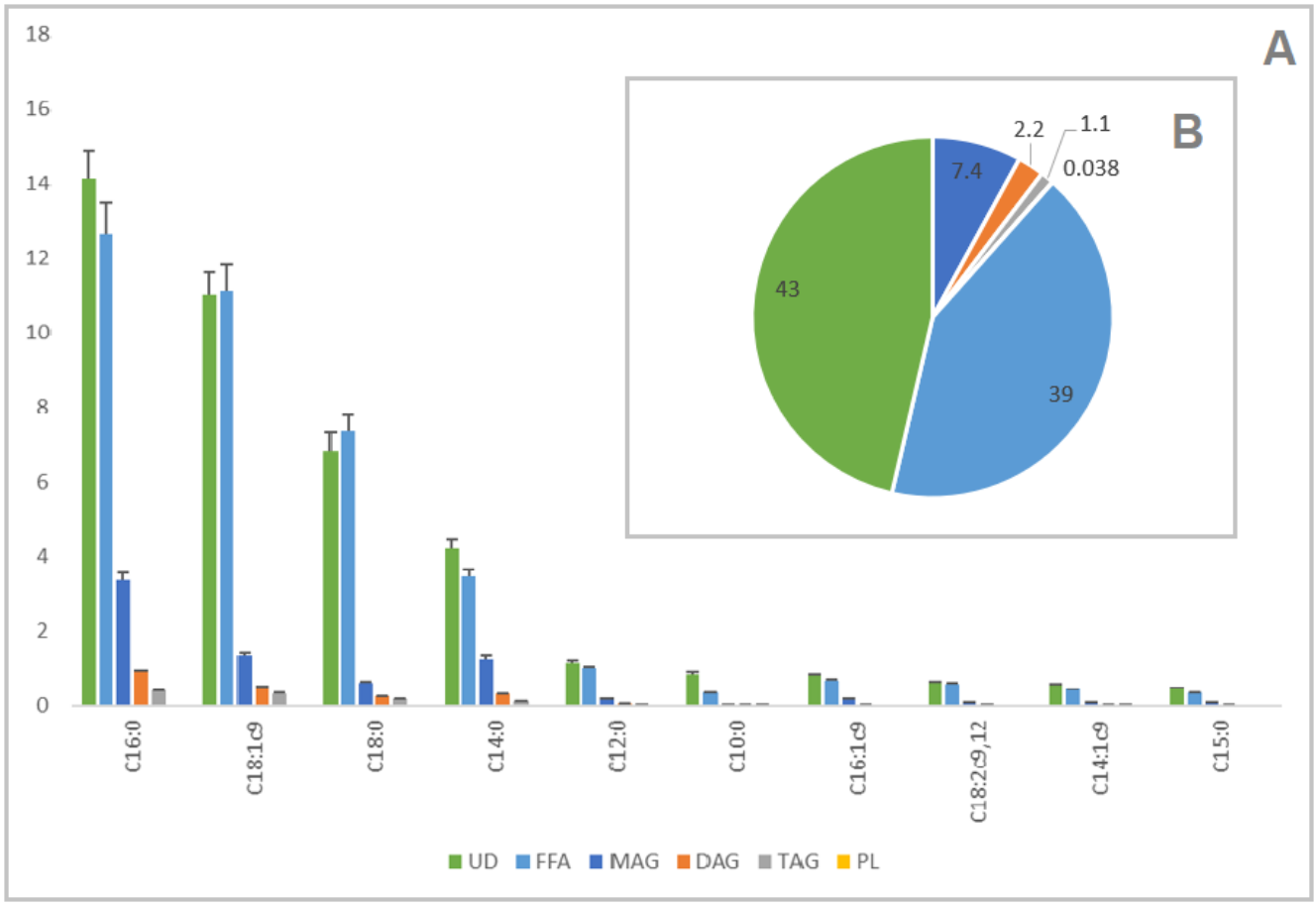

Figure 16: FA composition (mg/g ± SD, n = 3) for whole cream. A- The subset of most abundant FAs for each fraction is arranged in order of decreasing amount. B – Total amount of FAs for each fraction.

From figure 16, we can again see the same pattern discussed in figure 15. Again, there are most undigested FAs, with FFA, MAG, DAG, TAG and PL in decreasing amounts. Again, we can see the same FA profile as for gouda cheese. Here the difference lies in a slight elevation in FFAs; we can see that for C18:1 and C18:0, there is more FFA than undigested. This is anomalous and will be discussed further later.

From figures 14 – 16, the absolute amounts of each fraction can be discussed. Theoretically, one would expect equal amounts of undigested as the sum of the digested FA fractions since the sample amount is the same and *in vitro* digestion guarantees that no sample is absorbed into the body. However, for gouda cheese and cream cheese, the undigested fraction is slightly larger than the sum of digested fractions, see figure 14. This trend is typical, as shorter chain FAs are more polar and volatile and will thus be lost to evaporation, extraction, and in the SPE column, disproportionally compared to the internal standard. Interestingly, the undigested fraction for whole cream and sour cream is smaller than the sum of their respective digested fractions. Closer inspection reveals a comparatively higher concentration of FFA for whole cream and sour cream (40 – 42 %) than gouda cheese and cream cheese (32 – 37 %), but with the same or slightly higher amount of MAG and DAG. Considering the anomaly also observed in figure 16, there is more FFA for some FAs than theoretically possible. The undigested fraction contains all the FAs possible to observe in the combined digested fractions, so when there are larger amounts of digested FAs than undigested, it shows that there is something wrong with quantifying the FAs. Common problem is FAs that bleed from the SPE column, contaminating the samples, resulting in higher concentrations of FAs. In this method, however, blank samples have been analysed, and any contaminations are subtracted from the other sample analyses.

The standard deviation for all samples is mostly <5%. This reflects that the method's precision is excellent, and human error is minimal. Therefore, there must be some systematic error within the method that leads to wrong quantification, and the critical part of this is the concentration of the internal standards used. Most likely, the concentration of the internal standards is slightly lower than estimated, resulting in calculations from the chromatogram will be much higher than reality. In addition, the internal standard is sensitive to evaporation or deposition over time, lowering its concentration, so it must be made ready within a short time of the analysis. Due to this project's limited timeframe, however, rectifying this problem was not feasible for this thesis, and therefore any discussion on absolute amounts of FAs is omitted.

Glycerol analysis

If 2-MAG isomerises into 1(3)-MAG during digestion, the FA chain can be hydrolysed by the digestive enzymes. Evidence of this process will be in the presence of free glycerol in the samples after digestion, as this is the end product together with FFA. Therefore, the total amount of glycerol in the sample will indicate the degree of isomerization in the samples.

Even though the digested samples have been extracted with Folch's solution and washed with brine, glycerol was detected in all digested samples. The analyses for the undigested fractions have a large standard deviation compared to the digested counterparts, likely due to the concentrations being much lower and slightly above LOD for the method. The Megazyme® detection kit has a LOD of 0.3 µg/mL and a linear range from 0.8 – 35 µg glycerol in the sample, so if the concentration is lower, the analysis is too uncertain to quantify.

Glycerol is highly water soluble, and most of the glycerol will be dissolved in the water phase during Folch's extraction. However, any glycerol dissolved in the organic phase will again be extracted into the brine solution during subsequent washing. The minor presence of glycerol present thus indicates that there could be a substantial amount of free glycerol produced during digestion and that the current digestion model completely hydrolyses TAG into FFA and free glycerol.

Table 9. Glycerol analysis of digested and undigested dairy products (in µg glycerol per 1g 3.5 % fat, ± SD, n =3)

Glycerol analysis			
	Mean (µg/g)	STD (µg/g)	RSTD (%)
Gouda cheese			
Undigested	7.14	4.72	66
Digested	10.90	6.58	60
Cream cheese			
Undigested	<LOD	-	-
Digested	31.50	5.44	17
Whole cream			
Undigested	<LOD	-	-
Digested	16.50	4.98	30
Sour cream			
Undigested	10.70	21.70	202
Digested	5.68	3.23	56
Whole milk			

Undigested	<LOD	-	-
Digested	3620	200	5.6

Bovine whole milk (3.5 % fat) was digested and analysed for glycerol straight from the digestion solution, adhering to the same Megazyme® procedure. There was no glycerol in undigested milk and 3.62 mg/g glycerol after digestion. A 1 g sample of whole milk with 3.5 % fat is theoretically 35 mg fat in the sample. This means that at least 10 % of the lipid weight was hydrolysed into free glycerol.

Further work

Adjustments and improvements to this work should be forthcoming. Firstly, the FA analysis should be redone with more accurate internal standard concentrations to ensure accurate results. Secondly, glycerol analysis should be redone for digested dairy products before extraction with Folch's solution. This will allow for more accurate quantification of glycerol produced during digestion.

Although GC-MS works well for analysing FAMEs, other methods for analysing FAs should be employed. A natural next step is investigating methods for HPLC-MS as it can analyse more complex mixtures and a greater variety of products than GC. Using HPLC-MS, derivatization of FAs to FAMEs is not necessary. HPLC-MS will also allow for the analysis of the complete molecule (FA attached to the glycerol) and not just the FAMEs. TAG analysis will also allow for a better insight into the FA composition within the TAG molecule.

The 2-MAG spontaneous isomerization is a challenging process to analyse. An important question to try to solve is whether only 2-MAG is produced during digestion. Its spontaneity makes it challenging to control, and it will not be easy to measure the concentration of 2-MAG without it isomerizing. Also, the absorption and metabolic fate of MAG have not been studied extensively, and it will be pertinent to investigate the absorption of 2-MAG compared to 1-MAG. The MAG isomerization likely has many vital implications within the digestion and absorption of FAs *in vivo*, and its investigation will likely produce illuminating insight and unforeseen serendipitous results. *In vitro* digestion has limitations and does not give a completely accurate representation of lipid digestion *in vivo*. *In vivo* digestion is very complex, and there are many factors and variables that can influence the results, including practical and ethical limitations of using humans for experiments. *In vivo* experiments, however, should be the goal for researching lipid digestion.

Fatty acid chemistry, milk and dairy chemistry, digestion and MAG isomerization are very complex fields of study, and much territory remains unexplored.

Conclusion

Herein is a complete fatty acid analysis of four dairy products before and after digestion. Thirty-six unique FAs have been quantified, with an additional 11 identified using GC-MS. The analyses were done in triplicates, and the results are reported in mean concentration (mg/g) with acceptable standard deviations and good precision. The LOD and LOQ were calculated for FAME standards and are 0.06 – 1.1 µg/mL and 0.2 – 3.4 µg/mL, respectively, with $R^2 > 0.8$ for all.

The lipid composition and FA profiles are similar to previous reports on whole milk lipids, with approximately 70 % SFAs, 30 % MUFA and 1 – 2 % PUFA and >90 % comprised of C16:0, C18:1, C18:0, C14:0 and C12:0. Notwithstanding effects of season, feed, breed and various other factors outlined.

The results corroborate well-established literature on FA positions on the TAG molecule in dairy lipids. The results show that SCFA and MCFA are preferentially attached in the sn-3 position, and LCFA is in the sn-2 position. There is also a higher degree of saturation of FAs in the sn-2 position. This is illustrated in that the relative FA abundance increases for C16:0 and C14:0 while decreasing for C18:0, C18:1 and SCFAs in MAG.

The results show a significant degree of spontaneous isomerisation of the 2-MAG, resulting in proportionally higher amounts of FFA in the digested fractions and the presence of free glycerol in digested samples. Glycerol analysis of whole milk suggests that the INFOGEST 2.0 digestion model hydrolyses TAG completely into FFA and glycerol. Free glycerol after digestion is up to 10% of total lipid weight. Digestive enzymes are reported to be rate sensitive to concentrations of FFA, and in a closed *in vitro* system like here employed, FFA and MAG will not be absorbed from the matrix and will shift the equilibrium of the digestive enzymes, giving incomplete digestion. This is seen by the presence of DAG and TAG in the solution.

The analyses have excellent precision, with most standard deviations falling within 5 %. However, the method has been shown to report too high amounts of FAs, especially for FFA fractions. This results from the concentration of IS being lower than estimated, likely from evaporation or

deposition from the solution. Unfortunately, time constraints did not allow for rectifying this problem, and thus discussions on absolute amounts are omitted from this thesis.

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Supporting Information

Fatty acid analysis of dairy products after *in vitro* digestion by using offline SPE and GC-MS.

NMBU – Norwegian University of Life Sciences

August 2022 – April 2023

Einar Jonsson

Table of Figures

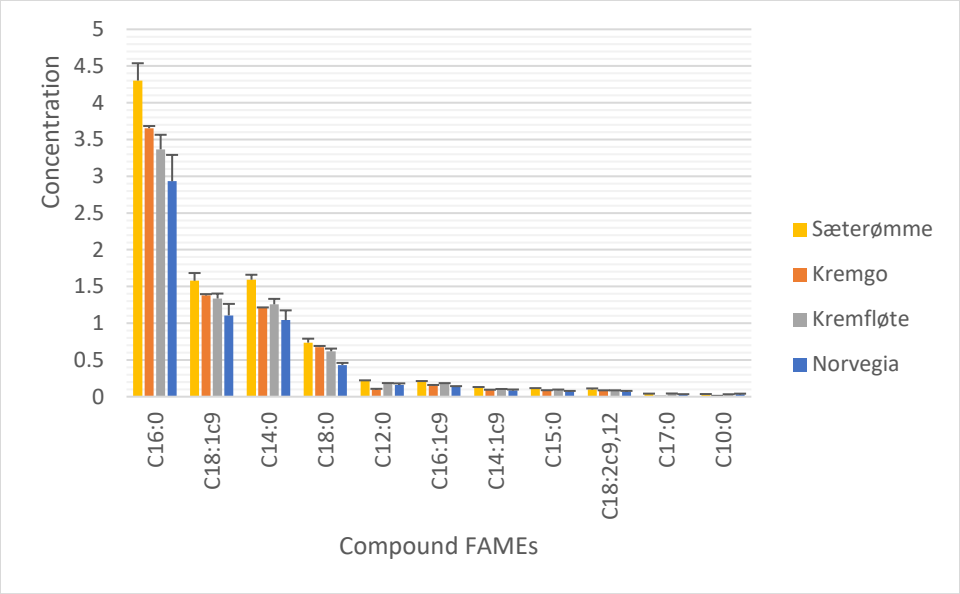

Figure 1. MAG profile for digested dairy products (mg/g sample)

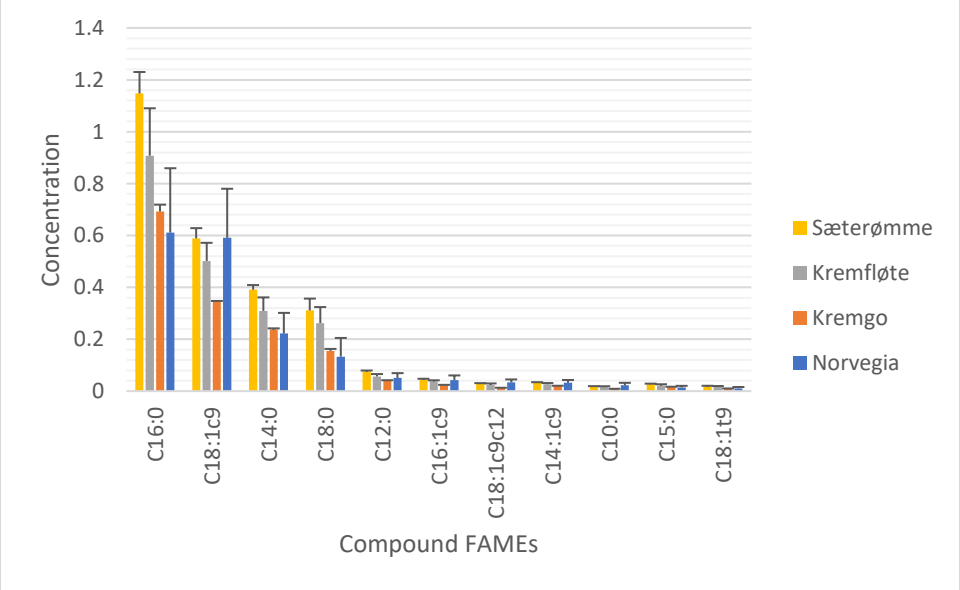

Figure 2. DAG profile for digested dairy products (mg/g sample)

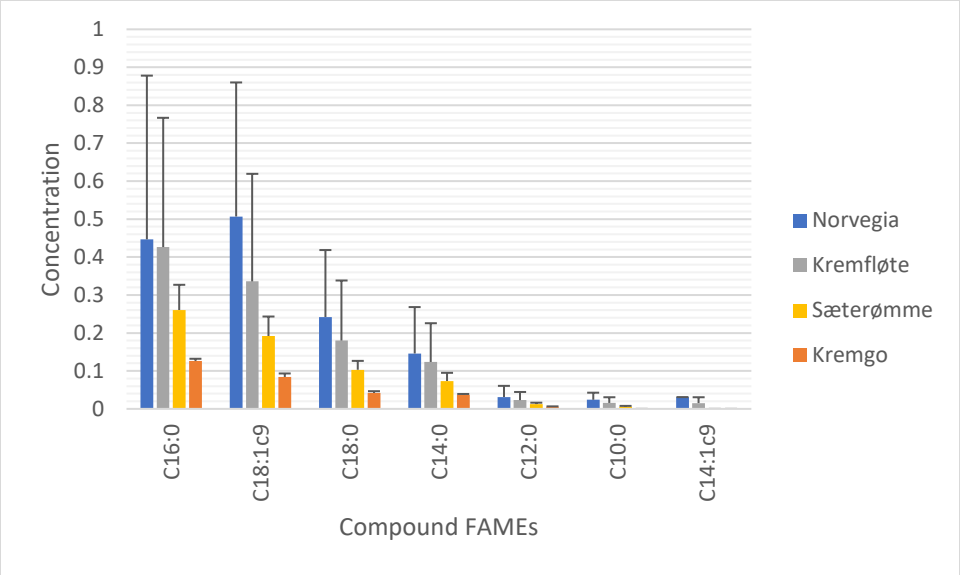

Figure 3. TAG profile for digested dairy products (mg/g sample)

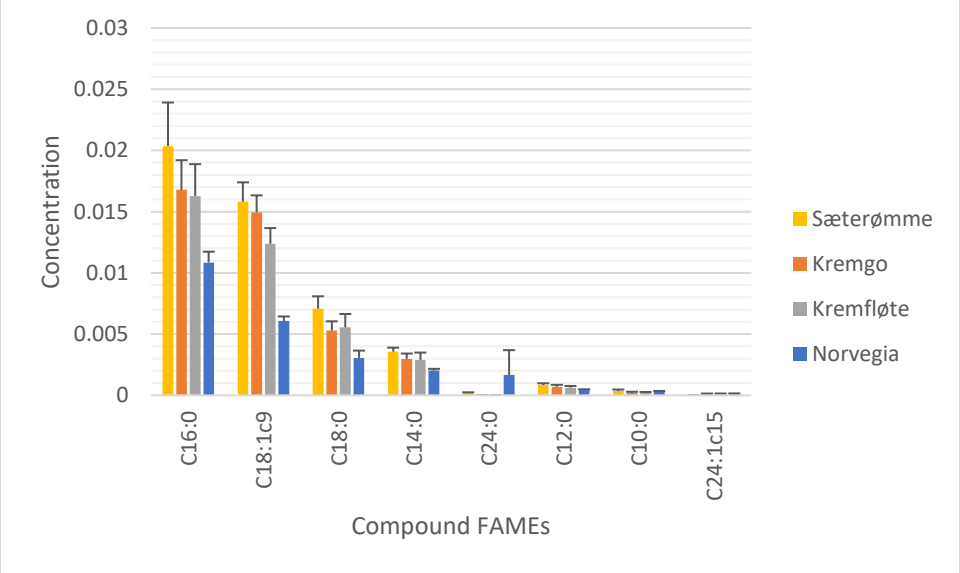

Figure 4. Polar lipid (PL) profile for digested dairy products (mg/g sample).

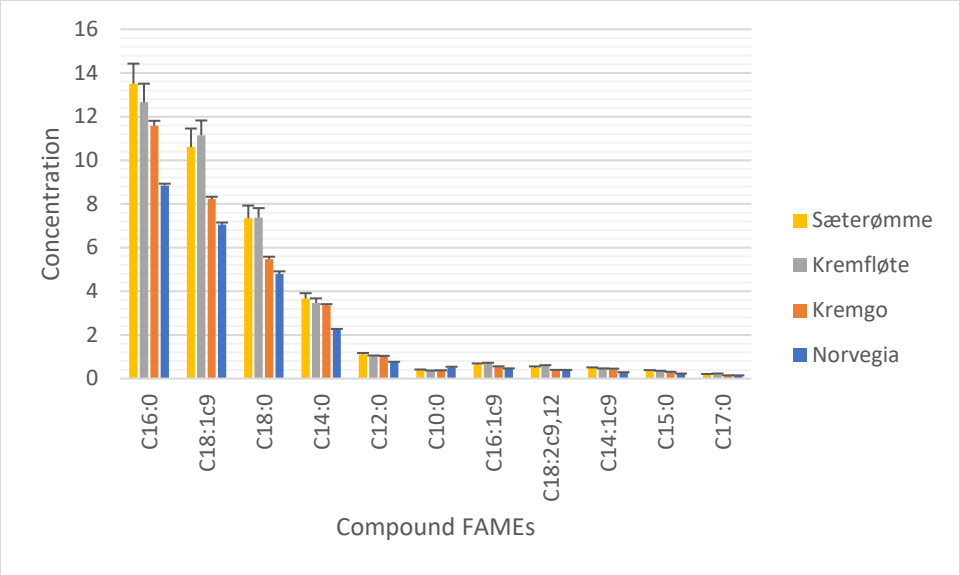

Figure 5. Free fatty acid (FFA) profile for digested dairy products (mg/g sample).

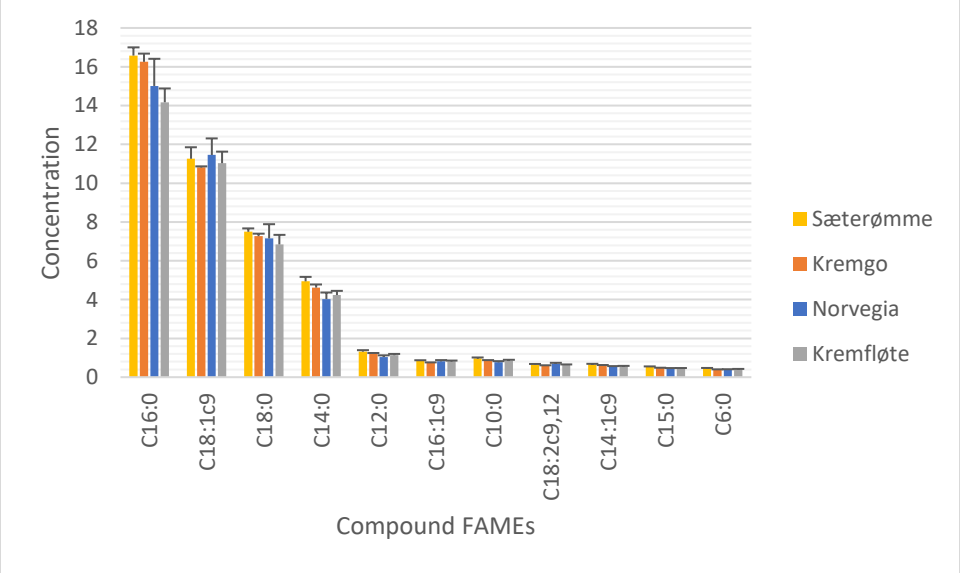

Figure 6. FA profile of undigested dairy products (mg/g sample)

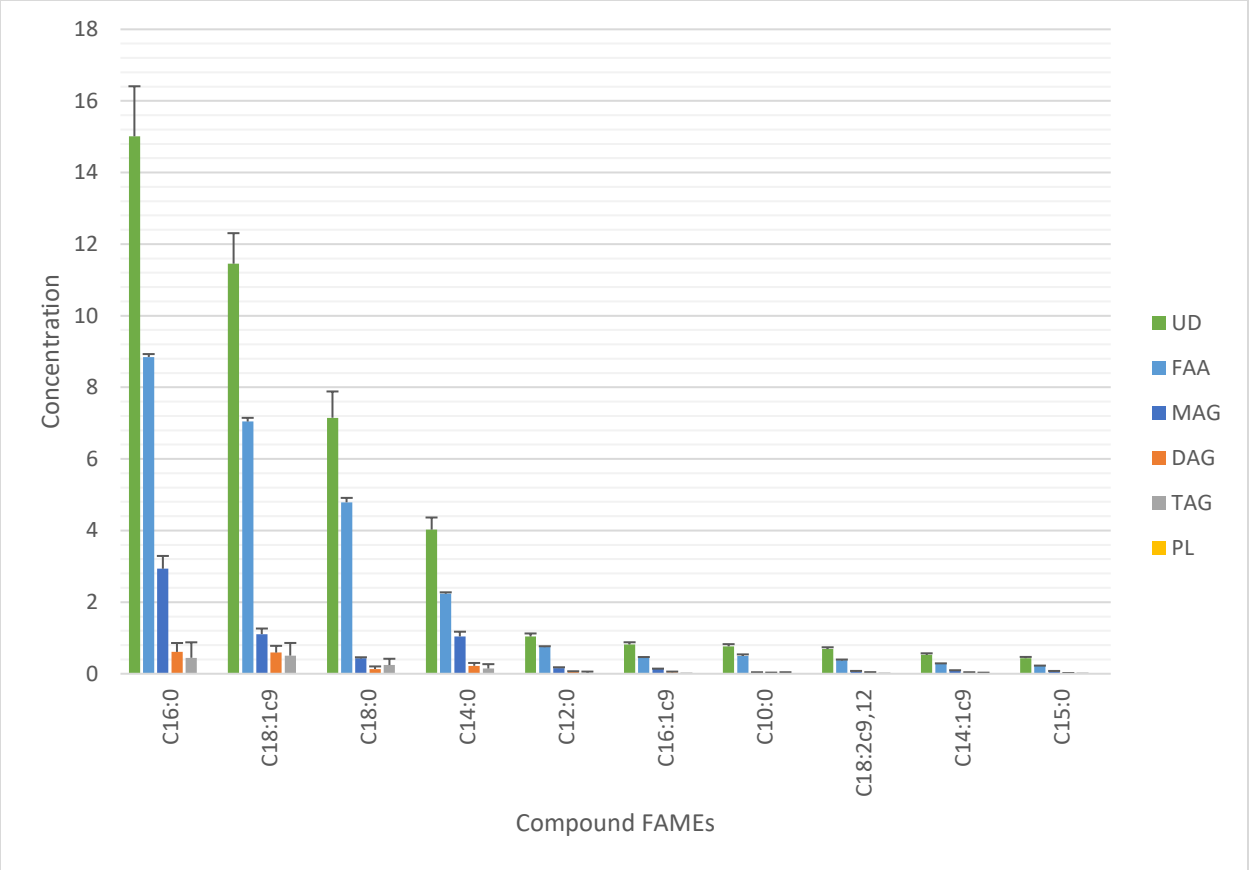

Figure 7. FA profile of gouda cheese (mg/g sample).

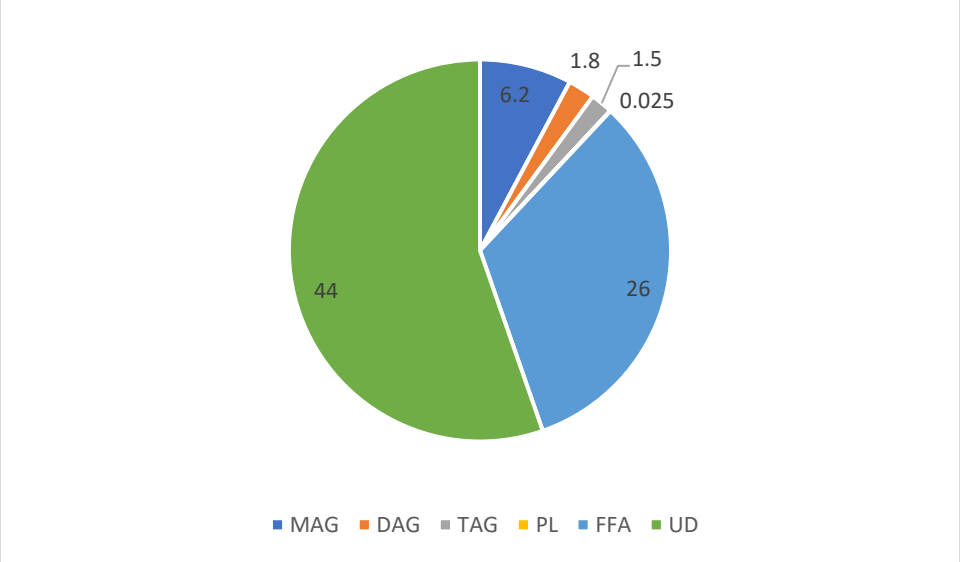

Figure 8. Total FA content for each fraction of gouda cheese (mg/g sample)

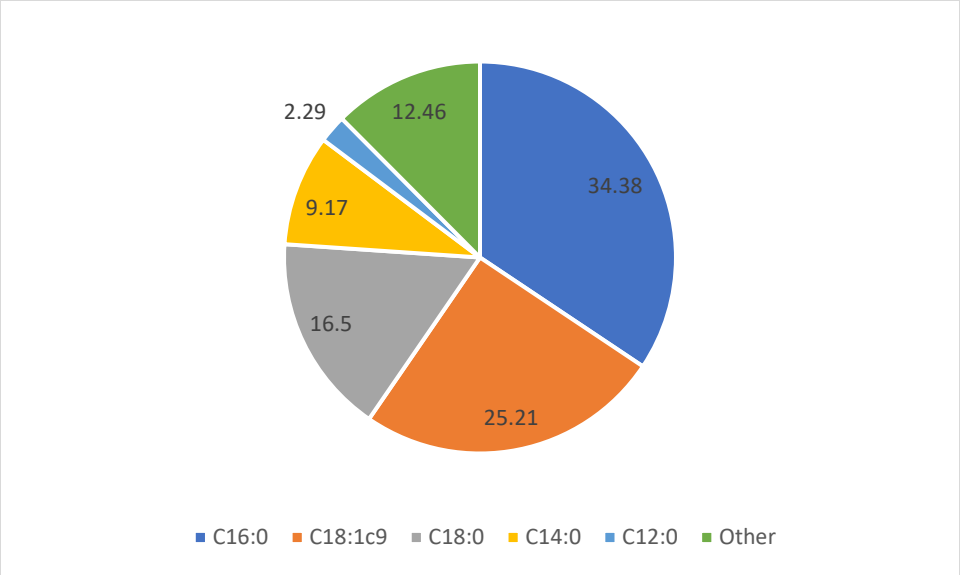

Figure 9. Relative FA composition of gouda cheese.

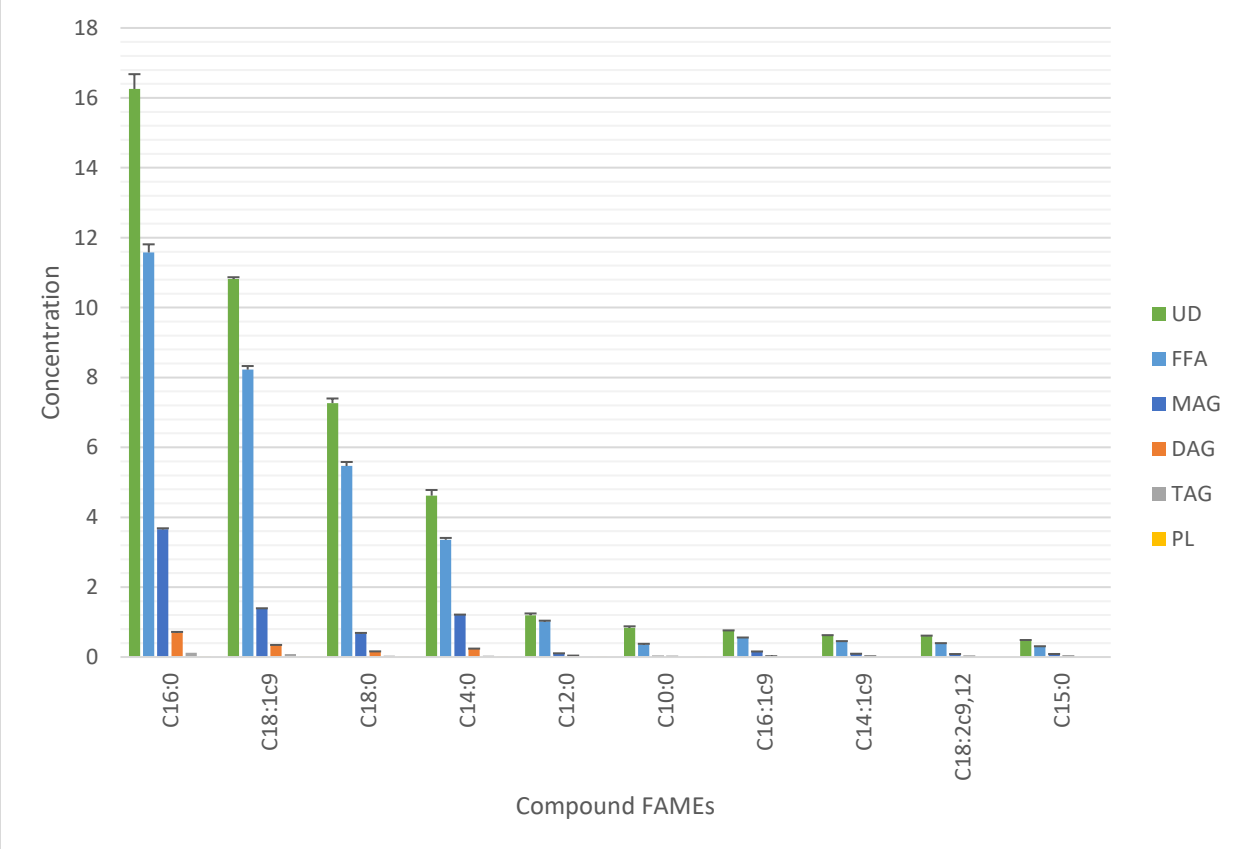

Figure 10. FA profile for cream cheese (mg/g sample).

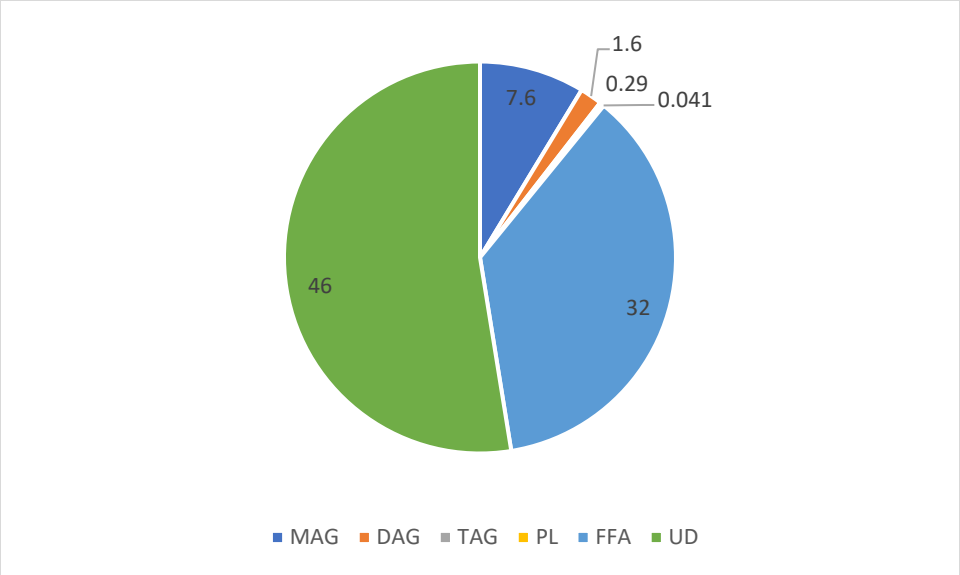

Figure 11. Total FA content for each fraction of cream cheese (mg/g sample).

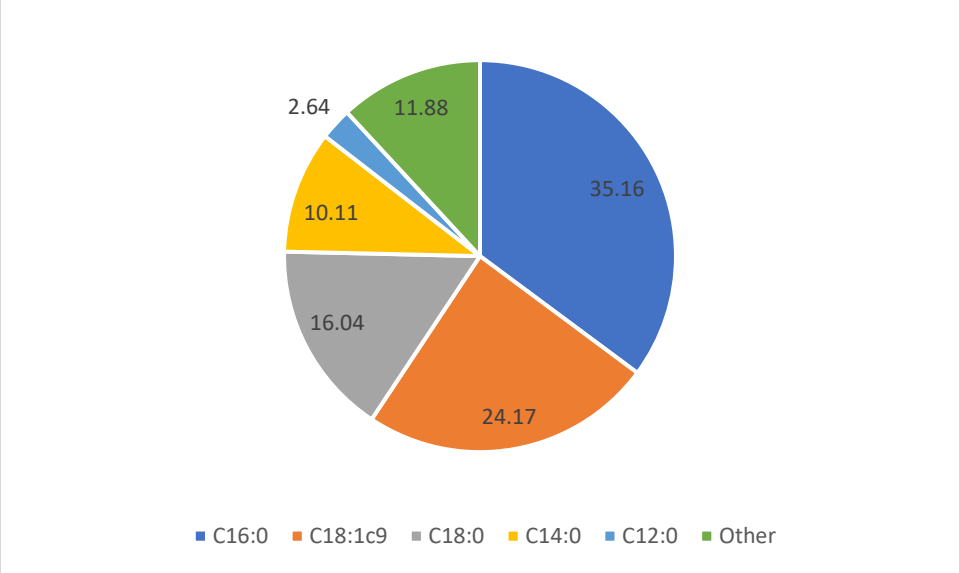

Figure 12. Relative FA composition of cream cheese.

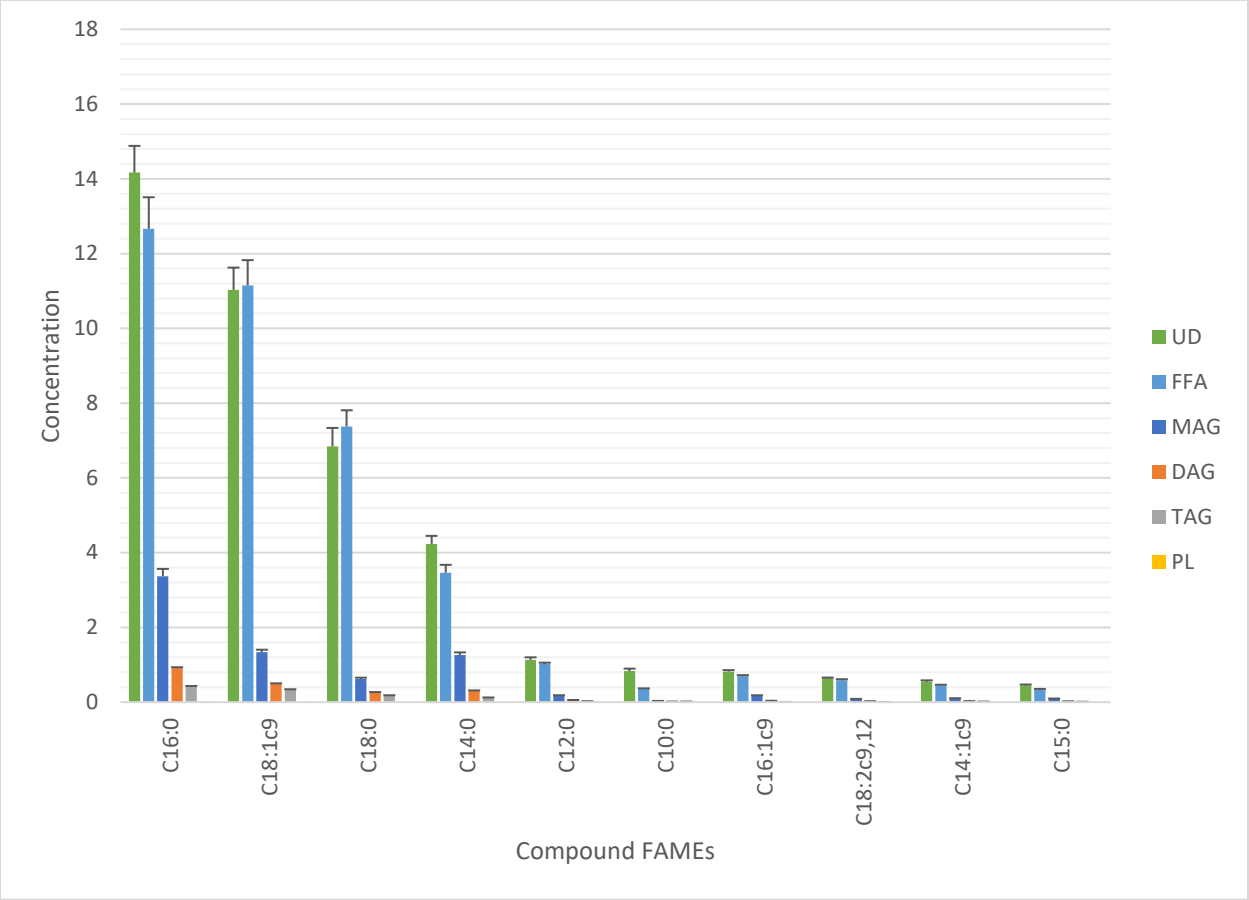

Figure 13. FA profile of whole cream (mg/g sample)

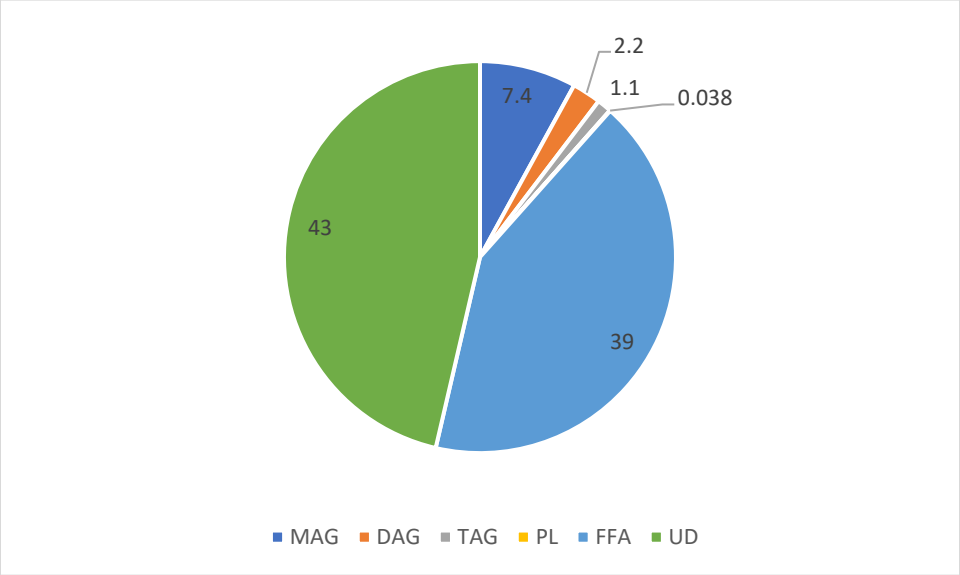

Figure 14. Total FA content in each fraction of whole cream (mg/g sample)

X

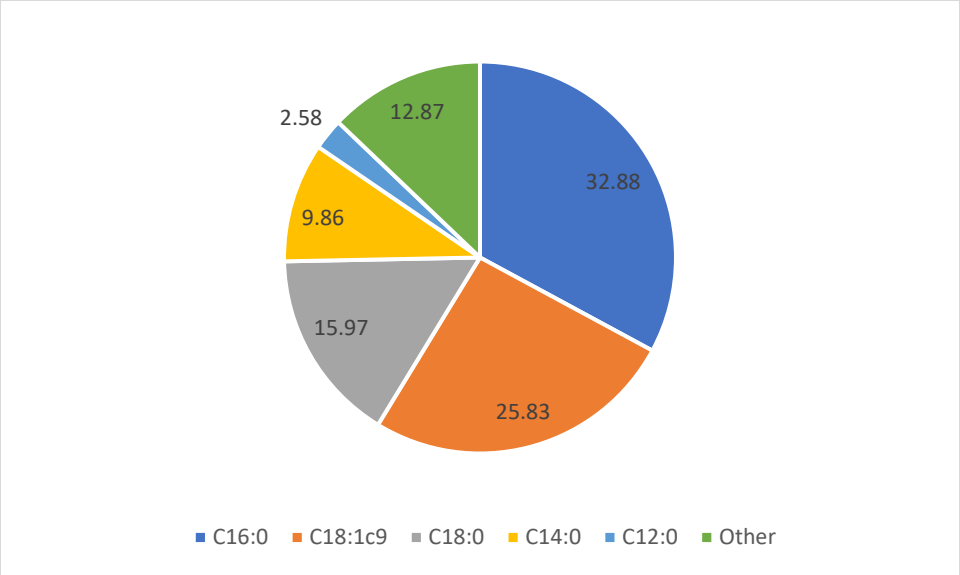

Figure 15. Relative FA composition of whole cream.

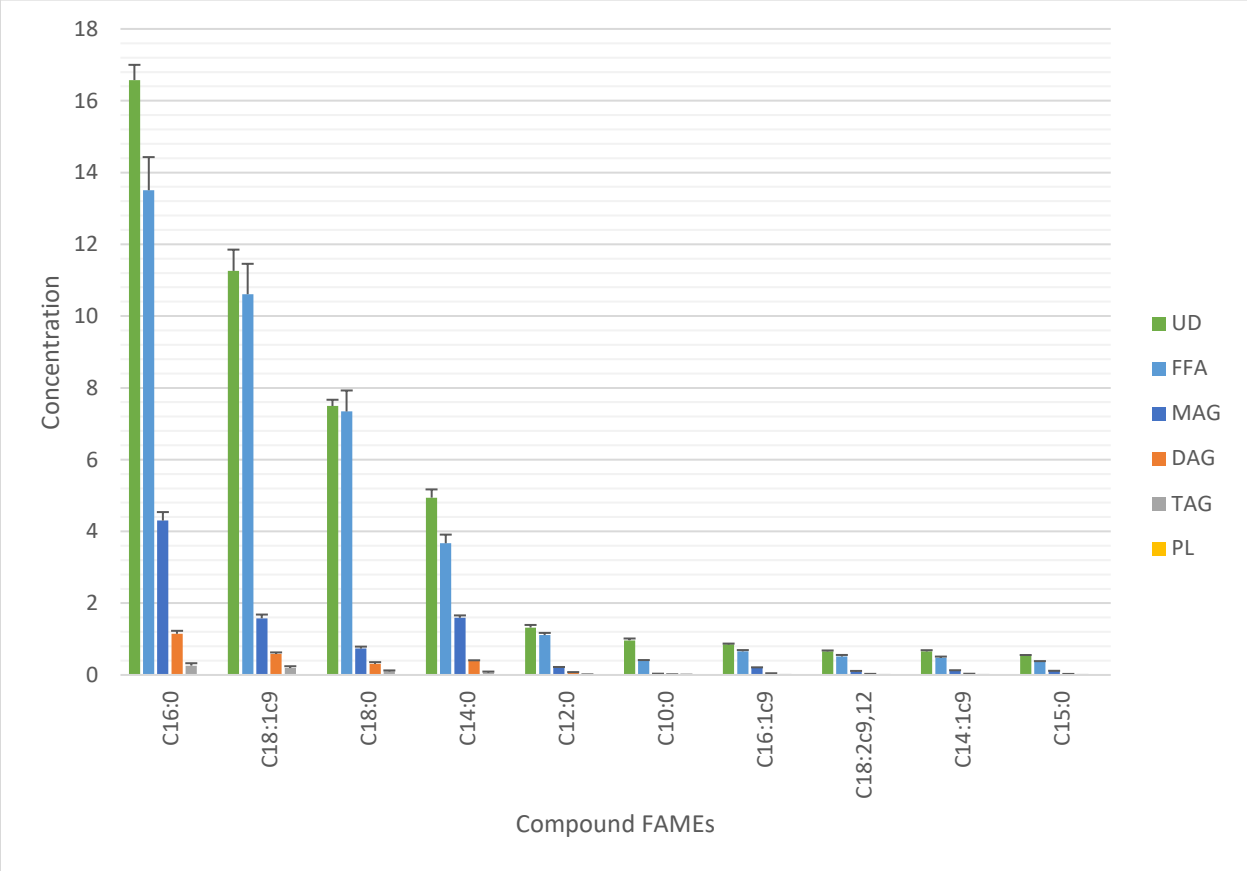

Figure 16. FA profile of sour cream.

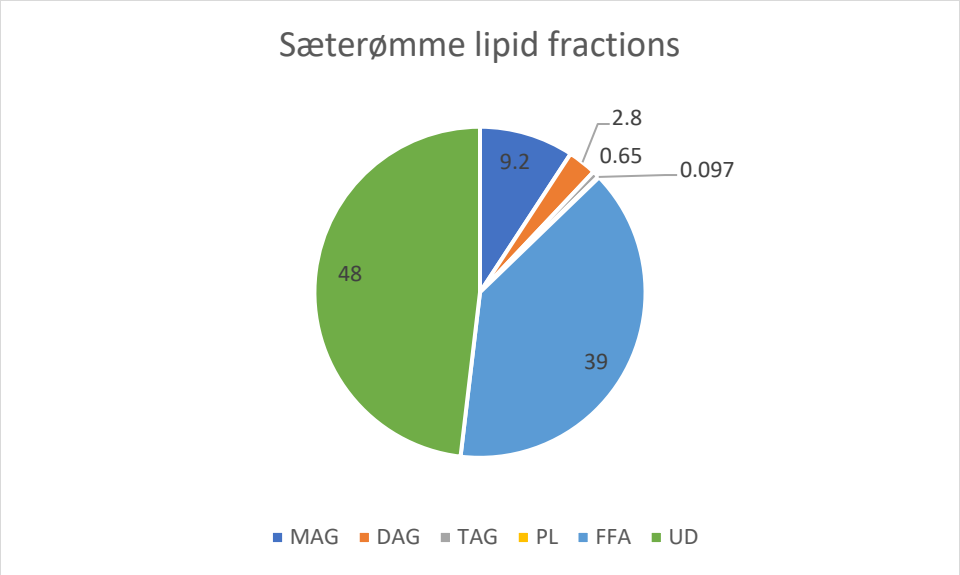

Figure 17. Total FA content in each fraction of sour cream (mg/g sample)

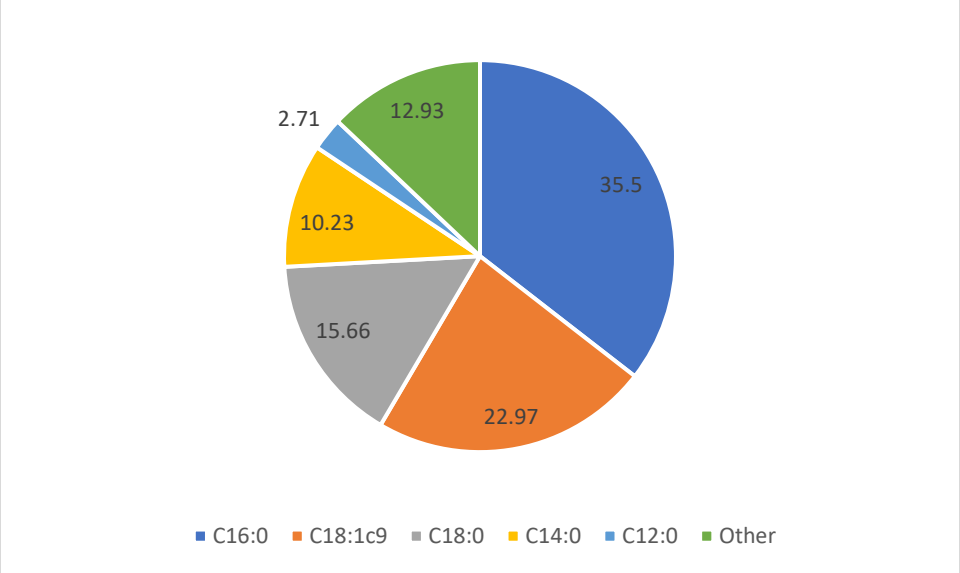

Figure 18. Relative FA composition of sour cream

Table 1. FAME standards used in GC-MS

RESTEK FOOD INDUSTRY FAME MIX (37 COMPONENTS)			
CHAIN	**Compound**	**CAS no.**	**% by weight**
C4:0	Methyl butyrate	623-42-7	4 %
C6:0	Methyl caproate	106-70-7	4 %
C8:0	Methyl caprylate	111-11-5	4 %
C10:0	Methyl decanoate	110-42-9	4 %
C11:0	Methyl undecanoate	1731-86-8	2 %
C12:0	Methyl dodecanoate	111-82-0	4 %
C13:0	Methyl tridecanoate	1731-88-0	2 %
C14:0	Methyl myristate	124-10-7	4 %
C14:1 (CIS-9)	Methyl myristoleate	56219-06-8	2 %
C15:0	Methyl pentadecanoate	7132-64-1	2 %
C15:1 (CIS-10)	Methyl pentadecenoate	90176-52-6	2 %
C16:0	Methyl palmitate	112-39-0	6 %
C16:1 (CIS-9)	Methyl palmitoleate	1120-25-8	2 %
C17:0	Methyl heptadecanoate	1731-92-6	2 %
C17:1	Methyl heptadecenoate	75190-82-8	2 %
C18:0	Methyl stearate	112-61-8	4 %
C18:1 (TRANS-9)	Methyl octadecenoate	1937-62-8	2 %
C18:1 (CIS-9)	Methyl oleate	112-62-9	4 %
C18:2 (ALL-TRANS-9,12)	Methyl linolelaidate	2566-97-4	2 %
C18:2 (ALL-CIS-9,12)	Methyl linoleate	112-63-0	2 %
C18:3 (ALL-CIS-6,9,12)	Methyl linolenate	16326-32-2	2 %
C18:3 (ALL-CIS-9,12,15)	Methyl linolenate	301-00-8	2 %
C20:0	Methyl arachidate	1120-28-1	4 %
C20:1 (CIS-11)	Methyl eicosenoate	2390-09-2	2 %
C20:2 (ALL-CIS-11,14,)	Methyl eicosadienoate	2463-02-7	2 %
C20:3 (ALL-CIS-8,11,14)	Methyl eicosatrienoate	21061-10-9	2 %
C20:3 (ALL-CIS-11,14,17)	Methyl eicosatrienoate	55682-88-7	2 %
C20:4 (ALL-CIS-5,8,11,14)	Methyl arachidonate	2566-89-4	2 %
C20:5 (ALL-CIS-5,8,11,14,17)	Methyl eicosapentaenoate	2734-47-6	2 %
C21:0	Methyl heneicosanoate	6064-90-0	2 %
C22:0	Methyl behenate	929-77-1	4 %
C22:1 (CIS-13)	Methyl erucate	1120-34-9	2 %
C22:2 (ALL-CIS-13,16)	Methyl docosadienoate	61012-47-3	2 %
C22:6 (ALL-CIS-4,7,10,13,16,19)	Methyl docosahexaenoate	2566-90-7	2 %
C23:0	Methyl tricosanoate	2433-97-8	2 %
C24:0	Methyl lignocerate	2442-49-1	4 %
C24:1 (CIS-15)	Methyl nervonate	2733-88-2	2 %

INDUVIDUAL FAME STANDARDS BY LARODAN			
CHAIN	**Compounds**	**CAS no.**	**ammount**
C7:0	Methyl heptanoate	106-73-0	100mg
C9:0	Methyl nonanoate	1731-84-6	100mg
C19:0	Methyl nonadecanoate	1731-94-8	100mg
11ME-C12:0 (13C) ISO	Methyl 11-Methyldodecanoate	5129-57-7	25mg
10ME-C12:0 (13C) ANTEISO	Methyl 10-Methyldodecanoate	5129-65-7	25mg
12ME-C13:0 (14C) ISO	Methyl 12-methyltridecanoate	21-1312-5	10mg
13ME-C14:0 (15C) ISO	Methyl 13-Methyltetradecanoate	21-1413-7	25mg
14ME-C15:0 (16C) ISO	Methyl 14-Methylpentadecanoate	21-1514-5	10mg
14ME-C16:0 (17C) ANTEISO	Methyl 14-Methylhexadecanoate	21-1614-5	10 mg
C16:1CIS-7	Methyl 7(Z)-Hexadecenoate	20-1607-5	10 mg i 0,4mL EtOH
C18:1CIS-13	Methyl 13(Z)-Octadecenoate	20-1815-4	5 mg i 0,5mL MeOH
C22:5C7,10,13,16,19	Methyl 7(Z),10(Z),13(Z),16(Z),19(Z)-Docosapentaenoate	20-2205-9	100mg
C16:1CIS-9	trans-9-Hexadecanoic acid ME	10030-74-7	100mg
C19:1CIS-10	cis-10-Nonadecanoate ME	19788-74-0	25mg
C18:2CIS-9 TRANS-12	cis-9-,trans-12-Octadienoic acid ME	20221-26-5	2mg
C26:0	Methyl Hexacosanoate	5802-82-4	100mg
3,7,11,15ME-C16:0	Methyl 3,7,11,15-Tetramethylhexadecanoate	1118-77-0	10mg

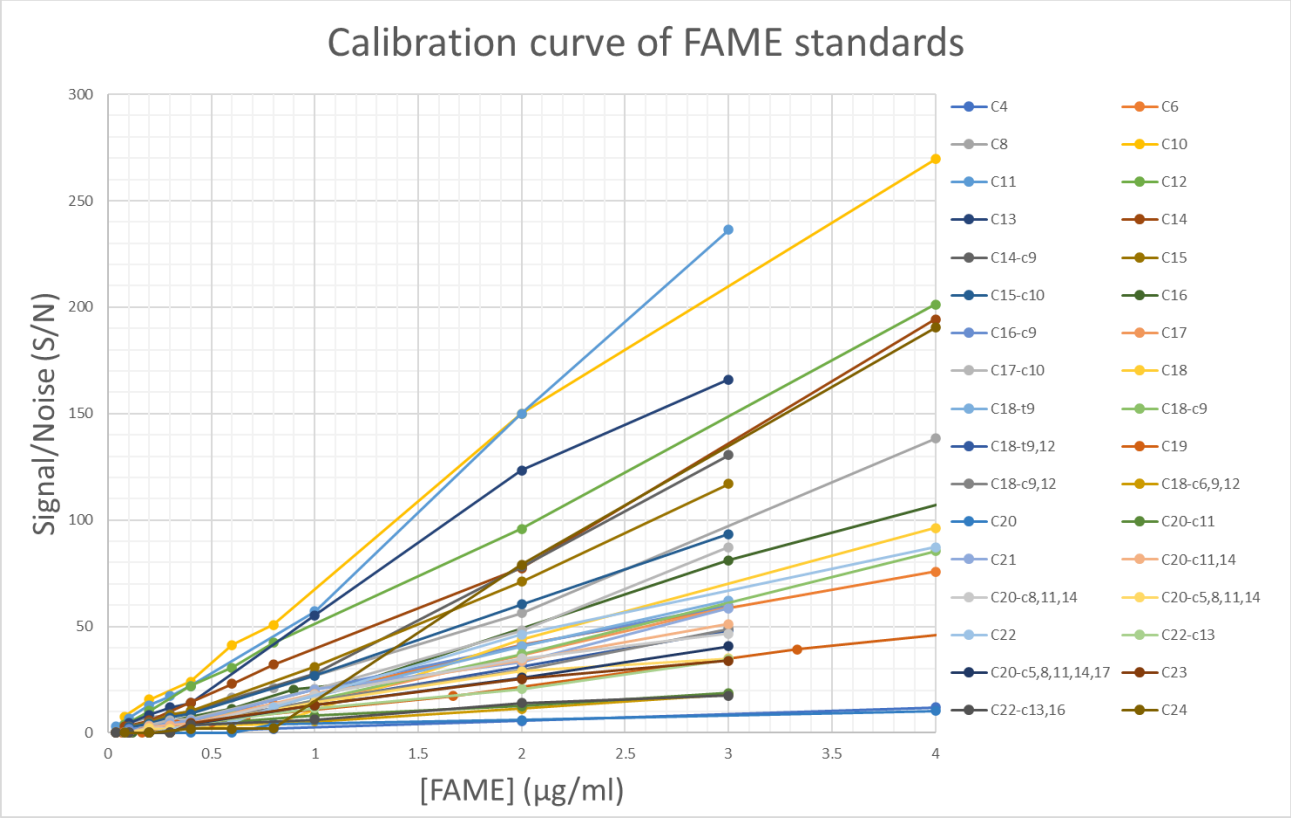

Figure 19. Calibration curve of FAME standards of known concentration.

Table 2. Limit of detection (LOD) and limit of quantification (LOQ) for FAME standards calculated from calibration curve.

Compound	LOD (µg/mL)	LOQ (µg/mL)	R^2
C4:0	1.13	3.36	0.999806
C6:0	0.172	0.536	0.995753
C8:0	0.183	0.388	0.996131
C10:0	0.0577	0.16	0.996045
C11:0	0.116	0.205	0.992697
C12:0	0.146	0.299	0.995492
C13:0	0.115	0.235	0.993027
C14:0	0.192	0.335	0.996584
C14:1-c9	0.211	0.37	0.988128
C15:0	0.182	0.361	0.995371
C15:1-c10	0.173	0.395	0.998422
C16:0	0.269	0.528	0.999063
C16:1-c9	0.195	0.537	0.997935
C17:0	0.294	0.641	0.994866
C17:1-c10	0.272	0.511	0.986093
C18:0	0.595	0.839	0.996582
C18:1-t9	0.272	0.596	0.998841
C18:1-c9	0.349	0.674	0.998065
C18:2-t9,12	0.282	0.706	0.999918
C19:0	0.294	0.909	0.997487
C18:2-c9,12	0.292	0.715	0.996823
C18:3-c6,9,12	0.678	1.94	0.978313
C20:0	1.32	2.45	0.810633
C20:1-c11	0.824	2.06	0.994658
C21:0	0.233	0.594	0.989475
C20:2-c11,14	0.235	0.633	0.996858
C20:3-c8,11,14	0.264	0.681	0.98509
C20:3-c5,8,11,14	0.263	0.812	0.970808
C22:0	0.37	0.675	0.995991
C22:1-c13	0.299	0.914	0.991997
C20:5-c5,8,11,14,17	0.304	0.809	0.999375
C23:0	0.355	0.971	0.989701
C22:2-c13,16	0.782	2.03	0.977477
C24:0	0.576	0.712	0.994186
C20:6-c4,7,10,13,16,19	0.218	0.361	0.997432

Table 3. FA profile of gouda cheese (mg/g).

Gouda cheese	MAG		DAG		TAG		PL		FFA		UD	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
C4:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28	0.033
C6:0	n.d.	n.d.	n.d.	n.d.	0.028	n.d.	n.d.	n.d.	n.d.	n.d.	0.37	0.036
C8:0	n.d.	n.d.	n.d.	n.d.	0.011	n.d.	n.d.	n.d.	0.042	0.009	0.28	0.033
C10:0	0.033	0.0083	0.022	0.0097	0.024	0.019	<LOQ	-	0.5	0.046	0.76	0.062
C12:0	0.16	0.022	0.051	0.019	0.031	0.029	<LOQ	-	0.74	0.025	1	0.086
C13:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.0016	0.025	0.000041
C14:0	1	0.13	0.22	0.079	0.15	0.12	0.002	0.00014	2.2	0.033	4	0.34
C14:1c9	0.087	0.011	0.032	0.011	0.031	n.d.	n.d.	n.d.	0.28	0.0063	0.53	0.04
C15:0	0.071	0.0081	0.015	0.0059	n.d.	n.d.	n.d.	n.d.	0.22	0.0058	0.43	0.036
C16:0	2.9	0.36	0.61	0.25	0.45	0.43	0.011	0.00089	8.8	0.081	15	1.4
C16:1c9	0.13	0.009	0.043	0.018	n.d.	n.d.	n.d.	n.d.	0.44	0.028	0.82	0.063
C17:0	0.034	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.0097	0.3	0.02
C18:0	0.43	0.029	0.13	0.072	0.24	0.18	0.0031	0.0006	4.8	0.12	7.2	0.74
C18:1t9	n.d.	n.d.	0.011	0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:1c9	1.1	0.16	0.59	0.19	0.51	0.35	0.0061	0.00037	7	0.1	11	0.85
C18:2c9,12	0.067	0.013	0.034	0.011	n.d.	n.d.	n.d.	n.d.	0.38	0.02	0.69	0.051
C18:3c9,12,15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.0053
C20:3c8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.023	0.004
C20:4c5,8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.039	0.0051
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0017	0.002	n.d.	n.d.	0.0096	0.0011
C24:1c15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	-	n.d.	n.d.	n.d.	n.d.
t-4-C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.025	0.0036	n.d.	n.d.
10me-C12:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0092	0.00042	0.0093	0.00084
11me-C12:0	0.0069	0.00051	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.016	0.0009	0.027	0.0017
12me-C13:0	0.0087	0.00038	0.0028	0.00075	n.d.	n.d.	n.d.	n.d.	0.014	0.00051	0.036	0.0027
13me-C14:0	0.016	0.0009	0.0041	0.0016	n.d.	n.d.	n.d.	n.d.	0.042	0.00091	0.094	0.0067
c-11-C14:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.011	0.00082
14me-C15:0	0.017	0.0022	0.0033	0.002	n.d.	n.d.	n.d.	n.d.	0.045	0.001	0.1	0.0078

c-7-C16:1	0.016	0.0011	0.0041	0.0018	n.d.	n.d.	n.d.	n.d.	0.035	0.0013	0.071	0.0074
14me-C16:0	0.027	0.0078	0.0061	0.0019	n.d.	n.d.	n.d.	n.d.	0.1	0.0042	0.18	0.013
t-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.096	0.0011	n.d.	n.d.
c-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.011
c-10-C19:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.018	0.0025
c-7,10,13,16,19-C22:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.016	0.0017
C26:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0072	0.001
3,7,11,15me-C16:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑SFA	4.8	0.57	1.1	0.44	0.93	0.78	0.018	0.0037	18	0.34	30	2.8
∑MUFA	1.3	0.18	0.68	0.22	0.54	0.35	0.0062	0.00041	7.9	0.14	13	0.98
∑PUFA	0.067	0.013	0.034	0.011	0	0	0	0	0.38	0.02	0.9	0.067
∑ω-3	0	0	0	0	0	0	0	0	0	0	0.16	0.0078
∑ω-6	0.067	0.013	0.034	0.011	0	0	0	0	0.4	0.024	0.75	0.06
∑FA	6.2	0.76	1.8	0.68	1.5	1.1	0.025	0.0042	26	0.51	44	3.9
IA	5.1		2.2		2		3.1		2.2		2.3	
IT	6.2		2.7		3.1		5.1		3.8		3.4	

Table 4. FA profile of cream cheese (mg/g).

Cream cheese	**MAG**		**DAG**		**TAG**		**PL**		**FFA**		**UD**	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
C4:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.26	0.023
C6:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.37	0.033
C8:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.019	0.00042	0.29	0.015
C10:0	0.0097	0.0018	0.0086	0.00048	n.d.	n.d.	<LOQ	-	0.36	0.015	0.84	0.043
C12:0	0.1	0.007	0.041	0.0016	0.0055	0.0011	<LOQ	-	1	0.02	1.2	0.053
C13:0	0.0063	0.00096	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.021	0.0023	0.029	0.0036
C14:0	1.2	0.0064	0.24	0.0049	0.037	0.0022	0.003	0.00044	3.4	0.053	4.6	0.16
C14:1c9	0.092	0.003	0.02	0.00044	n.d.	n.d.	n.d.	n.d.	0.43	0.024	0.61	0.02

C15:0	0.083	0.005	0.016	0.0011	n.d.	n.d.	n.d.	n.d.	0.3	0.0094	0.47	0.015
C16:0	3.7	0.029	0.69	0.026	0.13	0.0061	0.017	0.0024	12	0.23	16	0.42
C16:1c9	0.15	0.01	0.024	0.00042	n.d.	n.d.	n.d.	n.d.	0.55	0.014	0.75	0.015
C17:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.0064	0.26	0.025
C18:0	0.67	0.016	0.16	0.0074	0.042	0.0043	0.0053	0.00075	5.5	0.12	7.3	0.13
C18:1t9	n.d.	n.d.	0.0098	0.00052	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:1c9	1.4	0.018	0.35	0.0021	0.084	0.0092	0.015	0.0014	8.2	0.1	11	0.052
C18:2c9,12	0.079	0.0072	0.013	0.00027	n.d.	n.d.	n.d.	n.d.	0.39	0.0084	0.6	0.013
C18:3c9,12,15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13	0.012
C20:3c8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.027	0.0031
C20:4c5,8,11,14-ME	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.041	0.0018
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.011	0.0034
C24:1c15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	-	n.d.	n.d.	n.d.	n.d.
t-4-C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.0013	n.d.	n.d.
10me-C12:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.011	0.002	0.0093	0.00012
11me-C12:0	0.0067	0.00035	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.023	0.00046	0.031	0.0014
12me-C13:0	0.0092	0.00061	0.002	0.00031	n.d.	n.d.	n.d.	n.d.	0.021	0.00035	0.036	0.0014
13me-C14:0	0.018	0.0014	0.0038	0.00012	n.d.	n.d.	n.d.	n.d.	0.056	0.0011	0.1	0.0039
c-11-C14:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.00086
14me-C15:0	0.021	0.0019	0.0038	0.00068	n.d.	n.d.	n.d.	n.d.	0.054	0.0024	0.1	0.0044
c-7-C16:1	0.02	0.0007	0.0029	0.00087	n.d.	n.d.	n.d.	n.d.	0.037	0.001	0.061	0.0023
14me-C16:0	0.031	0.0022	0.0057	0.00015	n.d.	n.d.	n.d.	n.d.	0.11	0.00043	0.16	0.0023
t-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11	0.0035	n.d.	n.d.
c-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.0012
c-10-C19:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.017	0.00094
c-7,10,13,16,19-C22:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.016	0.00073
C26:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0088	0.00018
3,7,11,15me-C16:0	0.011	0.00059	0.025	0.00021	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑SFA	5.8	0.073	1.2	0.043	0.21	0.014	0.026	0.0039	23	0.46	32	0.94
∑MUFA	1.6	0.031	0.4	0.0044	0.084	0.0092	0.015	0.0014	9.4	0.15	12	0.093
∑PUFA	0.079	0.0072	0.013	0.00027	0	0	0	0	0.39	0.0084	0.81	0.03
∑ω-3	0	0	0	0	0	0	0	0	0	0	0.16	0.013

∑ω-6	0.079	0.0072	0.013	0.00027	0	0	0	0	0.41	0.0097	0.67	0.018
∑FA	7.6	0.11	1.6	0.048	0.29	0.023	0.041	0.0053	32	0.62	46	1.1
IA	5		4		3.3		1.9		2.7		2.7	
IT	6.4		5.2		4.9		3.3		4.2		3.9	

Table 5. FA profile of whole cream (mg/g).

Whole cream	MAG		DAG		TAG		PL		FFA		UD	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
C4:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.26	0.048
C6:0	0.0062	0.00015	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.39	0.041
C8:0	0.0019	0.0002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.018	0.00073	0.29	0.027
C9:0	0.0044	0.00022	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C10:0	0.03	0.0031	0.015	0.0036	0.016	0.015	<LOQ	-	0.36	0.0094	0.83	0.065
C12:0	0.18	0.0094	0.057	0.0092	0.023	0.022	<LOQ	-	1	0.038	1.1	0.068
C13:0	0.0073	0.00081	0.0077	0.0003	n.d.	n.d.	n.d.	n.d.	0.023	0.0015	0.028	0.00063
C14:0	1.3	0.074	0.31	0.052	0.12	0.1	0.0029	0.0006	3.5	0.21	4.2	0.22
C14:1c9	0.099	0.006	0.027	0.0046	0.015	0.015	n.d.	n.d.	0.45	0.018	0.55	0.031
C15:0	0.089	0.0075	0.021	0.0054	0.011	0.01	n.d.	n.d.	0.34	0.016	0.45	0.023
C16:0	3.4	0.2	0.91	0.18	0.43	0.34	0.016	0.0026	13	0.84	14	0.71
C16:1c9	0.17	0.0097	0.037	0.0046	n.d.	n.d.	n.d.	n.d.	0.69	0.028	0.82	0.038
C17:0	0.039	0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21	0.021	0.25	0.013
C18:0	0.62	0.037	0.26	0.062	0.18	0.16	0.0055	0.0011	7.4	0.43	6.8	0.49
C18:1t9	n.d.	n.d.	0.018	0.0017	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:1c9	1.3	0.068	0.5	0.07	0.34	0.28	0.012	0.0013	11	0.68	11	0.6
C18:2c9,12	0.081	0.0056	0.024	0.0053	n.d.	n.d.	n.d.	n.d.	0.58	0.03	0.65	0.011
C18:3c9,12,15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17	0.0052
C20:3c8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	0.00098
C20:4c5,8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.037	0.0029
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.011	0.0029
C24:1c15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	-	n.d.	n.d.	n.d.	n.d.
t-4-C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.014	0.00083	n.d.	n.d.
10me-C12:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.001	0.0097	0.00051
11me-C12:0	0.0088	0.00072	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.024	0.0012	0.03	0.0011
12me-C13:0	0.011	0.0008	0.0024	0.00021	n.d.	n.d.	n.d.	n.d.	0.022	0.0013	0.036	0.0011
13me-C14:0	0.021	0.0018	0.0047	0.0013	n.d.	n.d.	n.d.	n.d.	0.066	0.005	0.099	0.0062
c-11-C14:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.013	0.0006

14me-C15:0	0.02	0.0014	0.004	0.00093	n.d.	n.d.	n.d.	n.d.	0.059	0.0021	0.094	0.0034
c-7-C16:1	0.018	0.0019	0.004	0.00085	n.d.	n.d.	n.d.	n.d.	0.052	0.0048	0.067	0.001
14me-C16:0	0.035	0.0018	0.0078	0.0018	n.d.	n.d.	n.d.	n.d.	0.15	0.0046	0.17	0.0036
t-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.0084	n.d.	n.d.
c-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.011
c-10-C19:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.017	0.00095
c-7,10,13,16,19-C22:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	0.0022
C26:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.007	0.00078
3,7,11,15me-C16:0	0.0037	0.001	0.018	0.0019	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑SFA	5.7	0.34	1.6	0.32	0.78	0.65	0.026	0.0045	26	1.6	29	1.7
∑MUFA	1.6	0.085	0.59	0.082	0.35	0.3	0.013	0.0013	12	0.74	13	0.68
∑PUFA	0.081	0.0056	0.024	0.0053	0	0	0	0	0.58	0.03	0.89	0.023
∑ω-3	0	0	0	0	0	0	0	0	0	0	0.2	0.008
∑ω-6	0.081	0.0056	0.024	0.0053	0	0	0	0	0.6	0.031	0.7	0.015
∑FA	7.4	0.44	2.2	0.41	1.1	0.95	0.038	0.0058	39	2.4	43	2.4
IA	5		3.6		2.7		2.3		2.1		2.4	
IT	6.1		4.8		4.2		4		3.6		3.3	

Table 6. FA profile of sour cream (mg/g).

Sour cream	**MAG**		**DAG**		**TAG**		**PL**		**FFA**		**UD**	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
C16:0	4.3	0.24	1.1	0.083	0.26	0.066	0.02	0.0036	14	0.92	17	0.42
C14:0	1.6	0.067	0.39	0.018	0.073	0.022	0.0036	0.00033	3.7	0.24	4.9	0.23
C18:1c9	1.6	0.1	0.59	0.04	0.19	0.051	0.016	0.0016	11	0.85	11	0.59
C18:0	0.74	0.053	0.31	0.046	0.1	0.023	0.0071	0.001	7.3	0.59	7.5	0.17
C12:0	0.22	0.0068	0.076	0.0039	0.013	0.0037	<LOQ	-	1.1	0.062	1.3	0.072
C16:1c9	0.21	0.0061	0.045	0.0032	n.d.	n.d.	n.d.	n.d.	0.66	0.036	0.86	0.018
C14:1c9	0.12	0.0071	0.033	0.0018	n.d.	n.d.	n.d.	n.d.	0.48	0.035	0.66	0.032

C15:0	0.11	0.0023	0.027	0.0017	n.d.	n.d.	n.d.	n.d.	0.36	0.024	0.54	0.019
C18:2c9,12	0.1	0.012	0.027	0.0036	n.d.	n.d.	n.d.	n.d.	0.51	0.044	0.66	0.021
C18:1t9	0.046	0.0016	0.018	0.0025	n.d.	n.d.	0.049	n.d.	n.d.	n.d.	n.d.	n.d.
C17:0	0.041	0.00081	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.19	0.019	0.31	0.069
14me-C16:0	0.04	0.0033	0.01	0.00061	n.d.	n.d.	n.d.	n.d.	0.15	0.017	0.19	0.02
C10:0	0.035	0.0015	0.018	0.00098	0.0061	0.0017	<LOQ	-	0.4	0.011	0.96	0.051
13me-C14:0	0.025	0.0013	0.0061	0.00035	n.d.	n.d.	n.d.	n.d.	0.066	0.0037	0.12	0.0049
14me-C15:0	0.024	0.00071	0.0053	0.00035	n.d.	n.d.	n.d.	n.d.	0.06	0.0029	0.1	0.006
c-7-C16:1	0.021	0.002	0.0045	0.0006	n.d.	n.d.	n.d.	n.d.	0.047	0.0044	0.068	0.00084
12me-C13:0	0.012	0.00093	0.0028	0.00051	n.d.	n.d.	n.d.	n.d.	0.021	0.0011	0.039	0.0021
11me-C12:0	0.01	0.00034	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.027	0.0013	0.037	0.0023
C13:0	0.0092	0.00036	0.084	0.0027	n.d.	n.d.	n.d.	n.d.	0.023	0.0024	0.034	0.00099
3,7,11,15me-C16:0	0.0061	0.001	0.029	0.0032	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C4:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.32	0.01
C6:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.46	0.017
C8:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.021	0.00052	0.35	0.024
C9:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3-c9,12,15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16	0.0031
C20:3-c8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.021	0.0045
C20:4-c5,8,11,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.041	0.0057
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0096	0.001
C24:1c15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	-	n.d.	n.d.	n.d.	n.d.
t-4-C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.016	0.00048	n.d.	n.d.
10me-C12:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.0013	0.01	0.0004
c-11-C14:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.015	0.001
t-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.15	0.012	n.d.	n.d.
c-13-C18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18	0.0079
c-10-C19:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.017	0.00068
C22:5-c7,10,13,16,19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.022	0.00079

C26:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0081	0.00065
∑SFA	7.2	0.38	2.1	0.16	0.46	0.12	0.032	0.0052	27	1.9	34	1.1
∑MUFA	2	0.12	0.69	0.048	0.19	0.051	0.065	0.0016	12	0.93	13	0.65
∑PUFA	0.1	0.012	0.027	0.0036	0	0	0	0	0.51	0.044	0.91	0.035
∑ω-3	0	0	0	0	0	0	0	0	0	0	0.2	0.0049
∑ω-6	0.1	0.012	0.027	0.0036	0	0	0	0	0.53	0.045	0.72	0.031
∑FA	9.2	0.51	2.8	0.21	0.65	0.17	0.097	0.0068	39	2.9	48	1.8
IA	5.2		3.9		3		0.55		2.3		2.7	
IT	6.4		5.2		4.6		0.95		3.9		3.7	

Table 7. Unquantified FAs in gouda cheese.

Gouda cheese	**FFA**		**UF**		**MAG**	
Compound	RT	SI	RT	SI	RT	SI
cis-5-dodecanoic acid ME	13:38	874	13:41	895	n.d.	n.d.
Cyclopropenoic acid ME	15:37	889	13:91	849	n.d.	n.d.
cis-10-pentadecanoic acid ME	n.d.	n.d.	21:80	845	n.d.	n.d.
trans-7-hexadecanoic acid ME	28:56	891	26:66	915	n.d.	n.d.
Hexadecanoic acid,14-methyl-,ME	30:49	835	30:57	845	30:42	778
cis-11-hexadecanoic acid ME	31:45	875	31:57	827	28:56	857
cis-10-heptadecanoic acid ME	36:84	808	36:96	908	n.d.	n.d.
cis-8-heptadecanoic acid ME	37:77	850	37:25	881	37:16	738
11-cyclohexyl undecanoic acid ME	47:29	795	47:37	802	n.d.	n.d.
Cyclopropane octanoic acid,2-oktyl-, ME	n.d.	n.d.	53:80	753	n.d.	n.d.
10-oxo-octadecanoicacid ME	97:92	760	n.d.	n.d.	n.d.	n.d.

Table 8. Unquantified FAs in cream cheese.

Cream cheese	FFA		UF		MAG	
Compound	RT	SI	RT	SI	RT	SI
cis-5-dodecanoic acid ME	13:37	870	13:41	880	n.d.	n.d.
Cyclopropenoic acid ME	13:88	830	13:90	848	n.d.	n.d.
cis-10-pentadecanoic acid ME	n.d.	n.d.	21:80	850	n.d.	n.d.
trans-7-hexadecanoic acid ME	28:56	860	26:66	914	n.d.	n.d.
Hexadecanoic acid,14-methyl-,ME	30:49	850	30:56	848	30:41	719
cis-11-hexadecanoic acid ME	31:45	890	31:56	826	28:53	736
cis-10-heptadecanoic acid ME	36:84	790	36:96	907	n.d.	n.d.
cis-8-heptadecanoic acid ME	37:75	832	37:25	879	n.d.	n.d.
11-cyclohexyl undecanoic acid ME	47:29	820	47:36	804	n.d.	n.d.
Cyclopropane octanoic acid,2-oktyl-, ME	n.d.	n.d.	53:79	755	n.d.	n.d.
10-oxo-octadecanoicacid ME	97:90	799	n.d.	n.d.	n.d.	n.d.

Table 9. Unquantified FAs in whole cream.

Whole cream	FFA		UF		MAG	
Compound	RT	SI	RT	SI	RT	SI
cis-5-dodecanoic acid ME	13:40	875	13:40	895	n.d.	n.d.
Cyclopropenoic acid ME	13:88	769	13:90	849	n.d.	n.d.
cis-10-pentadecanoic acid ME	n.d.	n.d.	21:80	845	n.d.	n.d.
trans-7-hexadecanoic acid ME	28:54	890	26:66	915	n.d.	n.d.
Hexadecanoic acid,14-methyl-,ME	30:49	843	30:56	845	30:41	770
cis-11-hexadecanoic acid ME	31:47	869	31:56	827	28:52	700
cis-10-heptadecanoic acid ME	36:82	810	36:96	908	n.d.	n.d.
cis-8-heptadecanoic acid ME	37:77	855	37:25	881	37:16	730

11-cyclohexyl undecanoic acid ME	47:28	802	47:36	802	n.d.	n.d.
Cyclopropane octanoic acid,2-oktyl-, ME	n.d.	n.d.	53:80	753	n.d.	n.d.
10-oxo-octadecanoicacid ME	97:94	720	n.d.	n.d.	n.d.	n.d.

Table 10. Unquantified FAs in sour cream .

Sour cream	**FFA**		**UF**		**MAG**	
Compound	RT	SI	RT	SI	RT	SI
cis-5-Dodecanoic acid ME	13:38	873	13:41	896	n.d.	n.d.
Cyclopropenoic acid ME	13:88	877	13:90	851	n.d.	n.d.
cis-10-Pentadecanoic acid ME	n.d.	n.d.	21:80	840	n.d.	n.d.
trans-7-Hexadecanoic acid ME	28:56	893	26:65	919	n.d.	n.d.
Hexadecanoic acid,14-methyl-,ME	30:49	833	30:58	849	30:40	763
cis-11-Hexadecanoic acid ME	31:45	866	31:57	833	28:52	714
cis-10-Heptadecanoic acid ME	36:84	810	36:97	914	n.d.	n.d.
cis-8-Heptadecanoic acid ME	37:77	849	37:24	890	n.d.	n.d.
11-cyclohexyl Undecanoic acid ME	47:29	811	47:39	810	n.d.	n.d.
Cyclopropane Octanoic acid,2-oktyl-, ME	n.d.	n.d.	53:81	750	n.d.	n.d.
10-oxo-Octadecanoicacid ME	97:92	760	n.d.	n.d.	n.d.	n.d.

Table 10. Glycerol analysis of dairy products prior- and post-digestion.

	Glycerol analysis		
	Mean (µg/g)	STD (µg/g)	RSTD (%)
Gouda cheese			
Undigested	7.14	4.72	66
Digested	10.90	6.58	60
Cream cheese			
Undigested	<LOD	-	-
Digested	31.50	5.44	17
Whole cream			
Undigested	<LOD	-	-
Digested	16.50	4.98	30
Sour cream			
Undigested	10.70	21.70	202
Digested	5.68	3.23	56
Whole milk			
Undigested	<LOD	-	-
Digested	3620	200	5.6

Table 11. Analysis of variance. One-way ANOVA with tukey test n=3. Computed by Rstudio.

FAME	ANOVA P-VALUE	GROUP 1	GROUP 2	TUKEY P-VALUE	TUKEY SIGNIFICANT
MAG C12:0	0,0000339	Kremfløte	Kremgo	0,000456	***
MAG C12:0	0,0000339	Kremfløte	Norvegia	0,446	ns
MAG C12:0	0,0000339	Kremfløte	Seterømme	0,0231	*
MAG C12:0	0,0000339	Kremgo	Norvegia	0,0024	**
MAG C12:0	0,0000339	Kremgo	Seterømme	0,0000211	****
MAG C12:0	0,0000339	Norvegia	Seterømme	0,00313	**
MAG C14:0	0,000271	Kremfløte	Kremgo	0,891	ns
MAG C14:0	0,000271	Kremfløte	Norvegia	0,0531	ns
MAG C14:0	0,000271	Kremfløte	Seterømme	0,00497	**
MAG C14:0	0,000271	Kremgo	Norvegia	0,144	ns
MAG C14:0	0,000271	Kremgo	Seterømme	0,00215	**
MAG C14:0	0,000271	Norvegia	Seterømme	0,000184	***
MAG C9-C14:1	0,001	Kremfløte	Kremgo	0,666	ns
MAG C9-C14:1	0,001	Kremfløte	Norvegia	0,264	ns
MAG C9-C14:1	0,001	Kremfløte	Seterømme	0,0137	*
MAG C9-C14:1	0,001	Kremgo	Norvegia	0,835	ns
MAG C9-C14:1	0,001	Kremgo	Seterømme	0,00321	**
MAG C9-C14:1	0,001	Norvegia	Seterømme	0,00125	**
MAG C15:0	0,000165	Kremfløte	Kremgo	0,637	ns
MAG C15:0	0,000165	Kremfløte	Norvegia	0,0247	*
MAG C15:0	0,000165	Kremfløte	Seterømme	0,00489	**
MAG C15:0	0,000165	Kremgo	Norvegia	0,135	ns
MAG C15:0	0,000165	Kremgo	Seterømme	0,00121	**
MAG C15:0	0,000165	Norvegia	Seterømme	0,000113	***
MAG C16:0	0,000689	Kremfløte	Kremgo	0,485	ns
MAG C16:0	0,000689	Kremfløte	Norvegia	0,192	ns
MAG C16:0	0,000689	Kremfløte	Seterømme	0,00562	**
MAG C16:0	0,000689	Kremgo	Norvegia	0,0239	*
MAG C16:0	0,000689	Kremgo	Seterømme	0,0406	*
MAG C16:0	0,000689	Norvegia	Seterømme	0,000474	***
MAG C9-C16:1	0,000043	Kremfløte	Kremgo	0,0352	*
MAG C9-C16:1	0,000043	Kremfløte	Norvegia	0,00227	**
MAG C9-C16:1	0,000043	Kremfløte	Seterømme	0,00958	**
MAG C9-C16:1	0,000043	Kremgo	Norvegia	0,217	ns
MAG C9-C16:1	0,000043	Kremgo	Seterømme	0,000225	***
MAG C9-C16:1	0,000043	Norvegia	Seterømme	0,0000389	****
MAG C18:0	0,0000393	Kremfløte	Kremgo	0,293	ns
MAG C18:0	0,0000393	Kremfløte	Norvegia	0,00109	**
MAG C18:0	0,0000393	Kremfløte	Seterømme	0,0169	*
MAG C18:0	0,0000393	Kremgo	Norvegia	0,00017	***
MAG C18:0	0,0000393	Kremgo	Seterømme	0,241	ns

MAG C18:0	0,0000393	Norvegia	Seterømme	0,0000327	****
MAG C9-C18:1	0,003	Kremfløte	Kremgo	0,953	ns
MAG C9-C18:1	0,003	Kremfløte	Norvegia	0,0888	ns
MAG C9-C18:1	0,003	Kremfløte	Seterømme	0,071	ns
MAG C9-C18:1	0,003	Kremgo	Norvegia	0,0429	*
MAG C9-C18:1	0,003	Kremgo	Seterømme	0,147	ns
MAG C9-C18:1	0,003	Norvegia	Seterømme	0,00192	**
MAG C9,C12-C18:2	0,019	Kremfløte	Kremgo	0,995	ns
MAG C9,C12-C18:2	0,019	Kremfløte	Norvegia	0,336	ns
MAG C9,C12-C18:2	0,019	Kremfløte	Seterømme	0,16	ns
MAG C9,C12-C18:2	0,019	Kremgo	Norvegia	0,44	ns
MAG C9,C12-C18:2	0,019	Kremgo	Seterømme	0,116	ns
MAG C9,C12-C18:2	0,019	Norvegia	Seterømme	0,013	*
DAG C12:0	0,021	Kremfløte	Kremgo	0,314	ns
DAG C12:0	0,021	Kremfløte	Norvegia	0,907	ns
DAG C12:0	0,021	Kremfløte	Seterømme	0,202	ns
DAG C12:0	0,021	Kremgo	Norvegia	0,646	ns
DAG C12:0	0,021	Kremgo	Seterømme	0,0152	*
DAG C12:0	0,021	Norvegia	Seterømme	0,0798	ns
DAG C14:0	0,01	Kremfløte	Kremgo	0,328	ns
DAG C14:0	0,01	Kremfløte	Norvegia	0,202	ns
DAG C14:0	0,01	Kremfløte	Seterømme	0,24	ns
DAG C14:0	0,01	Kremgo	Norvegia	0,981	ns
DAG C14:0	0,01	Kremgo	Seterømme	0,019	*
DAG C14:0	0,01	Norvegia	Seterømme	0,0116	*
DAG C15:0	0,02	Kremfløte	Kremgo	0,464	ns
DAG C15:0	0,02	Kremfløte	Norvegia	0,285	ns
DAG C15:0	0,02	Kremfløte	Seterømme	0,327	ns
DAG C15:0	0,02	Kremgo	Norvegia	0,975	ns
DAG C15:0	0,02	Kremgo	Seterømme	0,04	*
DAG C15:0	0,02	Norvegia	Seterømme	0,0227	*
DAG C16:0	0,014	Kremfløte	Kremgo	0,408	ns
DAG C16:0	0,014	Kremfløte	Norvegia	0,185	ns
DAG C16:0	0,014	Kremfløte	Seterømme	0,326	ns
DAG C16:0	0,014	Kremgo	Norvegia	0,922	ns
DAG C16:0	0,014	Kremgo	Seterømme	0,034	*
DAG C16:0	0,014	Norvegia	Seterømme	0,0146	*
DAG C18:0	0,009	Kremfløte	Kremgo	0,138	ns
DAG C18:0	0,009	Kremfløte	Norvegia	0,0668	ns
DAG C18:0	0,009	Kremfløte	Seterømme	0,683	ns
DAG C18:0	0,009	Kremgo	Norvegia	0,952	ns
DAG C18:0	0,009	Kremgo	Seterømme	0,0284	*
DAG C18:0	0,009	Norvegia	Seterømme	0,0141	*
DAG T9-C18:1	0,013	Kremfløte	Kremgo	0,039	*
DAG T9-C18:1	0,013	Kremfløte	Norvegia	0,0776	ns

DAG T9-C18:1	0,013	Kremfløte	Seterømme	0,999	ns
DAG T9-C18:1	0,013	Kremgo	Norvegia	0,959	ns
DAG T9-C18:1	0,013	Kremgo	Seterømme	0,0329	*
DAG T9-C18:1	0,013	Norvegia	Seterømme	0,0653	ns
PL C12:0	0,031	Kremfløte	Kremgo	0,908	ns
PL C12:0	0,031	Kremfløte	Norvegia	0,365	ns
PL C12:0	0,031	Kremfløte	Seterømme	0,249	ns
PL C12:0	0,031	Kremgo	Norvegia	0,153	ns
PL C12:0	0,031	Kremgo	Seterømme	0,544	ns
PL C12:0	0,031	Norvegia	Seterømme	0,0222	*
PL C14:0	0,012	Kremfløte	Kremgo	0,994	ns
PL C14:0	0,012	Kremfløte	Norvegia	0,117	ns
PL C14:0	0,012	Kremfløte	Seterømme	0,262	ns
PL C14:0	0,012	Kremgo	Norvegia	0,0825	ns
PL C14:0	0,012	Kremgo	Seterømme	0,358	ns
PL C14:0	0,012	Norvegia	Seterømme	0,00756	**
PL C16:0	0,018	Kremfløte	Kremgo	0,855	ns
PL C16:0	0,018	Kremfløte	Norvegia	0,166	ns
PL C16:0	0,018	Kremfløte	Seterømme	0,359	ns
PL C16:0	0,018	Kremgo	Norvegia	0,0545	ns
PL C16:0	0,018	Kremgo	Seterømme	0,776	ns
PL C16:0	0,018	Norvegia	Seterømme	0,0146	*
PL C18:0	0,02	Kremfløte	Kremgo	0,964	ns
PL C18:0	0,02	Kremfløte	Norvegia	0,132	ns
PL C18:0	0,02	Kremfløte	Seterømme	0,462	ns
PL C18:0	0,02	Kremgo	Norvegia	0,0686	ns
PL C18:0	0,02	Kremgo	Seterømme	0,718	ns
PL C18:0	0,02	Norvegia	Seterømme	0,0157	*
PL C9-C18:1	0,000046	Kremfløte	Kremgo	0,131	ns
PL C9-C18:1	0,000046	Kremfløte	Norvegia	0,00115	**
PL C9-C18:1	0,000046	Kremfløte	Seterømme	0,0385	*
PL C9-C18:1	0,000046	Kremgo	Norvegia	0,000107	***
PL C9-C18:1	0,000046	Kremgo	Seterømme	0,822	ns
PL C9-C18:1	0,000046	Norvegia	Seterømme	0,0000534	****
FFA C8:0	0,000503	Kremfløte	Kremgo	0,995	ns
FFA C8:0	0,000503	Kremfløte	Norvegia	0,00084	***
FFA C8:0	0,000503	Kremfløte	Seterømme	0,876	ns
FFA C8:0	0,000503	Kremgo	Norvegia	0,00107	**
FFA C8:0	0,000503	Kremgo	Seterømme	0,953	ns
FFA C8:0	0,000503	Norvegia	Seterømme	0,00188	**
FFA C10:0	0,000535	Kremfløte	Kremgo	0,996	ns
FFA C10:0	0,000535	Kremfløte	Norvegia	0,00073	***
FFA C10:0	0,000535	Kremfløte	Seterømme	0,199	ns
FFA C10:0	0,000535	Kremgo	Norvegia	0,000923	***
FFA C10:0	0,000535	Kremgo	Seterømme	0,269	ns

FFA C10:0	0,000535	Norvegia	Seterømme	0,00937	**
FFA C12:0	0,0000186	Kremfløte	Kremgo	1	ns
FFA C12:0	0,0000186	Kremfløte	Norvegia	0,000134	***
FFA C12:0	0,0000186	Kremfløte	Seterømme	0,0856	ns
FFA C12:0	0,0000186	Kremgo	Norvegia	0,000124	***
FFA C12:0	0,0000186	Kremgo	Seterømme	0,0975	ns
FFA C12:0	0,0000186	Norvegia	Seterømme	0,000016	****
FFA C13:0	0,003	Kremfløte	Kremgo	0,793	ns
FFA C13:0	0,003	Kremfløte	Norvegia	0,00579	**
FFA C13:0	0,003	Kremfløte	Seterømme	0,978	ns
FFA C13:0	0,003	Kremgo	Norvegia	0,0193	*
FFA C13:0	0,003	Kremgo	Seterømme	0,576	ns
FFA C13:0	0,003	Norvegia	Seterømme	0,00358	**
FFA C14:0	0,0000215	Kremfløte	Kremgo	0,854	ns
FFA C14:0	0,0000215	Kremfløte	Norvegia	0,0000762	****
FFA C14:0	0,0000215	Kremfløte	Seterømme	0,454	ns
FFA C14:0	0,0000215	Kremgo	Norvegia	0,000148	***
FFA C14:0	0,0000215	Kremgo	Seterømme	0,166	ns
FFA C14:0	0,0000215	Norvegia	Seterømme	0,0000239	****
FFA C9-C14:1	0,0000296	Kremfløte	Kremgo	0,83	ns
FFA C9-C14:1	0,0000296	Kremfløte	Norvegia	0,000108	***
FFA C9-C14:1	0,0000296	Kremfløte	Seterømme	0,428	ns
FFA C9-C14:1	0,0000296	Kremgo	Norvegia	0,000226	***
FFA C9-C14:1	0,0000296	Kremgo	Seterømme	0,143	ns
FFA C9-C14:1	0,0000296	Norvegia	Seterømme	0,0000312	****
FFA C15:0	0,0000191	Kremfløte	Kremgo	0,0504	ns
FFA C15:0	0,0000191	Kremfløte	Norvegia	0,0000653	****
FFA C15:0	0,0000191	Kremfløte	Seterømme	0,365	ns
FFA C15:0	0,0000191	Kremgo	Norvegia	0,00124	**
FFA C15:0	0,0000191	Kremgo	Seterømme	0,005	**
FFA C15:0	0,0000191	Norvegia	Seterømme	0,0000183	****
FFA C16:0	0,000101	Kremfløte	Kremgo	0,235	ns
FFA C16:0	0,000101	Kremfløte	Norvegia	0,000373	***
FFA C16:0	0,000101	Kremfløte	Seterømme	0,421	ns
FFA C16:0	0,000101	Kremgo	Norvegia	0,00346	**
FFA C16:0	0,000101	Kremgo	Seterømme	0,0248	*
FFA C16:0	0,000101	Norvegia	Seterømme	0,0000896	****
FFA C9-C16:1	0,0000138	Kremfløte	Kremgo	0,000804	***
FFA C9-C16:1	0,0000138	Kremfløte	Norvegia	0,0000165	****
FFA C9-C16:1	0,0000138	Kremfløte	Seterømme	0,429	ns
FFA C9-C16:1	0,0000138	Kremgo	Norvegia	0,00682	**
FFA C9-C16:1	0,0000138	Kremgo	Seterømme	0,00489	**
FFA C9-C16:1	0,0000138	Norvegia	Seterømme	0,0000518	****
FFA C17:0	0,00047	Kremfløte	Kremgo	0,00147	**
FFA C17:0	0,00047	Kremfløte	Norvegia	0,000987	***

FFA C17:0	0,00047	Kremfløte	Seterømme	0,348	ns
FFA C17:0	0,00047	Kremgo	Norvegia	0,982	ns
FFA C17:0	0,00047	Kremgo	Seterømme	0,0125	*
FFA C17:0	0,00047	Norvegia	Seterømme	0,00779	**
FFA C18:0	0,0000478	Kremfløte	Kremgo	0,00111	**
FFA C18:0	0,0000478	Kremfløte	Norvegia	0,000133	***
FFA C18:0	0,0000478	Kremfløte	Seterømme	0,999	ns
FFA C18:0	0,0000478	Kremgo	Norvegia	0,197	ns
FFA C18:0	0,0000478	Kremgo	Seterømme	0,00125	**
FFA C18:0	0,0000478	Norvegia	Seterømme	0,000147	***
FFA C9-C18:1	0,0000447	Kremfløte	Kremgo	0,000818	***
FFA C9-C18:1	0,0000447	Kremfløte	Norvegia	0,0000745	****
FFA C9-C18:1	0,0000447	Kremfløte	Seterømme	0,641	ns
FFA C9-C18:1	0,0000447	Kremgo	Norvegia	0,111	ns
FFA C9-C18:1	0,0000447	Kremgo	Seterømme	0,00309	**
FFA C9-C18:1	0,0000447	Norvegia	Seterømme	0,000206	***
FFA C9,C12-C18:2	0,0000532	Kremfløte	Kremgo	0,000157	***
FFA C9,C12-C18:2	0,0000532	Kremfløte	Norvegia	0,0001	****
FFA C9,C12-C18:2	0,0000532	Kremfløte	Seterømme	0,0653	ns
FFA C9,C12-C18:2	0,0000532	Kremgo	Norvegia	0,949	ns
FFA C9,C12-C18:2	0,0000532	Kremgo	Seterømme	0,00335	**
FFA C9,C12-C18:2	0,0000532	Norvegia	Seterømme	0,00182	**
UF C6:0	0,034	Kremfløte	Kremgo	0,957	ns
UF C6:0	0,034	Kremfløte	Norvegia	0,0455	*
UF C6:0	0,034	Kremfløte	Seterømme	1	ns
UF C6:0	0,034	Kremgo	Norvegia	0,0922	ns
UF C6:0	0,034	Kremgo	Seterømme	0,975	ns
UF C6:0	0,034	Norvegia	Seterømme	0,0516	ns
UF C8:0	0,031	Kremfløte	Kremgo	0,998	ns
UF C8:0	0,031	Kremfløte	Norvegia	0,074	ns
UF C8:0	0,031	Kremfløte	Seterømme	0,92	ns
UF C8:0	0,031	Kremgo	Norvegia	0,0938	ns
UF C8:0	0,031	Kremgo	Seterømme	0,855	ns
UF C8:0	0,031	Norvegia	Seterømme	0,0307	*
UF C10:0	0,014	Kremfløte	Kremgo	1	ns
UF C10:0	0,014	Kremfløte	Norvegia	0,0868	ns
UF C10:0	0,014	Kremfløte	Seterømme	0,442	ns
UF C10:0	0,014	Kremgo	Norvegia	0,0765	ns
UF C10:0	0,014	Kremgo	Seterømme	0,488	ns
UF C10:0	0,014	Norvegia	Seterømme	0,01	**
UF C12:0	0,007	Kremfløte	Kremgo	0,701	ns
UF C12:0	0,007	Kremfløte	Norvegia	0,204	ns
UF C12:0	0,007	Kremfløte	Seterømme	0,104	ns
UF C12:0	0,007	Kremgo	Norvegia	0,044	*
UF C12:0	0,007	Kremgo	Seterømme	0,435	ns

UF C12:0	0,007	Norvegia	Seterømme	0,00534	**
UF C14:0	0,008	Kremfløte	Kremgo	0,293	ns
UF C14:0	0,008	Kremfløte	Norvegia	0,424	ns
UF C14:0	0,008	Kremfløte	Seterømme	0,0705	ns
UF C14:0	0,008	Kremgo	Norvegia	0,0316	*
UF C14:0	0,008	Kremgo	Seterømme	0,733	ns
UF C14:0	0,008	Norvegia	Seterømme	0,0079	**
UF C9-C14:1	0,005	Kremfløte	Kremgo	0,223	ns
UF C9-C14:1	0,005	Kremfløte	Norvegia	0,282	ns
UF C9-C14:1	0,005	Kremfløte	Seterømme	0,0737	ns
UF C9-C14:1	0,005	Kremgo	Norvegia	0,015	*
UF C9-C14:1	0,005	Kremgo	Seterømme	0,853	ns
UF C9-C14:1	0,005	Norvegia	Seterømme	0,00538	**
UF C15:0	0,004	Kremfløte	Kremgo	0,649	ns
UF C15:0	0,004	Kremfløte	Norvegia	0,0538	ns
UF C15:0	0,004	Kremfløte	Seterømme	0,298	ns
UF C15:0	0,004	Kremgo	Norvegia	0,0106	*
UF C15:0	0,004	Kremgo	Seterømme	0,888	ns
UF C15:0	0,004	Norvegia	Seterømme	0,00431	**
UF C16:0	0,026	Kremfløte	Kremgo	0,0629	ns
UF C16:0	0,026	Kremfløte	Norvegia	0,964	ns
UF C16:0	0,026	Kremfløte	Seterømme	0,335	ns
UF C16:0	0,026	Kremgo	Norvegia	0,0326	*
UF C16:0	0,026	Kremgo	Seterømme	0,628	ns
UF C16:0	0,026	Norvegia	Seterømme	0,182	ns
UF C9-C16:1	0,049	Kremfløte	Kremgo	0,184	ns
UF C9-C16:1	0,049	Kremfløte	Norvegia	0,036	*
UF C9-C16:1	0,049	Kremfløte	Seterømme	0,202	ns
UF C9-C16:1	0,049	Kremgo	Norvegia	0,663	ns
UF C9-C16:1	0,049	Kremgo	Seterømme	1	ns
UF C9-C16:1	0,049	Norvegia	Seterømme	0,625	ns
UF C9,C12-C18:2	0,031	Kremfløte	Kremgo	0,288	ns
UF C9,C12-C18:2	0,031	Kremfløte	Norvegia	0,118	ns
UF C9,C12-C18:2	0,031	Kremfløte	Seterømme	0,0232	*
UF C9,C12-C18:2	0,031	Kremgo	Norvegia	0,909	ns
UF C9,C12-C18:2	0,031	Kremgo	Seterømme	0,33	ns
UF C9,C12-C18:2	0,031	Norvegia	Seterømme	0,665	ns
UF C9,C12,C15-C18:3	0,000202	Kremfløte	Kremgo	0,000312	***
UF C9,C12,C15-C18:3	0,000202	Kremfløte	Norvegia	0,0014	**
UF C9,C12,C15-C18:3	0,000202	Kremfløte	Seterømme	0,38	ns
UF C9,C12,C15-C18:3	0,000202	Kremgo	Norvegia	0,488	ns
UF C9,C12,C15-C18:3	0,000202	Kremgo	Seterømme	0,00179	**
UF C9,C12,C15-C18:3	0,000202	Norvegia	Seterømme	0,0108	*

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