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Abstract

The production of cheese is in continuous development with objectives to increase productivity, achieve changes in shelf life and functional properties of the products. One concept in the development is to substitute milk fat with vegetable fats and oils in the manufacture of cheese.

The objective of this study was to investigate effects on cheese yield and cheese quality of 4 different semi-hard analogue cheese products produced by substituting milk fat with vegetable fat, each analogue cheese with a specific mixture of vegetable fat. As reference a cheese produced with anhydrous milk fat was used.

The yield and recovery of fat and protein were determined by recording the weights of each cheese direct after press and after 20±2 days of storage and analysis of the composition of the cheeses after 20±2 days of storage. The cheese was further subjected for texture profile analysis in a TA-XT Plus Texture Analyser and analysed with Differential Scanning Calorimetry (DSC) to examine whether the fat behaves similar in the cheese as in pure form in respect to its thermal properties.

The cheese yield was not significantly different when using different fats. Different amounts of water were absorbed during the water cooling. The fat C cheese absorbed significantly less water than the other cheeses. The cheese made of fat C was significantly harder than the other cheeses. The springiness as well as the cohesiveness of the different types of cheeses was similar even though significant differences occurred. DSC showed that the fat behaved similarly in the cheese and in pure form in all types of cheeses in respect to melting and crystallization pattern of the fat. Guiding water contents for all types of cheeses, and representative fat contents for cheeses made of fat A and C could be determined from the DSC spectrums. In conclusion, regarding fat and protein content of the cheese it is possible to produce similar kinds of cheeses, using different kinds of fats. Using different kinds of vegetable fats or milk fat does not result in significant differences in yield by using the same manufacturing process.

Sammanfattning

Ostproduktion är i konstant utveckling med objektivet att öka lönsamheten, upnå ändringar i produkternas hållbarhet, följt av att kunna ändra funktionella egenskaper hos produkterna. En riktning i utvecklingen är att byta ut mjölkfettet mot vegetabiliskt fett och vegetablisk olja i ostproduktionen. .

Syftet med den här studien var att undersöka effekten på ostutbyte och kvalité i 4 olika semi-hårda analoga ostprodukter när mjölkfett ersattes av vegetabilisk fett, där varje analog ostprodukt hade en typ av vegetabiliskt fett. En referens ost av anhydrerat mjölkfett användes.

Utbytet och andelen fett och protein kvar i ostarna bestämdes genom väga varje ost efter press och efter 20±2 dagars lagring följt av att bestämma kompositionen i ostarna efter 20±2 dagars lagring. Efter 20±2 dagars lagring analyserades texturegenskaper med en TA-XT Plus Texture Analyser, och en Differential Scanning Calorimetry (DSC) användes för att undersöka om fettets smält- och kristalliseringsegenskaper skiljde sig från när fettet är i osten till ren form av fettet.

Ostutbytet skiljde sig inte signifikant åt oavsett vilket fett som användes till osttillverkningen. Olika mängd vatten absorberades under kylningen i vattenbad. Osten gjord av fett C absorberade signifikant mindre vatten än de andra ostarna. Osten gjord av fett C var signifikant hårdare än de andra ostarna. Elasticiteten och sammanhållningen var liknande mellan de olika ostarna även om vissa signifikanta skillnader fanns. DSC visade att fettets smält- och kristalliseringsegenskaper inte skiljde sig när det var inkorporerat i ost som när i ren form. Representativa vattenhalter från alla typer av ostar och representativa fetthalter från ost gjord fett A och C kunde utläsas från DSC diagrammen. Som en slutsats är det möjligt att med avseende på fett och protein innehåll producera liknande ostar med olika typer av vegetabiliskt fett. Tillverkningen med olika typer av vegetabiliskt fett eller med mjölkfett resulterar inte i varierande ostutbyte när samma tillverkningsprocess används.

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1 Introduction

1.1 Background

Milk and therefore also cheese is considered a wholesome food since it contains an adequate source of valuable macro- and micronutrients. Cheese has been produced for over several thousands of years and has in many cultures been developed to an important part of the diet. In manufacture of cheese, milk is coagulated by addition of enzyme or through acidification. The coagulum is cut to drain off the whey and consequently most of the water-soluble components. Depending on the type of cheese, the curd is then shaped, moulded, salted and ripened until it develops to the final product (Fox & McSweeney, 1998). The European consumption of cheese per capita was in 2012 17.2 kg and of all milk produced in Sweden in 2013, 25.7 % was used for cheese production (LRF, 2014).

During recent years consumers have become increasingly aware of the importance of maintaining adequate nutrition. One approach would be to formulate foods with ingredients that help in lowering health risks. With this objective, products have been developed in which saturated milk fat has been replaced by vegetable fats and oils, for instance in cheese production (Erickson & Frey, 1994). A limitation of milk as a raw material is its perishability, since its excellent feature as growth medium for micro-organisms. Traditionally, cheese production was therefore a way of preserving the milk for a longer time. However, the perishability is nowadays overcome by application of various heat treatments, based on the combined effect of temperature and time (Fox & McSweeney, 1998).

The production of cheese is continuously developing to increase productivity, reduce microbiological risks. The dairy industry is also developing to create cheese with new functional properties. Milk concentrates are used, however also non-dairy ingredients find a critical role in synergy with the chemical constituents of milk in order to alter the sensory and nutritional profile (Lobato-Calleros *et al.*, 2002; Yu & Hammond, 2000) and at the same time possibly reduce the cost of the final product (Hesseltine & Wang, 1980). Replacing milk fat with vegetable fat, usage of enzymes and an increasing number of processed cheese varieties has been in development over the last decades.

The composition and functional properties of vegetable fats vary naturally, i.e., a fat with a higher degree of unsaturation is less solid at a specific temperature than a fat with a lower level of unsaturation. In addition, the vegetable fat may be even more diversified by industrial processes which modify the composition. As a result of the vast variation of vegetable fats, their applications in cheese give rise to different properties (Wennermark *et al.*, 2014).

Substituting milk fat with vegetable fat changes the nutritional profile of the cheese. The cheese becomes lower in cholesterol and the composition of saturated and unsaturated fatty acids changes. Lobato-Calleros *et al.* (2002) and Yu and Hammond (2000) produced and investigated functional properties of ripened cheese analogues using emulsifiers. The instrumental and sensory textural characteristics were analysed and evaluated to study the correlation between different parameters. They found no significant differences in texture between use of high-oleic sunflower oil (HOSO) and milk fat except in cohesiveness. Dinkçi *et al.* (2011) produced Turkish Kashar cheese and compared the physical properties of products with vegetable fat and milk fat. They showed significant differences in texture and colour. The milk fat product perceived higher sensory scores even though the vegetable fat product was organoleptic and texturally acceptable. Yu and Hammond (2000) used varied proportions and types of HOSO in their production and presented it to be economically advantageous as a replacement for butterfat in cheese production. The commercial feasibility accounted for the cost of the HOSO, the modification of it and the additional revenue from when using butterfat in alternative products.

The yield (kg cheese/kg milk) in cheese manufacturing is of particular interest since it affects the commercial feasibility. The probable single most important factor affecting cheese yield is milk composition and in particular its concentration and composition of fat and protein (Fox *et al.*, 2000).

Several techniques have been assessed for increasing the cheese yield. Huppertz *et al.* (2004) show that high-pressure treatment of milk prior cheese making increases the yield through enhanced incorporation of denatured whey proteins in the curd. Heat treatment has a similar effect but the resulting cheese show textural and flavour defects and increased water content (Huppertz *et al.*, 2004). Further, the cheese yield may be enhanced by adding calcium in the form of calcium chloride ($CaCl_2$) to the milk. Wolfschoon-Pombo (1997) indicated a slightly higher transfer of milk fat and milk solids non-fat into the curd when adding $CaCl_2$.

1.2 Hypothesis

The composition of the cheese milk is one of the most important factors affecting the cheese yield and the cheese properties i.e. texture and other organoleptic attributes. Substituting the milk fat with vegetable fat will have an effect on the yield and the functional properties. Fats with higher melting points are assumed have more rigid fat globules due to their higher amount of saturated fatty acids. This will make the formation of a well-functioning casein network more difficult. On the other hand, more liquid fats with softer fat globules are assumed to be able to be deformed in the network, thus making it possible for the casein to form a stronger network. In a system with 30 % fat the distance between the globules is short and the rheological properties of the globules are expected to greatly influence the systems textural properties. This is the case with a 30 % fat cheese where most of the fat is still in emulsified form.

2 Literature review

2.1 Dispersed systems

2.1.1 Emulsions

Most foods are in fact dispersions of different kinds. Dispersions consist of either solid or liquid particles which are homogenously distributed in a continuous phase. One type of dispersion is emulsions, and it is common to distinguish between two types of emulsions. In “water-in-oil emulsions” water droplets are dispersed in a fat matrix, butter being an example. Mayonnaise is an example of an oil-in-water emulsion, in which oil droplets are dispersed in water. An emulsion is rarely stable on its own. Through coalescence of the dispersed phase it will strive to form distinct layers of oil and water. The stability is increased by surfactants; amphiphilic molecules, often proteins which are incorporated in the emulsion forming a surface layer on the droplets (Walstra *et al.*, 1999).

2.1.2 Gels

Gels, soft solids, are other kind of dispersions, where the dispersed phase forms a cohesive network in the dispersant. Gels are formed in a process called gelation which may occur by altered pH, heating, cooling or by other agents, such as enzymes. The process can be irreversible, such as heated egg white, or reversible, which is the case with gelatine. Gelatine forms gel structures at low temperatures but melts when temperature is increased again (McClements, 1999). The properties of a gel are dependent on the concentration, rheological properties and filler-gel interactions of the dispersants (van Vliet, 1988). The formation of the gel entraps components such as water or oil, which are dispersed within the matrix.

Gels act elastically to a certain amount of stress. On the other hand if the stress is too great, the gels deform and become viscoelastic. An important gel property is the ability to retain liquids. A strong ability would cause swelling while a weak would cause expelling, syneresis, of liquid (Walstra *et al.*, 1999). Enzymatic or acid-coagulated milk gels in cheese production are quite stable if left undisturbed, but when they are cut or broken or subjected to external pressure, the aqueous phase, whey, is expelled from the gel (Fox *et al.*, 2000).

2.2 Components and properties of milk

One typical emulsion is milk; a dilute emulsion of lipids in milk serum mainly consisting of water, in the case of bovine milk 85.3-88.7 % w/w. The dry matter of milk contains among other lactose, 3.8-5.3 % w/w, fat, 2.5-5.5 % w/w, protein, 2.3-4.4 % w/w, and mineral substances, 0.57-0.83 % w/w (Walstra *et al.*, 1999).

2.2.1 Milk lipids

Nearly all lipids in milk are contained in milk fat globules and consist of a mixture of triglycerides. Milk lipids are also found in the fat globule membrane and free in milk serum. The milk fat globules are enclosed by a membrane built up by phospholipids, proteins and glycoproteins, which prevent aggregation and coalescence. The chemical and physical properties of a lipid depend on the kind of lipid molecule (triglyceride, phospholipid, sterol etc.) but also participating fatty acid, suggested as the chain length, level of unsaturation, position of double bonds etc. (Walstra *et al.*, 1999). Table 1 demonstrates the composition of saturated and unsaturated fatty acids in milk fat.

Table 1. *Fatty acid type composition of milk fat (Lindmark Månsson, 2010)*

Fatty acid	%
Saturated	70.4
Unsaturated	29.0
-Monounsaturated	24.4
-Polyunsaturated	2.1
- Trans	2.5
- Cis	26.5
Other	0.6
Sum	100.0

The great variety of lipids in milk explains to the wide melting range for milk fat spanning from -40˚C to +40˚C. In this interval some lipids will be liquid while others will be crystallized (Walstra *et al.*, 1999). The fat globules are the largest particles in milk ranging in size from 0.1 to 20 µm in diameter. Homogenization i.e. pumping milk at high pressure through a narrow opening disrupts the fat globules and smaller ones are formed. The disruption of the globules increases the interface between fat and milk serum. The smaller fat globule is, however, stabilized by coating proteins, mainly caseins in the form of micelles or sub-micelles (Walstra *et al.*, 1999).

2.2.2 Casein

Casein is the most abundant protein in milk, precipitating at a pH near 4.6. Casein includes four main components, αs1-, αs2-, β- and κ-casein and none of them is particularly heat-sensitive. The reason is the high proline content of caseins resulting in little secondary and tertiary structure. On the other hand, α- and β-caseins precipitate in the presence of calcium. In their natural environment, they are stabilized in micelles by κ-casein which not is sensitive to calcium. These features are widely used in cheese production in which the rennet enzyme chymosin attacks and splits up a portion of the κ-casein which consequently loses its property as non-sensitive to calcium with a concurrent destabilisation of the paracasein micelles. The splitting causes flocculation of the paracasein micelles, due to van der Waals attraction. The calcium ions neutralize the negative charges of the micelles and diminish the electrostatic repulsion. Furthermore, the calcium ions creates salt linkages between the negative sites on the paracasein micelles (Walstra *et al.*, 1999).

2.3 Cheese production and quality

2.3.1 Manufacture

Milk is often subject to pasteurization prior to the cheese production, e.g. high temperature during short time (HTST), 72˚C 15 s, to kill pathogenic and spoilage bacteria. In addition, pasteurization may increase the final cheese yield due to the denaturation of serum protein that during the process will be incorporated into the cheese curd. The manufacturing protocols for various cheese varieties differ in detail even though they have many elements in common (Fox *et al.*, 2000).

As a general simplification of the manufacture, after pasteurization and cooling to approximately 30˚C a starter culture is added and let to grow. To start coagulation a coagulant, i.e. rennet, is added. During the coagulation, the κ-casein will be hydrolysed by action of the coagulating enzymes chymosin and pepsin, which destabilizes the casein micelles by reducing solubility and capacity to repel other micelles. The remaining casein, paracasein, will begin to form aggregates. These aggregates will form a larger network and entrap fat globules and milk serum. When coagulation has occurred, the gel is cut into small cubes, 8-15 mm in size, and subjected to stirring and cooking (~35˚C). This promotes syneresis and results in a curd and expelled liquid, i.e. the whey. Whey is drained off and the curd is subjected to a scalding procedure. The whey is replaced with warm water to enhance the syneresis process, to extract part of the lactose from the curd in order to control the final pH of the cheese (IDF, 1994). The water is drained off and stirring is stopped, permitting the curd to sediment. The curd is formed and placed into moulds for pressing and further syneresis. Finally the cheese is placed in a brine solution. Besides from adding taste, texture and microbial protection the salt decreases the net weight since water is lost (Fox & McSweeney, 1998). After a time in the brine, the cheese is ready for storage. For example gouda-type cheese is ripened for at least 40 days to undergo glycolysis, lipolysis and proteolysis, as well as other catabolic changes, in order to obtain the characteristic mild flavour and aroma (Bertola *et al.*, 2000).

2.3.2 Cheese yield

The manufacturing process of a particular cheese is highly affecting the cheese yield. The most important factor affecting the yield is the composition of the milk, particularly the concentrations of fat and caseins. Fox *et al.* (2000) demonstrate a linear correlation between yield and the concentration of fat and casein in Cheddar cheese. Moreover, it was indicated that casein in general contributes significantly more to cheese yield than fat. Casein forms the continuous paracasein network and occludes the fat and moisture phases. Moisture contributes directly to cheese yield by the weight and indirectly by containing dissolved solids. The contribution from fat is that moisture content increases with increased fat content. Fat on its own has low water-holding capacity, but impedes syneresis when occluded in the casein network. The fat globules physically limit aggregation of the paracasein, resulting in less matrix contraction and less moisture expulsion (Fox *et al.*, 2000).

Also pH will have an influence on the yield. This can be explained with the effect pH has on the casein matrix. At pH values above 5.1 the matrix is more or less intact whereas at pH below 4.8, the casein sub-micelles have to a greater extent disintegrated. A disintegrated or looser matrix has the possibility to influence the yield as well as the texture if the water content is high, this because a higher ability to retain water (IDF, 1994).

Actual cheese yield (Y_a) is simply expressed as a percent yield (kg cheese/ 100 kg milk). However, this definition is not completely suitable when, like in this study, evaluating the inconstant composition of fat. Since the fat composition differs, meaning differs with type of fat; the yield should be defined as kg of cheese type per 100 kg milk, with specified content of protein and content and type of fat (IDF, 1991). The determination of the actual yield requires measurement of the weight of inputs and outputs in the production (Fox *et al.*, 2000). The actual yield may then be calculated using following equation:

$$Y_a = 100 \times \frac{weight\ of\ cheese}{(weight\ of\ milk + starter\ culture + salt)}$$ (Fox *et al.*, 2000)

The recovery of particular components from cheese milk to cheese, in other words to which degree the components are transferred to the cheese, affects the cheese yield and also determines the efficiency of the cheese making operation. Additionally, information on the component recovery may help trace the cause of high fat losses or poor cheese yield. Component losses which will be showed if analysing the whey. Hence, the amounts of fat and protein recovery can be used as an indirect measure of yield (Fox *et al.*, 2000). The recovery of components such as fat may be calculated with the equation:

$$fat\ recovery = 100 \times \frac{weight\ of\ cheese \times fat\ content\ of\ cheese}{weight\ of\ milk \times fat\ content\ of\ milk}$$ (Fox *et al.*, 2000)

2.3.3 Cheese composition and structure

A cheese is basically a gel matrix of caseins in which various components as fat, milk serum, minerals and microbes are entrapped (Everett & Auty, 2008). Moisture and fat composition influence the functional properties of cheese. Milk fat is distributed in the casein network and may be present as single globules or as aggregates. Moreover there is free fat, with the function to fill gaps in the casein network. The final structure and texture of the cheese is influenced, among other things, by how the fat interacts with other components (Michalski *et al.*, 2007). At storage temperatures and up to 41˚C the milk fat in cheese is partially crystallized. Using different temperatures during maturation will lead to formation of different kinds of crystals (Lopez *et al.*, 2006).

Within the gel matrix water is present in the form of milk serum. The water exists as free pools in serum channels in the matrix as well as bound tightly to casein proteins. The casein dehydrates over time, reducing the amount of free water. Dehydration occurs faster in salted cheese than in unsalted (Everett & Auty, 2008).

Reducing the fat content of cheese products affects the content of other components, the yield and functional properties of the cheese according to Caro *et al.* (2011) and Guinee *et al.* (2000). Milk fat plays an essential role in cheese quality since it acts as a plasticizer and affects cheese texture. Since it is a source of for example short-chain fatty acids it also gives rise to typical cheese flavour compounds. Low-fat cheese products may therefore show a reduced texture and flavour quality (Fox *et al.*, 2000).

2.3.4 Vegetable fat substitution

The lipid composition, including the fatty acid composition, of vegetable fats and oils differs from that of milk fat which results among other in a different melting and crystallisation range (Lobato-Calleros *et al.*, 1997). Vegetable fat is used in many food products and to achieve suitable characteristics of the final products, several steps of refining and modification is performed.

The crude oil, which is one of the resulting products from the extraction of the raw material, contains various components from the raw material. This includes free fatty acids, oxidation residues, colour compounds etc. which might reduce the shelf-life of the oil and the final products. The refining process aims to reduce these impurities. Refined oil should be pure and bright with a neutral taste and good shelf life (Wennermark *et al.*, 2014).

Food applications often require functionality that fats and oils are unable to provide in their natural state. To fulfil the requirements, the physical properties may be modified without compromising the nutritional profile (Wennermark *et al.*, 2014).

When substituting the milk fat with vegetable fat the composition of other components will not be significantly affected according to Dinkçi *et al.* (2011). However, the fat globule distribution in the casein matrix is affected. Cheese produced with vegetable fat instead of milk fat showed a more compact network. The vegetable fat globules were smaller and more uniform in size. According to Everett and Auty (2008) this is explained by the fact that the homogenisation of vegetable fat cheese milk creates smaller fat globules, which are more evenly dispersed, resulting in a more compact network.

2.3.5 Cheese sensory properties

The sensory properties of food are affected by the chemical and physical properties. Objective human sensory evaluation with trained taste panels such as quantitative descriptive analysis is the main method to analyse sensory properties of food. However the properties may also be measured instrumentally, even though the results are of restricted value in predicting the characteristics of the product as perceived by consumers (Ak & Gunasekaran, 2003). With measurements of e.g. the cheese texture and how the fat behaves at specific temperatures it is possible to indicate how the cheese will be perceived by the consumer.

Texture properties

The texture development in cheese is due to factors such as composition and structure which in turn is related to the manufacture e.g. properties of the milk and the curd. There are many terms used to describe food texture, for instance terms as adhesiveness; stickiness of food in mouth throughout chewing, creaminess; the extent to which the texture has broken down to a creamy, semiliquid texture during chewing, etc. (Ak & Gunasekaran, 2003).

Texture profile analysis (TPA), the time-force profile of an imitative two steps uniaxial compression test is a widely used instrumental texture measurement. A two-step compression is used to imitate a chewing's first two bites of a food. These two first bites give rise to a TPA force displacement curve, (Figure 1) from which one can read and calculate mechanical properties such as hardness, fracturability, cohesiveness springiness, adhesiveness and gumminess (Ak & Gunasekaran, 2003).

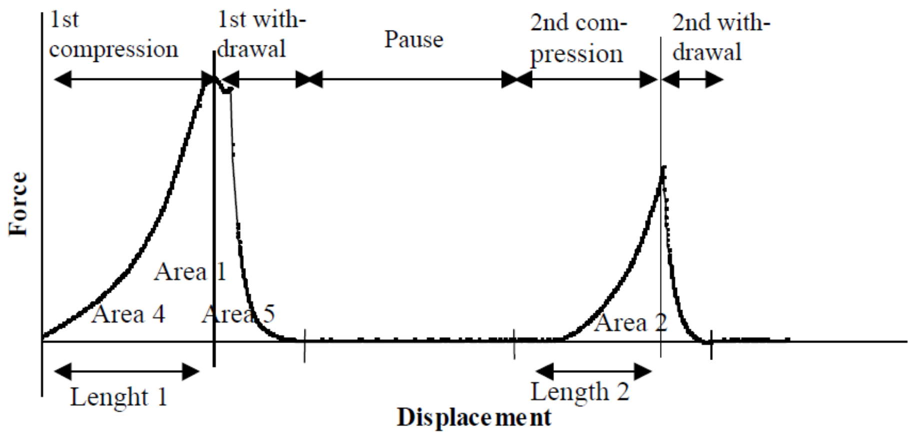

Figure 1. TPA force displacement curve with practical definitions to calculate textural properties of a sample.

Hardness is defined as the initial force needed to compress a tested sample and it is usually substituted by firmness in sensory analyses. Fracturability is defined as the force at the first significant break of the sample. Semisolid products, such as cheese, may not always show a first break, since it has an even texture throughout the product. Cohesiveness is obtained by dividing area 2 by area 1 (figure 1) and it is defined as the ratio of the force required for the second compression to the force required for the first compression. It is the measure how well the material holds together. Springiness or elasticity is obtained by dividing length 2 by length 1 and is related to the height the sample recovers between the two compressions. Gumminess is measured in terms of the energy required to chew a semisolid food and is obtained by multiplying hardness and cohesiveness. This is one of the most difficult characteristics to measure since chewing not only involves a compression, but also shearing, piercing, grinding etc. along with adequate lubrication by saliva at body temperatures (Ak & Gunasekaran, 2003).

Thermal properties

Fat plays an important role in determining specific properties of fat-rich food products such as cheese. Determining the thermal properties and therefore also the physical properties of fat, such as the liquid solid phase transition and the crystal polymorphism, may give indications of the rheological, sensorial and nutritional properties of the cheese (Lopez *et al.*, 2006).

The thermal properties of fat are usually studied by differential scanning calorimetry (DSC). DSC uses two cells, one reference cell and one sample cell. The two cells are heated simultaneously according to a pre-programmed temperature sequence. During the temperature-induced process in the sample cell, the calorimeter supplies more or less electrical energy (depending on whether the process of the components in the sample is endothermic or exothermic) to maintain the same temperature as the reference cell (Chowdhry & Cole, 1989). The DSC results in a spectrum as in figure 2. The x-axis demonstrates the time and the temperature programme and the movements of the curve vertically is whether the process is endothermic or exothermic. The endothermic demonstrates the melting and the exothermic the crystallization; reactions of fat as well as water. It is possible to calculate the water content since the reaction to crystallize 100 % of water corresponds to 295 J/g according to the DSC for a pure water sample. Fat content may be calculated by registering the changes in energy, and comparing cheese samples with the pure fat samples.

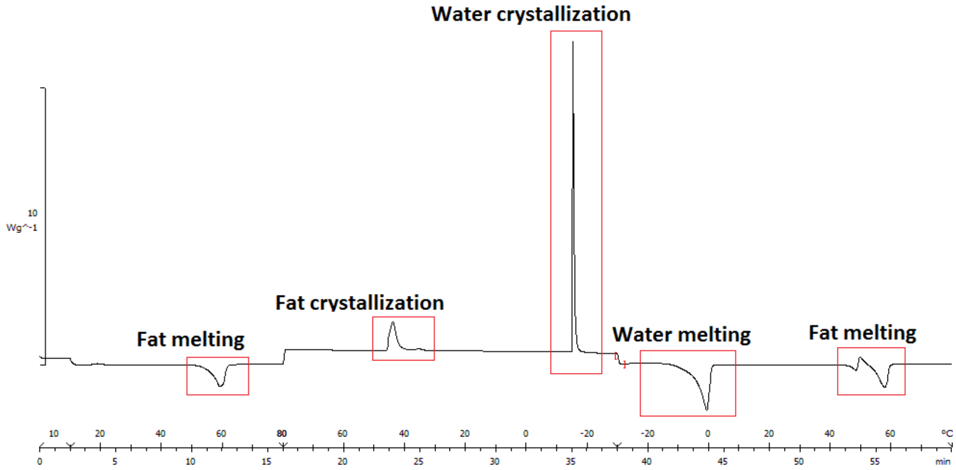

Figure 2. DSC spectrum with definitions of the endothermic (melting) and exothermic (crystallization) reactions

3 Objectives

The overall objective of this study was to investigate effects on cheese yield and cheese quality when milk fat was substituted by vegetable fat. The study included 5 different analogue semi-hard cheese products, each analogue cheese with a specific mixture of fat.

Specific aims of the study were to:

1) Determine cheese yield by measuring the weight of each cheese at specified time points.

2) Determine the composition of whey during the process and in the final cheese by analysing protein, fat, salt (NaCl), dry matter and pH.

3) Examine the texture and thermal properties of the final cheese.

Results are expected to extend the knowledge about the use of vegetable fats in cheese production. Further, the result will indicate promising formulas for fat mixtures to use in the development of vegetable fat cheeses.

4 Materials and methods

4.1 Experimental design

Five different types of gouda-type cheese products were produced in triplicates (at three different occasions) at the dairy pilot plant of AAK, Karlshamn, Sweden. Each cheese type was produced with one specific fat type in addition to the other ingredients, skim milk, beta carotene, starter culture, rennet, Calcium chloride ($CaCl_2$) and Sodium Nitrate ($NaNO_3$). The cheese products were produced in a randomized order and subjected for analyses as in table 2. The fat type is explained further in the cheese ingredient fat section and the different analyses are explained in the analyses section.

Table 2. *Experimental design of the study*

Experimental order	Fat	Analyses for all types and all replicates
1	A	**Whey:** Composition (protein, fat and dry matter) pH **Cheese (direct after production):** Weight **Cheese (20±2days of storage):** Weight Composition (protein, fat, NaCl, water) pH Textural properties (hardness, cohesiveness, springiness) Thermal properties (DSC)
7	A	
13	A	
3	B	
6	B	
11	B	
4	C	
14	C	
15	C	
2	D	
8	D	
10	D	
5	Anhydrous Milk Fat (AMF)	
9	AMF	
12	AMF	

4.2 Cheese ingredients

4.2.1 Milk

The bovine milk used in all types of cheese was standardised and low temperature (72-74˚C) pasteurised for <15 seconds, with a fat content <0.1 g /100 g was obtained in 1 litre containers from a local grocer and stored at temperatures <5˚C until processing. The time of storage did not exceed 4 days and the date for processing never exceeds the expiration date of the milk. The milk composition is shown in table 3.

Table 3. *Milk composition according to the manufacturer Skånemejerier (2014)*

Per 100 g	
Energy	150 kJ/35 kcal
Total fat	<0.1 g
-saturated fat	<0.1 g
Total carbohydrates	5 g
Protein	3.5 g

4.2.2 Milk fat and vegetable fat products

Five different types of fat products were used to produce the cheese. One of the fats was anhydrous milk fat (Arla Foods, Götene, Sweden), which was used as a standard. Beta carotene (DSM, Basel, Switzerland) was added to each fat before emulsification in order to obtain cheese with a more appreciable colour.

The fats were:

- A; a refined and modified fat with 99.8 % saturated fatty acids and a melting point between 30˚C and 35˚C (AAK, 2014a).
- B; refined and modified oil which is liquid at room temperature and down to 5˚C (AAK, 2014b).
- C; a fat with 99 % saturated fatty acids with high melting point at 60˚C (AAK, 2014c).
- D; refined oil which is liquid at room temperature and down to at least -30˚C (AAK, 2014d).
- Anhydrous Milk Fat (AMF); product derived exclusively from milk and/or products obtained from milk, resulting from a process which removes almost all water and non-fat solids. Minimum milk fat concentration 99.8 % (w/w) and maximum water concentration 0.1 % (w/w) (Codex, 1999).

Milk fat has a melting range between -40˚C and +40˚C (Lopez *et al.*, 2006). Fats B and D are liquid at 0˚C. Melting characteristics of fats A and C is shown in the solid fat content (SFC) curves (Figure 3). The characteristics of the fats are based on the fatty acid composition (see type of fatty acid in table 4).

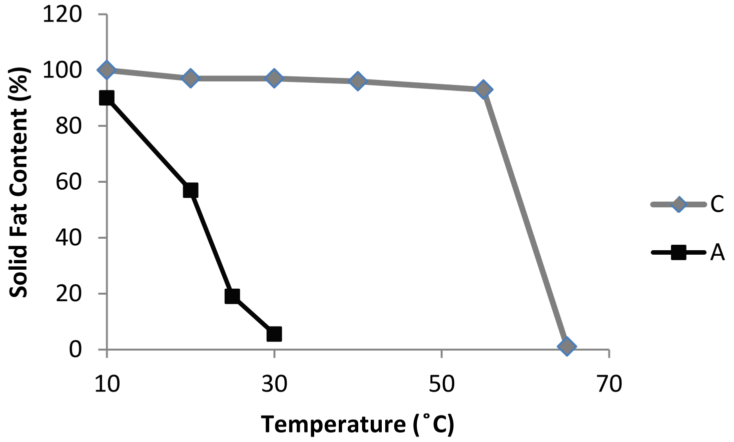

Figure 3.The solid fat content in percentage at different temperatures for fat A and C (AAK, 2014a; AAK, 2014c).

Table 4. *Fatty acid type composition of milk fat (MF) and vegetable fats A, B, C and D (AAK, 2014a; AAK, 2014b; AAK, 2014c; AAK, 2014d; Lindmark Månsson, 2010)*

	A	B	C	D	MF
Fatty acid	%	%	%	%	%
Saturated	99.8	32.2	99.0	6.9	70.4
Monounsaturated	0.1	58.4	0.5	62.7	24.4
Polyunsaturated	0.0	9.3	0.0	29.2	2.1
Trans	0.1	0.8	0.5	0.7	2.5
Cis	0.1	67.7	0.5	91.9	26.5
Unsaturated	0.2	68.5	1.0	92.6	29.0
Other					0.6
Sum	100.1	100.7	100.0	100.2	100.0

4.2.3 Starter culture

A mesophilic aromatic starter culture CHN-19 (Chr. Hansen A/S, Hoersholm, Denmark) was used in the manufacture. The culture was started 1 hour before it was added to the milk.

4.2.4 Rennet

Chy-Max® Plus coagulant, a chymosin produced by fermentation (Chr. Hansen A/S, Hoersholm, Denmark) was used in the manufacture. The manufacturer stated a minimum enzyme activity of ~200 IMCU/ml (International Milk Clotting Units). The coagulant was diluted 1:4 in water before added to the cheese milk.

4.2.5 Salts

Calcium chloride ($CaCl_2$) (VWR International AB, Stockholm, Sweden) was added to the cheese milk to enhance the flocculation of the casein. Sodium nitrate ($NaNO_3$) (VWR International AB, Stockholm, Sweden) was added as a preservation agent.

4.3 Incorporation of vegetable fat into cheese milk

The vegetable fat was incorporated into the milk by emulsification, resulting in a vegetable fat emulsion with a fat content of 30 %. The emulsification was carried out by heating both phases separately, i.e. milk and vegetable fat, to about 60˚C followed by vigorous blending for approximately 10 min until no fat droplets where visible. This was achieved in a multi-purpose processing vessel (FT40 Armfield Ltd, Ringwood, England), with a heavy duty laboratory mixer (Silverson L4R, Chesham, England). In the case of fat C, the emulsification temperature had to be increased to approximately 75˚C for both phases to prevent crystallization. Before the fat emulsion was added to a cheese vat, it was homogenised at 30/10 bar (GEA Niro Soavi S.P.A. NS1001 L, Parma, Italy) and finally pasteurised at 75˚C for 5 seconds using a plate heat exchanger (FT74P, Armfield Ltd, Ringwood, England) adjusted to 35 Hz.

4.3.1 Light microscopy observations of fat droplets

The fat emulsion was evaluated with microscope in order to control that the homogenisation had been performed properly and that fat droplets had similar sizes between the various types of vegetable fat used to produce the emulsions.

The fat emulsion were diluted 10 times and analysed with a Nikon OPTIPHOT-2 microscope (Nikon Instruments Europe BV, Amsterdam, Netherlands) in the x20 E Plan 20/0.4 160/0.17 Nikon objective, with a 12 V, 100 W halogen light source. The microscope had a camera, Infinity 2-2C (Lumenera Corporation, Ottawa, Canada) attached to capture photos of the samples. The diameter of randomly selected fat droplets in each photo was measured using Image-Pro 6.2 (Media Cybernetics ltd, Rockville, USA).

4.4 Cheese manufacture

The semi-hard cheese analogues was produced according to a protocol (table 5) elaborated by AAK to suit the available equipment. A vat machine (Invensys

APV, Silkeborg, Denmark) was filled with 89 L of milk. The 30 % fat emulsion was added to the milk in a 1:10 proportion, resulting in cheese milk with a fat content of 3 %. Together with 1 L milk for the starter culture the final cheese milk amount was 100 kg. A cheese press (Invensys APV, Silkeborg, Denmark) was used to press the cheese once the cheese curd was placed into moulds.

Table 5. *Production protocol for semi-hard analogue cheese manufacture (AAK)*

Operation	Time	Temperature (˚C)	Remarks
Fill vat		31	89 L Milk (<0.1 %)
Add fat emulsion		50-55	10 kg
Add starter, $CaCl_3$ and $NaNO_3$		31	10 g CH-N19 in 1 L milk
Ripening	40 min	31	
Coagulation	~30 min	31	30 g in 120 g water
Cutting and sedimentation	5 min	31	10 mm cubes
Stirring	~40 min	31	
Whey removal		31	30 L removed
Scalding	50 min	~38	Whey replaced with 30 L of 55-60˚C water
Vat press	20 min	~31	5,5 bar pressure
Drain whey			
Mould		<31	2* 25cm diameter circular shaped moulds
Press	3*20 min	<31	2, 4 and 5 bar pressure
Water bath	24 h	4	
Brine bath	20 h	4	
Packing			Vacuum
Maturation	20 ± 2 days	12	

Whey samples were taken before the scalding procedure and cheese samples were taken after storage for 20 ± 2 days and stored frozen until subjected for analyses.

4.5 pH

The pH was monitored throughout the process and in the stored products with a pH meter (Seven Easy, Mettler Toledo, Leicester, England). The pH was measured on the cheese milk just before adding the starter culture, on the cheese milk just before adding the rennet, on the curd just after cutting, on the whey at the first removal, on the whey at the second removal and on the cheese after 20±2 days of storage.

4.6 Weight of produced cheese and cheese yield

The two cheeses from each production were weighed with a scale (WBK 32 H, Adam Warrior, Scandinavian Scale Co AB, Hovmantorp, Sweden). The weights were registered as the cheeses leaved the press and after storage for 20±2 days according to IDF (1991). The weights were added together to obtain one total weight from each production. The final yield was calculated with the total weights registered after storage of each production, to include all factors of production. The weights registered when the cheese left the press was used to determine what occurred during the steps of water bath cooling and brining.

4.7 Compositional analysis of whey and cheese

Whey samples were analysed for content of crude protein, fat and dry matter (DM). Cheese samples were analysed for content of protein, fat, NaCl and water content. The compositional analyses were performed by AAK Analytic Centre (Karlshamn, Sweden); a SWEDAC accredited laboratory (Accreditation No. 1040). The protein content was determined by the Kjeldahl method, with conversion factor 6.25. The fat content was determined by a modified version of AOCS 963.15 (Analysis of total fat), a gravimetrical method. The dry matter and water content were determined gravimetrically with a drying method (AAK method no. 10R05M-1120). The NaCl content was determined by an AAK modified version of salt content by titration (AAK method no. 5015).

4.8 Texture analyses

Textural properties of the cheese were measured with TA-XT Plus Texture Analyser from Stable Micro Systems (Surrey, England) with a loading cell of 30 kg and a cylindrical probe, aluminium SMS P/25, 25 mm diameter and 30 mm height. Settings as in table 6 were used to obtain a texture profile analysis (TPA); the time-force profile of an imitative two step uniaxial compression test. The analysis gave rise to a graph from were hardness, cohesiveness and springiness could be determined.

Table 6. *Settings for the two steps compression test with the TA-XT Plus Texture Analyser*

Caption	Value	Unit
Test mode	Compression	
Pre-test speed	1.00	mm/sec
Test-speed	5.00	mm/sec
Post-test speed	5.00	mm/sec
Target mode	Distance	

Distance	10.00	mm
Time	5.00	sec
Trigger type	Auto (force)	
Trigger force	5.0	G
Tare mode	Auto	
Data acquisition rate	200	pps (points per second)

Cylindrical (24 mm diameter and 25 mm height) cheese samples were obtained from the cheese wheel. The samples were wrapped in plastic to prevent dehydration while adjusting to room temperature (18˚C).

4.9 Differential Scanning Calorimetry

Cheese samples and pure fats samples were analysed in DSC. The equipment used was a Mettler Toledo DSC 822^{e} with sample robot TSO 801R0 (Leicester, England) and temperature programmes I and II (Table 7). Cheese samples of fat A, B and D and pure fats were analysed with temperature program I. Cheese samples of fat C and pure fat C were analysed with the program with higher final temperature (II) counteract the greater melting temperature. The first temperature increase is to eliminate the thermal history of the different samples in order to standardize the method (Lopez *et al.*, 2006). When evaluating the spectrum, the samples were normalized by weight.

Table 7. *Differential Scanning Calorimetry temperature programmes I and II used in the study*

Segment	Temperature (˚C)	Time (min) / Rate (K/min)
I		
Initiation	10	2 min
Increase	70	5.00 K/min
Decrease	-30	-5.00 K/min
Increase	70	5.00 K/min
II		
Initiation	10	2 min
Increase	80	5.00 K/min
Decrease	-30	-5.00 K/min
Increase	80	5.00 K/min

4.10 Analysis of Variance (ANOVA) and Tukey's test

Using Excel and Minitab 16, results from cheese production, such as yield, weight difference from production to analyses, such as composition, were statistically analysed by one-way ANOVA test with significance level of 95 % to investigate whether the results were significantly different. Tukey's test was used at a significance level of 95 % to find out which of the results that differed significantly from the others.

5 Results

5.1 Light microscopy observation of fat droplets

The fat droplets were in movement when observed, and caption with the camera made it possible to measure them. The fat droplets were observed to have a diameter between 3.66 and 19.25 µm. In the fat emulsion made out of fat C and A as well as the milk fat there were signs of flocculation of the globules. Crystals inside the globules could be observed in the fat emulsion of fat C.

5.2 Cheese yields and weight increase

Using fat A resulted in the highest average cheese yield, 11.29 %, and using fat C resulted in the lowest average yield, 10.54 % when including all factors of production. However, differences between the different cheese types were not significant (p-value>0.05) (Table 8). From the time when the cheese left the press, the additional process steps of cooling in water bath and brining caused an increase in weight. The increase in weight after 20±2 days of storage was significantly lower for the cheese produced with fat C than the others (p-value<0.05) (table 8).

Table 8. *Average cheese yields and weight increase of the cheeses between the time when the cheeses leave the press and 20±2 days of storage. The average is calculated from the 3 productions of each type*

Type (n=3)	Yield (%)	Weight increase (%)
A	11.29±0.05	5.87±0.30[a]
B	10.97±0.45	6.06±0.89[a]
C	10.54±0.03	0.28±0.08[b]
D	10.86±0.71	5.60±2.67[a]
AMF	11.21±0.13	4.68±0.35[a]

5.3 pH and composition of whey and cheese

5.3.1 pH

The pH measured in the whey, curd and the stored cheese varied in a similar way regardless fat used in the production (Figure 4). More accurate figures of the average pH in the cheese after 20±2 days of storage are shown in table 10.

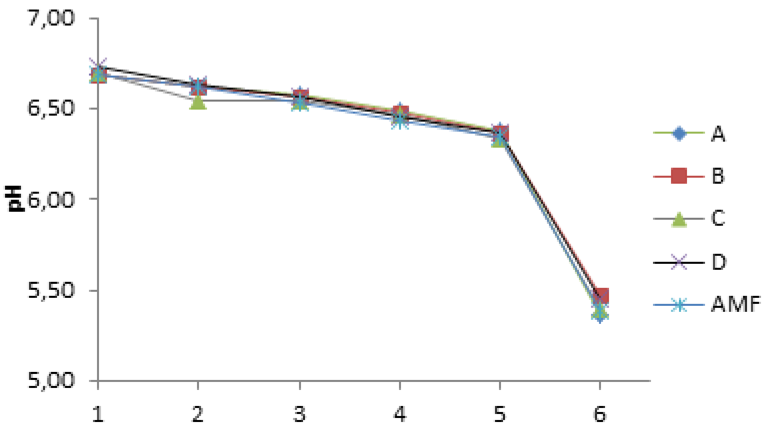

Figure 4. The pH measured during production (1-5) and in the stored products (6). Cheese milk just before adding the culture (1), cheese milk just before adding the rennet (2), of the curd just after cutting (3), of the whey at the first removal (4), of the whey at the second removal (5).

5.3.2 Whey composition

Table 9 demonstrates the average fat and protein content of the whey at the first whey removal. There were no significant differences in either fat or protein content in the whey between the different types of cheese.

Table 9. *Average composition of fat and protein in the whey at the first whey removal in the manufacture. The average is calculated from the 3 productions of each type*

Type (n=3)	Fat (%)	Protein (%)
A	0.27±0.08	1.00±0.00
B	0.21±0.09	0.93±0.06
C	0.25±0.06	0.93±0.06
D	0.23±0.06	0.97±0.06
AMF	0.17±0.06	1.00±0.00

5.3.3 Cheese composition

The average composition of the cheese is shown in table 10. Fat, protein and NaCl content did not differ significantly between the different cheese types. The water content of the fat A cheese was significantly different from fat B and C. The pH did not differ significantly between the types.

Table 10. *Average composition of the 20±2 days stored cheese. The average was calculated from the 3 productions of each type. All of the components were analyzed with one-way ANOVA. The analysis showed that only at least one of the content of water to be different from another. The other components were not significantly different when different fat used in production.*

Type (n=3)	Fat (%)	Fat in DM (%)	Water (%)	Protein (%)	NaCl (%)	pH
A	25.50±0.36	45.60±0.87	44.07±0.81[a]	21.17±2.36	1.63±0.15	5.36
B	25.67±2.76	42.38±3.35	39.53±2.10[b]	22.97±4.16	1.50±0.26	5.48
C	28.13±1.80	46.18±2.29	39.1±2.21[b]	22.03±1.45	1.17±0.06	5.39
D	24.73±1.16	42.19±3.42	41.27±1.77[ab]	22.63±3.01	1.53±0.40	5.45
AMF	25.06±0.75	44.30±1.65	43.40±0.75[ab]	21.43±1.78	1.70±0.26	5.38

5.3.4 Recovery of fat and protein

The average recovery of fat and protein i.e. the amount of the original content of fat and protein in the cheese milk recovered in the cheese is shown in table 11. There were no significant differences between the different types of cheese.

Table 11. *Average recovered fat and protein in the cheese based on the chemical composition of the 20±2 days stored cheese. The average recovery is calculated from the contents of fat and protein from the 3 productions of each type*

Type (n=3)	Fat (%)	Protein (%)
A	96.01±1.14	68.31±7.58
B	93.66±8.11	71.66±10.57
C	98.87±6.55	66.36±4.38
D	89.73±9.93	69.84±4.69
AMF	93.71±3.85	68.67±5.75

5.4 Texture properties

5.4.1 Hardness

The cheese made out of fat C was significantly harder than the cheeses made from any of the other fats (p-value<0.05), and could resist an average force of 11894±2608 g (Figure 5).

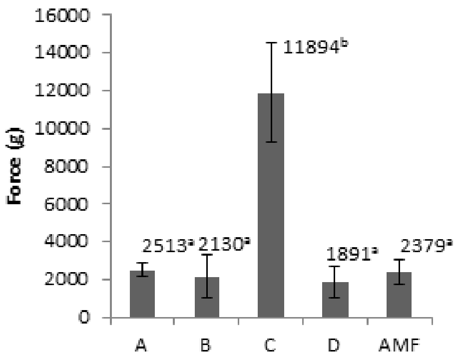

Figure 5. Average hardness obtained from the Texture Profile Analysis of the cheeses. The average was calculated from 9 measurements; 3 measurements of each replicate of cheese type.

5.4.2 Springiness

All cheeses showed elasticity. The average springiness was not lower than 80 %. Fat A was significantly different from fat D (p-value<0.05) (Figure 6).

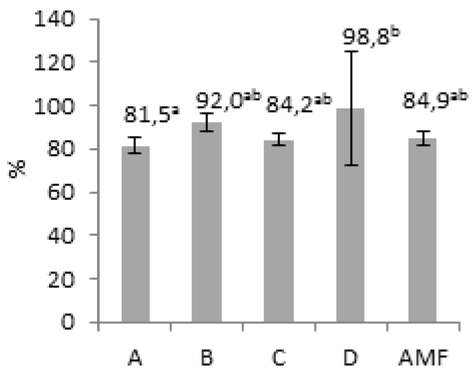

Figure 6. Average springiness obtained from the Texture Profile Analysis of the cheeses. The average was calculated from 9 measurements; 3 measurements of each replicate of cheese type.

5.4.3 Cohesiveness

Cheeses produced with fat B and D had significantly higher cohesiveness than the other cheeses, and the milk fat cheese had a significantly different cohesiveness than the vegetable fat cheeses (p-value<0.05) (Figure 7).

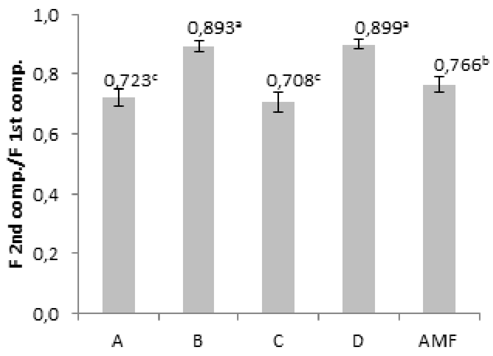

Figure 7. Average cohesiveness obtained from the Texture Profile Analysis of the cheeses. The average was calculated from 9 measurements; 3 measurements of each replicate of cheese type.

5.5 Thermal properties

The fat showed the same melting and crystallization pattern when incorporated in the cheese as when tested pure in DSC, which is illustrated for cheeses A and C in figures 8 and 9. The first dips in the curves at 60˚C (Figure 9) and at 20-35˚C (Figure 8) represent the melting of the fat (endothermic reaction). The green line, area and energy expenditure of the dip represent the pure form of the fat, while the blue, red and black represent the replicates of the test. From these comparable patterns a representative fat content could be calculated (Table 12). Water content in the cheeses could be calculated from the crystallisation of water (Appendix I).

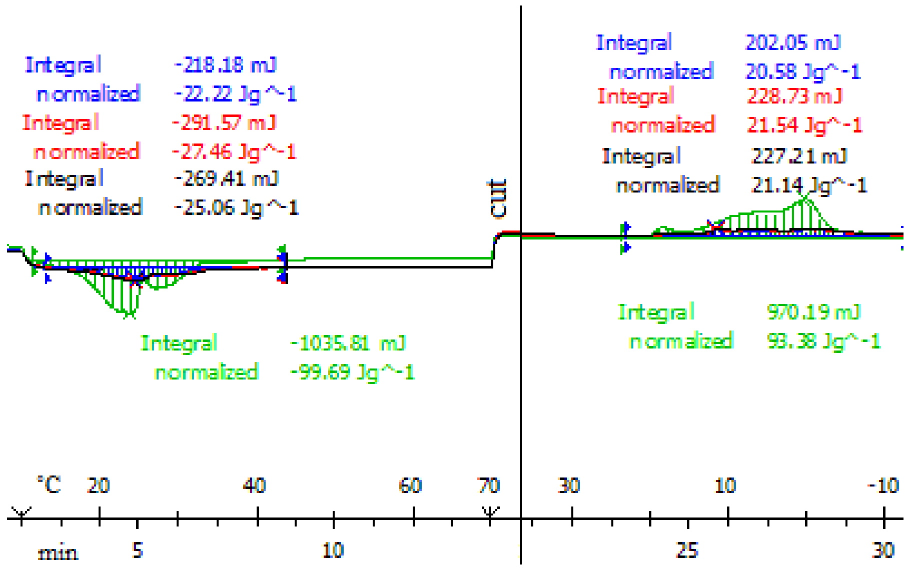

Figure 8. An enlargement of a section in a Differential Scanning Calorimetry spectrum of a fat A cheese and fat A in pure form (a section of approximately 6 min/ 30 ˚C decrease is cut off to fit the spectrum to the size of the figure).

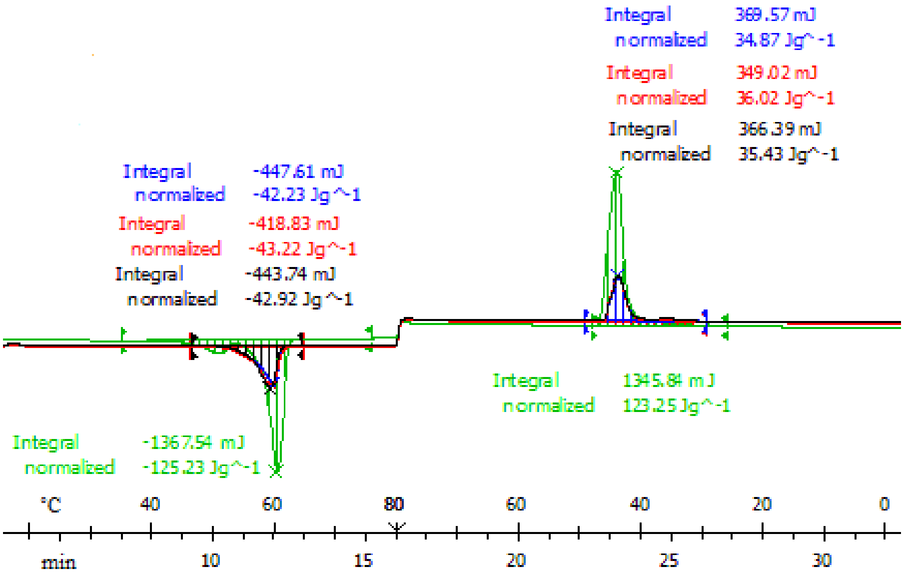

Figure 9. An enlargement of a section in a Differential Scanning Calorimetry spectrum of a fat C cheese and fat C in pure form.

Table 12. *Average water and fat content obtained by Differential Scanning Calorimetry. The average was calculated from 9 measurements; 3 measurements of each replicate of cheese type*

Fat (n=9)	Water (%)	Fat (%)
A	32.65±3.60	23.77±1.42
B	31.53±4.09	N.D.
C	28.28±1.86	29.43±0.83
D	28.80±6.24	N.D.
AMF	33.09±1.19	N.D.

N.D. = not determined

6 Discussion

The objectives of this study were to investigate effects on cheese yield and quality of 4 different analogue semi-hard cheese products, when the milk fat is substituted by vegetable fat. The most important factor affecting the cheese yield as well as the structural and functional properties is the composition of the cheese milk. Principally it is the concentration and detailed composition of fat and protein and how these components interact in the network. When using different types of vegetable fat instead of milk fat, the lipid composition changes, affecting the structure of the casein network. Differences in fatty acid composition between different vegetable fats and that of milk fat, give rise to different functional properties of the final cheeses.

6.1 Fatty acid composition

6.1.1 Melting point

The fats with higher content of saturated fatty acids, i.e. fat A (99.0 %) and C (99.8 %) (Table 4), are solid or in crystallized form until their melting point at 30˚C (fat A) and 60˚C (fat C). The fats with a lower content of saturated fatty acids, fat B (32.2 %) and D (6.9 %) (Table 4) and consequently a higher content of unsaturated fatty acids, are more or less liquid at room temperature and even below 0˚C (fat D). The physical form of a fat at specific temperatures will impact the manufacturing process and the resulting product.

The fact that fat C has a melting point of 60˚C means that as soon as the temperature decreases below 60˚C the liquid phase of the fat will begin to crystallize. As seen in the DSC-curve (Figure 3), fat C starts to crystallize at 40˚C. Even though fat C is in a stable emulsion with casein, it starts to crystallize when temperature decreases as the fat is added to the 31˚C temperature adjusted cheese vat. The matrix is still in movement even if the crystals are present in the globules according to the microscope observations. The partial crystallization of the fat before the actual rennet coagulation is something that will influence the curd formation and the final product. This characteristic, i.e. fat crystallization before the cheese is formed is not the case of fat B, D and milk fat since they start to crystallize at much lower temperatures (appendix I).

The properties of the finished products are dependent on the transition phase of the fat. Fat B is liquid throughout the process and does not start to crystallize until at refrigerated temperatures (<8˚C) according to the DSC spectrum in appendix I. Fat D which contains a larger degree of unsaturated fatty acids than fat B does not undergo phase transition even when the product is cooled to refrigerated temperatures (appendix I).

6.1.2 Structural properties

The content of saturated and unsaturated fatty acids, respectively, will additionally influence upon the structural properties of the fat globules. This is explained by the fact that unsaturated fatty acids consist of at least one double bond, thus have a more bendable structure than saturated fatty acids, with no double bonds and a more rigid structure. A larger amount of unsaturated fatty acids will result in a softer structure of the fat globule and vice versa. A softer or harder structure of the globule will probably have an effect on the structure of the final product. This since the casein network is dependent of the structure of the fat globules by its interaction with other components such as milk serum, minerals etc. when a gel network is formed (Michalski *et al.*, 2007).

6.2 Cheese yield

Even though different fats were used there was no significant difference in the final yield of the cheese (Table 8). The average cheese yield with fat C was lower than the others, but the difference was not statistically significant which might be due the higher variations among samples of fat B and D. However, there were notable differences in the manufacturing process of the cheeses that could have affected the yield. The water-bath cooling after the press made the cheeses of all fats, increase in weight. As seen in table 8, the increase in weight after the cooling of the cheese is due to water absorption. The increase in weight was significantly lower for the cheese produced with fat C (0.28±0.08 %) compared to the cheese produced with fat B, which showed the highest weight increase (6.06±0.89 %). Yet, the moisture content of the fat C cheese was only significantly lower than the fat A cheese and not drastically different to the cheeses produced with fat B, D or milk fat. During processing of fat C cheese the water will be encapsulated in the curd, which might be explained by the globules beginning to crystallize early in the process because of the temperature decrease. The crystals together with the caseins are probably creating a stiffer network hence the whey will not be released to the same extent during pressing. As a consequence, the weight increase will not be registered at the final weighing, because it already had occurred during the curd formation.

If the cheese not had been subjected to the water bath cooling, but air cooling, the yields would perhaps have been more even comparing the different types of cheese. However, it would result in lower water contents in the cheeses that increased during water bath cooling.

6.3 pH and composition of whey and cheese

The pH during the process and in the final product did not vary much (Figure 4; Table 10) despite differences in properties, e.g. hardness. If pH had varied more between the different types of cheeses it would perhaps had an effect on the yield and the structure of the casein network. If affecting the structure, it would probably also affect the retention of water (IDF, 1994).

The compositional analysis of the whey and the cheese showed that there were no significant differences in content and losses of fat and protein between the types of cheese. This indicates that the type of fat does not play a role in how much fat and protein in the cheese milk that will end up in the curd and finally in the cheese. The protein content was determined with Kjeldahl method with conversion factor for mixed foods (6.25). In the case of milk and milk products, a conversion factor of 6.38 is usually applied, and the usage of 6.25 results in a underestimation of the protein content (EC, 2005). The water content differed significantly between cheeses produced with fat A (44.07 %), fat B (39.53 %) and C (39.1 %), (Table 10). One explanation for this might be the early crystallization of fat C, with no absorption of additional water during the 24 h water bath cooling.

The average recovery of fat and protein did not differ significantly between the types of cheese. However, the average fat recovery of the fat C cheese was the highest while the protein recovery for the same cheese was the lowest (Table 11). The assumption was that cheese from fat C would have a lower fat recovery than the others, because of the higher crystallization temperature of fat C. The assumption was based on the fact that fat crystals would stick in the homogenizer or pasteurizer if the temperature not was kept above 60˚C during the mixing of the fat emulsion, without ending up in the cheese vat. The average protein recovery was between 66.36 % and 71.66 %. The protein network is mainly built up by casein and therefore it would have been interesting to investigate the casein-/whey protein ratio in whey and in the corresponding cheese. One could imagine that the cheese made of fat C, which presumably encapsulates whey when the globules crystallize in the cheese vat, would contain more whey protein. At the same time the fat C cheese has the lowest protein recovery (Table 11), which is contradicting this theory. The recovery might have differed more between the different types of cheeses and between the replicates if not using standardized contents of the cheese, since variations still can occur in standardized milk. The calculations with standardized contents could have resulted in either a higher or lower recovery than the cheeses truly had.

The similarity between results, or at least not significantly different values, for the content of different components (except the water content) indicates a reasonable reproducibility in the process of making the different types of cheese. If further tests will be performed with these or similar fats using the same equipment, it would probably not be a problem to reproduce similar cheeses.

6.4 Texture properties

6.4.1 Hardness

The cheese produced with fat C was harder and could withstand a significantly higher force than the other cheeses (Figure 5). This is explained by the high amount of saturated fatty acids (99.8 %) of fat C and an effect of its structural properties. More saturated fatty acids give a more rigid structure to the fat globules, and at the temperature of analysis (18˚C), almost all fat in the fat C cheese is fully crystallized according to the solid fat content curve (Figure 3). The rigid fat globules will also make it difficult for the other components of the network to perform any movement when force is applied to the cheese. A more rigid structure of the globules explains the differences among the cheeses, since type of fat is the only parameter that differs in their recipes. The theory is confirmed by the results for the cheeses of fat B and D, which have the highest proportions of unsaturated fatty acids, withstanding the lowest forces. Results for cheeses from fat B and D are similar to the ones from fat A and milk fat (Figure 5). Fat A, also containing a high amount of saturated fatty acids (99.0 %) does not withstand as high force as fat C. This is explained by the fact that at the analyzing temperature of 18˚C, it has a solid fat content of just approximately 30 % (Figure 3).

6.4.2 Springiness

Springiness is a measure of the extent to which the sample physically returns to its original height after the first compression. The cheese made of fat D showed to be the most elastic, because it went back to an average of 98.8±26 % of the original height after the first compression (Figure 6). However, it also had the largest fluctuations in springiness between replicates, raising the question if results are reliable. Fat B showed the second highest springiness (92±4.12 %) which indicates higher springiness in cheeses produced with fat higher in unsaturated fatty acids. The springiness of the cheeses produced with milk fat (84.9±2.8 %) and fat C (84.2±2.62 %) (Figure 6) was, however not significantly lower. Only the cheese made from fat A showed a significantly lower springiness than the cheese made of fat D.

6.4.3 Cohesiveness

Cohesiveness explains how well a sample withstands a second deformation relative to the first compression, and how well the material holds together. Cheeses made of fat B and D showed a significantly higher cohesiveness and held together better after the compressions than cheeses made of milk fat, fat A and C (Figure 7). This might be explained by the more flexible fat globules in fat B and D, due to the higher content of unsaturated fatty acids, which with a similar mechanism as for the springiness, these cheeses retain their form after the deformation. On the other hand fat globules with higher content of saturated fatty acids create a more stable, but more brittle network.

6.5 Thermal properties

DSC demonstrated that the fats incorporated in the cheese had the same melting and crystallizing patterns as the pure fat. The calculated water content of the cheeses from the crystallization peak of the spectrum (Appendix I) showed a lower content than the drying and gravimetrical method used in the composition analyses of the cheese. The fat contents obtained by DSC for fats A (23.77±1.42 %) and C (29.43±0.83 %) (Table 13) were in the same range as the contents obtained from the gravimetrical method (fat A 25.5±0.36 %; fat C 28.13±1.80 %), (Table 13). Since DSC is a rapid method when the equipment is at hand, the water and fat contents obtained by DSC can act as guidance to see if the manufacture was performed correctly.

Table 13. *Average water and fat content obtained by Differential Scanning Calorimetry (DSC) calculated from 9 measurements (3 measurements of each replicate of cheese type) and average water and fat content from the composition analysis calculated from the 3 productions of each type*

Fat (n=9)	Water (%) (DSC)	Water (%)	Fat (%) (DSC)	Fat (%)
A	32.65±3.60	44.07±0.81	23.77±1.42	25.50±0.36
B	31.53±4.09	39.53±2.10	N.D.	25.67±2.76
C	28.28±1.86	39.1±2.21	29.43±0.83	28.13±1.80
D	28.80±6.24	41.27±1.77	N.D.	24.73±1.16
AMF	33.09±1.19	43.40±0.75	N.D.	25.06±0.75

7 Conclusions

Cheese and cheese analogues were made where the only difference was the type of oil and fat used. One of the cheeses was made with milk fat and the other four with different vegetable oils and fats. The influence of oil and fat source on cheese properties was studied. All five cheeses where produced at three different occasions, and yield, composition, texture and thermal properties were analysed, giving rise to the following conclusions:

- Regarding fat and protein content of the cheeses it was possible to produce similar kind of cheeses, using different kinds of fat.

- Using vegetable fat in cheese production did not show significant differences in cheese yield compared to cheese made with milk fat, and there were no significant differences between any of the tested vegetable fats. To increase the yield, the process probably has to be optimized in respect to fat rather than solely substituting ingredients.

- Functional properties, e.g. hardness of the cheese will differ greatly depending on the type of fat used. The fat with the highest melting point resulted in the hardest cheese. This shows that the rheological properties (i.e. hardness) of the fat globules greatly affect the texture of the cheese. This is consistent with the assumption that most of the fat is in emulsion form, that globules in the casein network are closely packed and if they are rigid they greatly affect the texture. The liquid fats formed softer textures, suggesting that they did not contribute much to the overall strength of the cheese.

In order to eventually implement any of these vegetable fats and oils, or mixes of them, into cheese production more analyses should be performed. The cheeses should be subjected for a sensory analysis in order to obtain the whole spectra of distinct sensory properties that change, using different kinds of fat.

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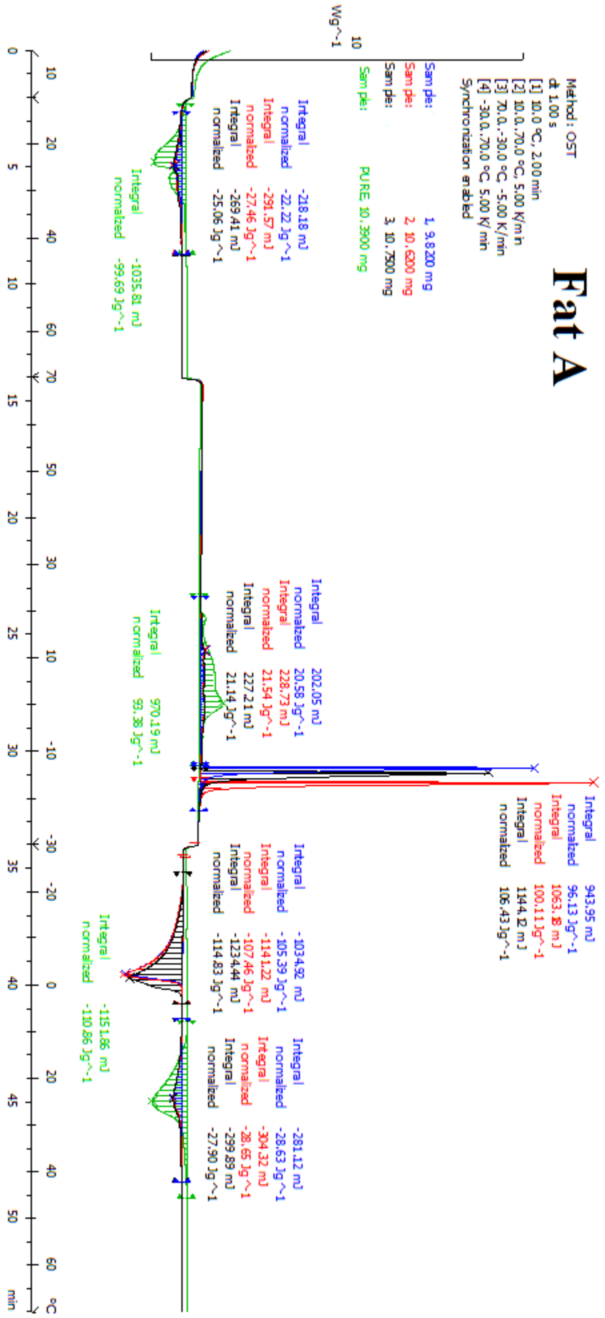
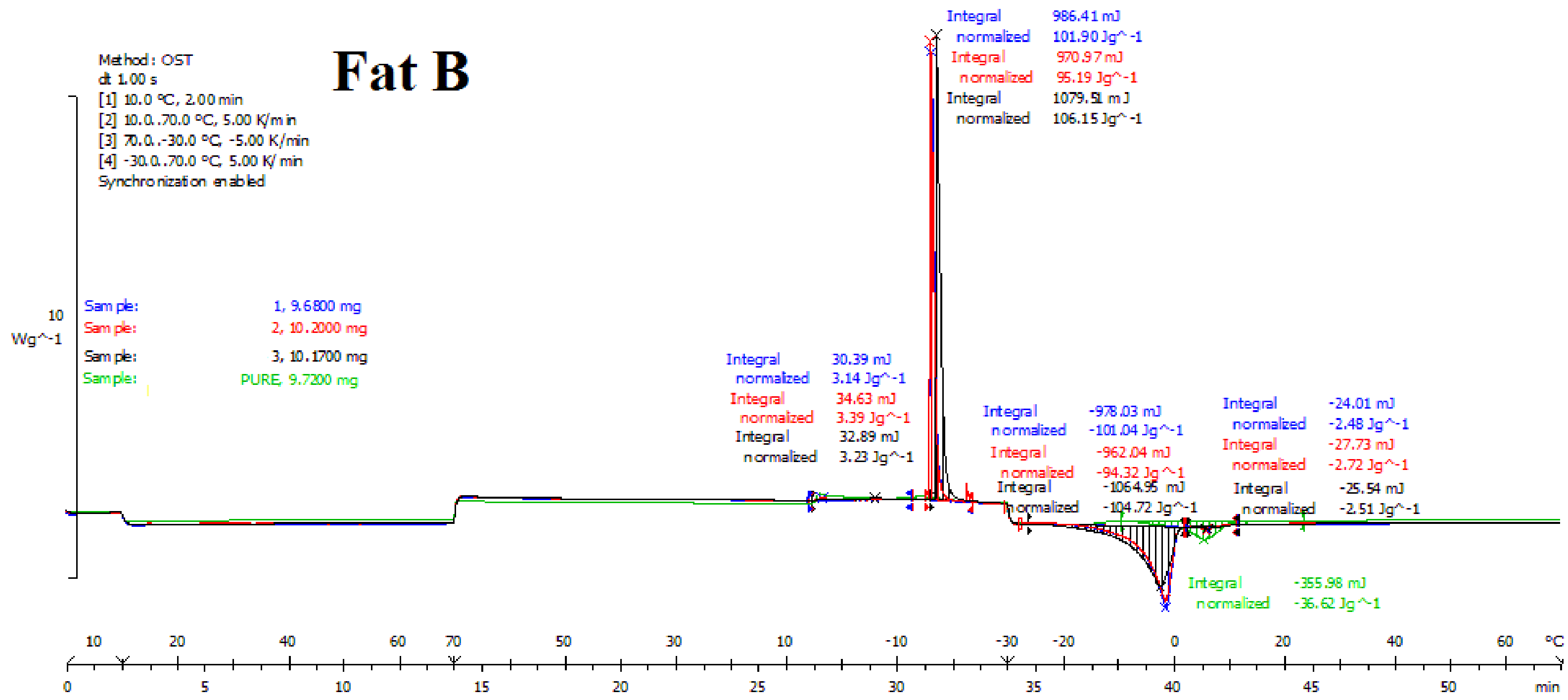
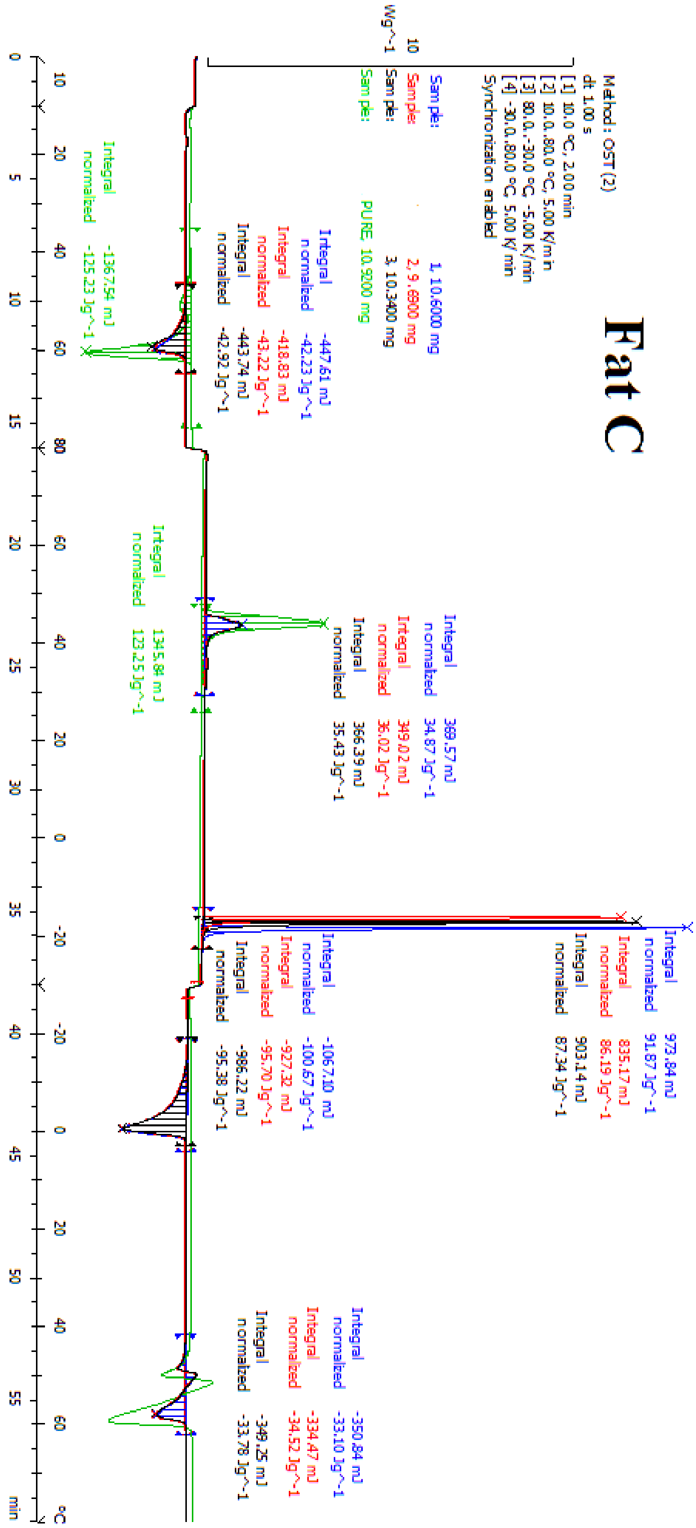
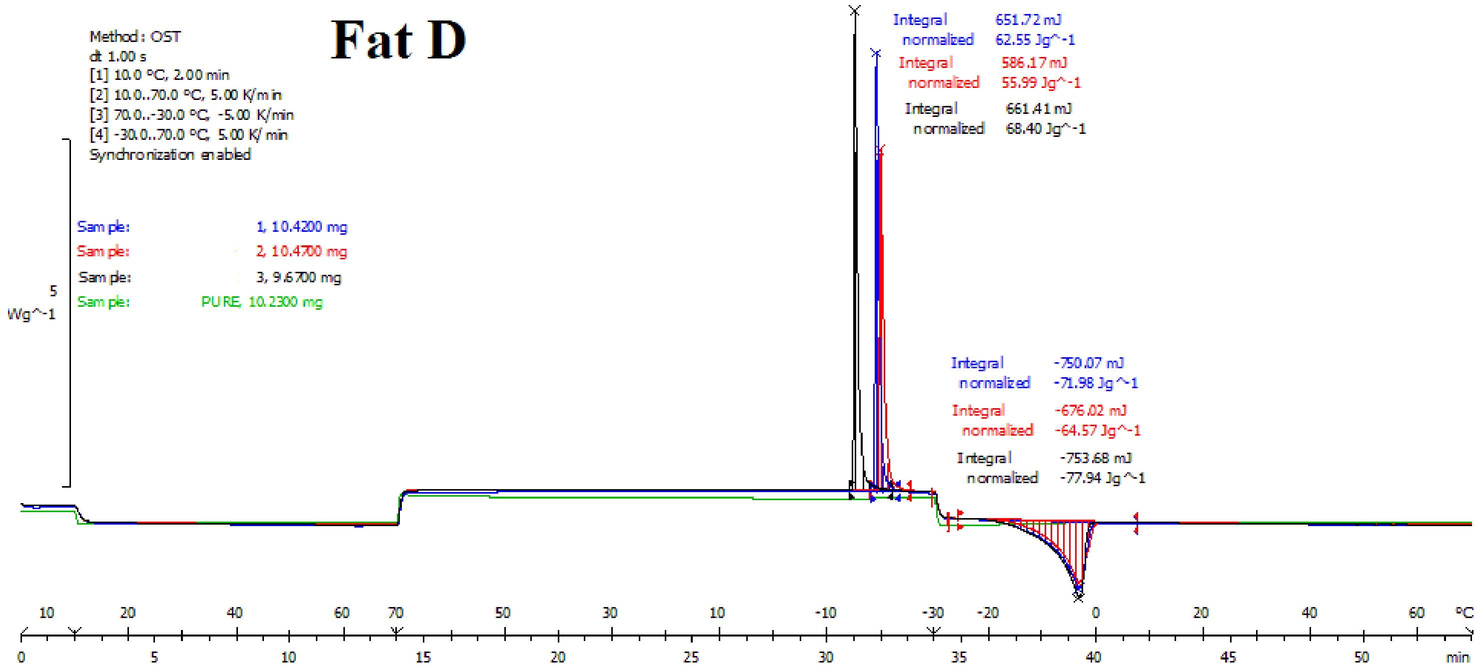
Appendix I: DSC spectrums

This appendix includes figures of DSC spectrums from cheese made of fat A, B, C, D and AMF. The blue, red and black lines and texts represent the 3 replicates of the cheese sample and the green line and text represents the pure fat.

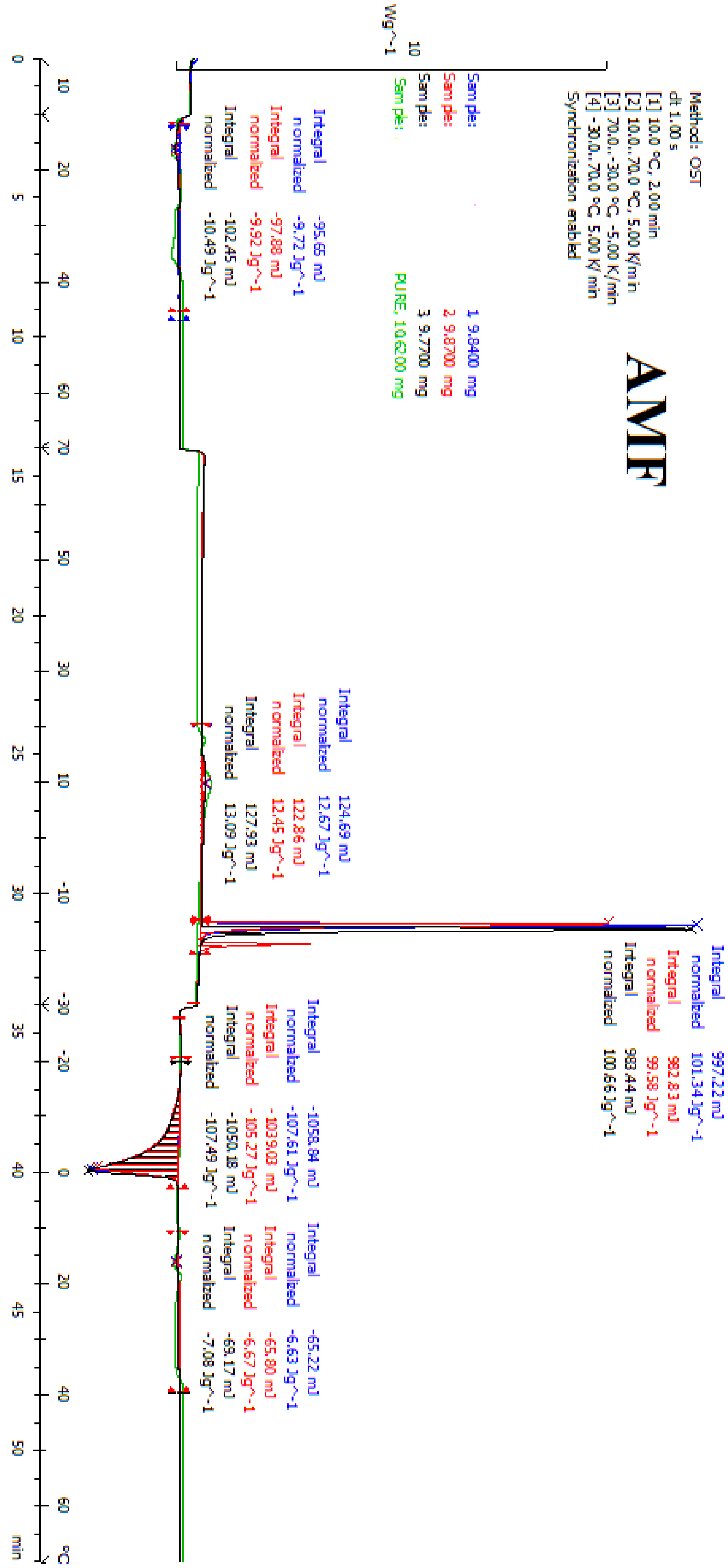



Fat B
Method: OST
dt 1.00 s
[1] 10.0 °C, 2.00 min
[2] 10.0..70.0 °C, 5.00 K/min
[3] 70.0..-30.0 °C, -5.00 K/min
[4] -30.0..70.0 °C, 5.00 K/min
Synchronization enabled
10
Wg^-1
Sample: 1, 9.6800 mg
Sample: 2, 10.2000 mg
Sample: 3, 10.1700 mg
Sample: PURE, 9.7200 mg
Integral 30.39 mJ
normalized 3.14 Jg^-1
Integral 34.63 mJ
normalized 3.39 Jg^-1
Integral 32.89 mJ
normalized 3.23 Jg^-1
Integral 986.41 mJ
normalized 101.90 Jg^-1
Integral 970.97 mJ
normalized 95.19 Jg^-1
Integral 1079.51 mJ
normalized 106.15 Jg^-1
Integral -978.03 mJ
normalized -101.04 Jg^-1
Integral -962.04 mJ
normalized -94.32 Jg^-1
Integral -1064.95 mJ
normalized -104.72 Jg^-1
Integral -24.01 mJ
normalized -2.48 Jg^-1
Integral -27.73 mJ
normalized -2.72 Jg^-1
Integral -25.54 mJ
normalized -2.51 Jg^-1
Integral -355.98 mJ
normalized -36.62 Jg^-1
10 20 40 60 70 50 30 10 -10 -30 -20 0 20 40 60 °C
0 5 10 15 20 25 30 35 40 45 50 min


Fat C
Method: OST (2)
dt 1.00 s
[1] 10.0 °C, 2.00 min
[2] 10.0..80.0 °C, 5.00 K/min
[3] 80.0..-30.0 °C, -5.00 K/min
[4] -30.0..80.0 °C, 5.00 K/min
Synchronization enabled
Sample: 1, 10.6000 mg
Sample: 2, 9.6900 mg
Sample: 3, 10.3400 mg
Sample: PURE, 10.9200 mg
Wg^-1
10
Integral -447.61 mJ
normalized -42.23 Jg^-1
Integral -418.83 mJ
normalized -43.22 Jg^-1
Integral -443.74 mJ
normalized -42.92 Jg^-1
Integral -1367.54 mJ
normalized -125.23 Jg^-1
Integral 369.57 mJ
normalized 34.87 Jg^-1
Integral 349.02 mJ
normalized 36.02 Jg^-1
Integral 366.39 mJ
normalized 35.43 Jg^-1
Integral 1345.84 mJ
normalized 123.25 Jg^-1
Integral 973.84 mJ
normalized 91.87 Jg^-1
Integral 835.17 mJ
normalized 86.19 Jg^-1
Integral 903.14 mJ
normalized 87.34 Jg^-1
Integral -1067.10 mJ
normalized -100.67 Jg^-1
Integral -927.32 mJ
normalized -95.70 Jg^-1
Integral -986.22 mJ
normalized -95.38 Jg^-1
Integral -350.84 mJ
normalized -33.10 Jg^-1
Integral -334.47 mJ
normalized -34.52 Jg^-1
Integral -349.25 mJ
normalized -33.78 Jg^-1
0 5 10 15 20 25 30 35 40 45 50 55 min
10 20 40 60 80 60 40 20 0 -20 -20 0 20 40 60 °C



Fat D
Method: OST
dt 1.00 s
[1] 10.0 °C, 2.00 min
[2] 10.0..70.0 °C, 5.00 K/min
[3] 70.0..-30.0 °C, -5.00 K/min
[4] -30.0..70.0 °C, 5.00 K/min
Synchronization enabled
Sample: 1, 10.4200 mg
Sample: 2, 10.4700 mg
Sample: 3, 9.6700 mg
Sample: PURE, 10.2300 mg
5
Wg^-1
Integral 651.72 mJ
normalized 62.55 Jg^-1
Integral 586.17 mJ
normalized 55.99 Jg^-1
Integral 661.41 mJ
normalized 68.40 Jg^-1
Integral -750.07 mJ
normalized -71.98 Jg^-1
Integral -676.02 mJ
normalized -64.57 Jg^-1
Integral -753.68 mJ
normalized -77.94 Jg^-1
10 20 40 60 70 50 30 10 -10 -30 -20 0 20 40 60 °C
0 5 10 15 20 25 30 35 40 45 50 min



AMF
Method: OST
dt 1.00 s
[1] 10.0 °C, 2.00 min
[2] 10.0..70.0 °C, 5.00 K/min
[3] 70.0..-30.0 °C, -5.00 K/min
[4] -30.0..70.0 °C, 5.00 K/min
Synchronization enabled
Sample: 1 9.8400 mg
Sample: 2 9.8700 mg
Sample: 3 9.7700 mg
Sample: PURE, 10.6200 mg
Wg^-1
10
Integral -95.65 mJ
normalized -9.72 Jg^-1
Integral -97.88 mJ
normalized -9.92 Jg^-1
Integral -102.45 mJ
normalized -10.49 Jg^-1
Integral 124.69 mJ
normalized 12.67 Jg^-1
Integral 122.86 mJ
normalized 12.45 Jg^-1
Integral 127.93 mJ
normalized 13.09 Jg^-1
Integral 997.22 mJ
normalized 101.34 Jg^-1
Integral 982.83 mJ
normalized 99.58 Jg^-1
Integral 983.44 mJ
normalized 100.66 Jg^-1
Integral -1058.84 mJ
normalized -107.61 Jg^-1
Integral -1039.03 mJ
normalized -105.27 Jg^-1
Integral -1050.18 mJ
normalized -107.49 Jg^-1
Integral -65.22 mJ
normalized -6.63 Jg^-1
Integral -65.80 mJ
normalized -6.67 Jg^-1
Integral -69.17 mJ
normalized -7.08 Jg^-1
min
°C

Popular summary

The cheese industry is in constant development to increase productivity, prolong the shelf-life and to alter characteristics of the cheese so that the occasions to consume cheese increases. One way to possibly achieve this is to replace milk fat with vegetable fats and oils in the manufacture of cheese.

This study has focused on productivity, to find out if it's possible to get out more or less cheese of the milk when using different kinds of vegetable fat, but also on cheese characteristics. To find out how and to what extent cheeses made of milk fat and different kinds of vegetable fat differs from each other. Four different vegetable fats, all with different characteristics were tested. Two of them were liquid at room temperatures while two were solid and had different melting temperatures. One milk fat product was used to see how an "ordinary" cheese behaved in a vegetable fat cheese production. From each kind of fat, three cheeses were produced.

The vegetable fat cheese in this study was produced by first mixing non-fat milk with vegetable fat so the cheese milk resembles ordinary cheese milk. At right temperature an active culture containing good bacteria was added that will begin to make the cheese milk sour. To the milk, rennet, a coagulation enzyme, was added to get the proteins of the milk to begin to attract to each other and forming a network, also called a cheese curd. Trapped in this network were fat, proteins, minerals such as calcium, and water. Whey, which is the water soluble proteins and water will be drained off when the cheese curd is cut. The whey was drained of letting the cheese curd which contains protein, fat, minerals and water, to be pressed to a cheese. The cheese was put in water to cool down, followed by salt water to add texture, flavour and protection against microbes to the final cheese. The cheeses were put in to storage for about 20 days.

To find out the yield of different cheeses, whether it's possible to get out more or less cheese of the milk when using the different kinds of fat, all cheeses were weighed, first before the cooling of the cheese and then after the storage, and compared to the original weight of the milk. With these weights, average yields of all cheese types could be determined and shown to not differ from each other, when using the same procedure for each fat.

Texture of the cheese, was studied with a specific instrument for measuring texture of materials by compressing them two times. These two bites represent the two first bites in chewing a food. The texture was shown to vary depending on which fat that was used in the production. When measuring the hardness of the cheeses, meaning how much pressure the cheeses could handle before collapsing, it was shown that the vegetable fat with highest melting point to be the hardest. All cheeses were shown to be elastic. They all had the possibility to keep a majority of their height after a compression. The cheeses produced with fats liquid at room temperature hold together better after a compression than cheeses produced with solid fats.

I was interesting to study how the fat in the cheese would behave in the mouth in respect to temperature. How the fats and the cheeses reacted to temperatures were examined using an instrument measuring how much the energy vary in different reactions that consequently happen at increasing and decreasing temperatures. The fats present in the cheeses reacted in the same way as it did in pure fat form.

The results showed that with the specific manufacturing procedure of this study no differences in yield could be shown and that the characteristics of the cheeses is by far depended on the characteristics of the fat used in the production. This means that it is a possibility to produce cheese with vegetable fat to increase productivity.