Variation of terpenes in milk and cultured cream from Norwegian alpine rangeland fed and in-door fed cows

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Abstract

The terpene content of milk and cream made from milk obtained from cows fed indoor, and early or late grazing in alpine rangeland farms in Norway were analysed for three consecutive years. The main terpenes identified and semi-quantified were the monoterpenes β-pinene, α-pinene, α-thujene, camphene, sabinene, δ-3-carene, D-limonene, γ-terpinene, camphor, β-citronellene, and the sesquiterpene β-caryophyllene. The average total terpene content increased five times during the alpine rangeland feeding period. The terpenes α-thujene, sabinene, γ-terpinene and β-citronellene were only detected in milk and cultured cream from the alpine rangeland feeding period and not in samples from the in-door feeding period. These four terpenes could be used as indicators to show that milk and cultured cream descend from the alpine rangeland feeding period. The terpenes did not influence the sensorial quality of the milk or the cultured cream.

Key words: alpine rangeland, grazing, terpenes, milk, cultured cream

1. Introduction

Terpenes are lipophilic aliphatic volatile compounds with the general chemical formula $(C_5H_8)_n$ particularly present in herb-like plants and synthesized and stored in plant vegetative organs (Croteau, 1987). More than 20,000 individual terpenes have been described (Connolly & Hill, 1991). The terpenes are important for the plant resistance to predations and infection (Croteau, 1987).

Terpenes constitute main components of essential oils, normally with certain aromatic properties designated as for instance “fresh”, “herbaceous”, “resin”, “lemon”, “coniferous”, “green/grassy”, “mint/chlorophyll” and “thyme/oregano” (Burt, 2004; Mariaca et al., 1997; Tornambé et al., 2008; Urbach, 1990). The content of terpenes in the forage will vary according to its plant composition (Fernández-García, Serrano, & Nuñez, 2002; Galina, Osnaya, Cuchillo, & Haenlein, 2007). Terpene content also varies depending on the stages of maturity of the plant and changes with change in environmental condition (Bugaud et al., 2000; Cornu et al., 2001; Mariaca et el., 1997; Tornambe et al., 2006). Chion et al. (2010) concluded that the milk produced on pasture obtained higher contents of terpenes than the milk from winter diets based on hay. The terpene molecules from plants in general appears in the rumen within 24h of grazing and then in raw milk used in the preparation of dairy products (Lejonklev et al., 2013). Terpenes have the potential to be used as markers to differentiate if the milk or cheese originate from from the herds fed on any grazing system or fed in-door (Cornu et al., 2001; Favaro, Magno, Boaretto, Bailoni & Mantovani, 2005; Viallon et et., 1999; Viallon et al., 2000). Morand-Fehr, Fedele, Berger, Le Du, & Spinnler (2007) claimed however that it is not easy to use terpenes as proof of different kinds of diets for sheep and goats.

Reports indicate that terpenes may also be formed by microorganisms in the milk or in the milk products and that terpenes may be changed as a result of microbial activity (Agrawal & Joseph, 2000; Fernández-García et al., 2002; Martin, Berger, Le Du, & Spinnler, 2001).

The objectives of this work was to compare the presence of terpenes in cow's milk from seven different farms produced at alpine rangeland in Norway with the presence of terpenes in milk from the in-door feeding period for the same farms. Furthermore, this study reports for the first time the presence and amounts of terpenes in cultured cream made from the milk collected from alpine rangeland farms. This study also evaluated if the content of terpenes in milk and cultured cream from alpine rangeland feeding period could be used to distinguish this milk and cultured cream from samples collected and produced during the in-door feeding period.

2. Materials and methods

2.1. Design of the investigation

Milk was sampled from seven farms situated in the area of Valdres, an alpine region situated in the central, southern part of Norway during three subsequent years (2007-2009). The selection of farmers to attend the investigation was done on the basis of several criteria, among these was that they should not practise concentrated calving. The average number of cows in the seven herds investigated was 14.4.

The summer farms are situated relatively close to each other at an altitude of approximately 900 m a.s.l., in the northern boreal vegetation zone. In this part of Norway this means close to the tree line. The bedrock in the area is phyllite, a schist-rich bedrock with a high weathering capacity giving soils of intermediate or good nutritional quality known to give species rich ranges. Sickel, Bilger, & Ohlson (2012) has investigated the wild grazing plant species in the same area in July and August 2009.

During the winter, the in-door feeding period, a standard feeding regime with conserved green fodder and concentrate was practiced at all farms included in the investigation. During summer-farming in the mountain the herds were grazing wild alpine plants during the day between morning milking and evening milking. After evening milking six of the herds were grazing in enclosure fields surrounding the summer farms. In one of the summer-farm (farm no. 3 in the results chapter) the herd was grazing wild alpine plants in the outlaying fields also during the night.

2.2 Milk sampling

During the in-door feeding period milk was sampled from each of the seven farms at calendar day no. 67 and 84 in year 1 and day no. 87 in year 3 of the investigation period. The first sampling during the summer-farming period in the alpine rangeland took place approximately one week after the start of summer-farming, in order to avoid any carry over effect from the feeding regime practiced until then. One sampling day (calendar day no. 191 in year 1, day no. 197 in year 2 and day no. 197 in year 3) in the early summer-farming period was practiced. In the late summer-farming period sampling took place four times (calendar days no. 220 and 248 in year 1, day no. 232 in year 2 and day no. 232 in year 3).

At each farm the milk was collected during three days before the milk sampling day and kept in a cooling tank (< 4 ºC) at the farm until it was collected and transported under refrigerated conditions to the central laboratory of the Norwegian dairy company TINE for sensorial grading and other quality analysis according to the internal control procedure of TINE. One milk sample from each farm at each sampling day was stored in plastic bottles at -80 °C until the chemical analysis performed at Nofima.

Milk for the production of cultured cream was collected from all seven farms and mixed in the same compartment on the milk tank lorry, and transported the day of collection to the pilot plant for food production at the Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences.

2.3. Production of cultured cream

Cultured cream was made twice from milk produced during the in-door feeding period and three times during the alpine rangeland feeding period for one year (2007). The production dates during the alpine rangeland feeding period were in the beginning of July, in the beginning of August and in the beginning of September. Cream from each day of milk delivery was divided into four batches of cream. Each batch was used for production of cultured cream, as four parallel productions.

The milk was separated in a Westfalia separator, type MS050-01-076 (Westfalia Separator AG, D 4740 Oelde, Germany) at approximately 60 °C. The cream was pasteurised at 74-75 °C in a plate heat exchanger (Alfa Laval, type M6-MFMC, Alfa Laval, Lund, Sweden) and cooled to < 4 °C. For each production day four batches of 4 L cream (38 % fat) were homogenized at 60 °C and 30 bar in a Rannie Homogenizer (type 16.50) (APV, Oslo, Norway). The homogenized cream was further heated to 90 °C for 5 minutes, cooled to the incubation temperature 20 °C before inoculation with 50 mL of a bulk starter of lactic acid bacteria (mesophilic mixed strains starter CHN-19 from Chr. Hansen, Hørsholm, Denmark). After thorough mixing of the inoculums in the cream the cream was distributed into disinfected plastic cups (200 mL) with lids. The cups were incubated at 20 °C in a temperature regulated water bath until pH 4.5-4.6 (approximately 18 hours after inoculation) and transferred to refrigerator room (2-4 °C) for storage. The cultured cream was analysed for the content of various terpenes and for sensorial quality 2-3 days after end of incubation (fresh product) and after approximately 3 weeks, which is the commercial shelf life of this type of products in Norway (stored product). Samples for analyses of terpenes were stored in plastic cups at -80 °C in darkness.

2.4. Analyses of terpenes

Frozen milk samples (bottles containing approximately 300 mL) were thawed at 4 °C in darkness overnight, resulting in a creamy upper layer. Samples of the lipid enriched layer was weighed into ultracentrifuge tubes and centrifuged at 28700 rpm (100.000 x *g* max, Ti 50.2 rotor) in a Beckman L-80 ultracentrifuge (Beckman Coulter Inc., Palo Alto, CA, USA) for 2 h at 26 °C. The milk lipid phase (yellow layer) was transferred by a Pasteur pipette to a 2 mL tube and re-centrifuged at 13000 rpm (16000 x *g*) in a Hereaus Biofuge Fresco (DJB Labcare Ltd., Buckinghamshire, England) to remove the last trace of water/protein phase contaminants. The lipid sample was stored in filled screw capped tubes at -80 °C until GC-MS analysis.

Milk and cultured cream fat samples were thawed to ambient temperature, and 0.400 g was weighed into 50 mL glass tubes with 7 mL Milli-Q water. Internal standard, 1 μL of a 40 μg/mL ethyl heptanoate (> 99 %, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) solution in methanol was added to each sample. The tubes were placed in a thermostat regulated water bath with a temperature of 70 °C. The volatiles from each milk fat sample were extracted by purging with ultrapure nitrogen gas, 100 mL min^{-1}, through a modified Drechsel head connected to a stainless steel tube packed with an adsorbent resin Tenax GR (mesh size 60/80, Alltech Associates Inc. Deerfield, IL, USA). Adsorbed water was removed by nitrogen flushing (50 mL min^{-1}) for 5 min in the opposite direction of sampling at room temperature. Three replicates were analysed of each sample. Trapped compounds were desorbed at 250 °C for 5 min in a Markes Thermal Desorber (Markes, Liantrisant, UK) and transferred to an Agilent 6890 GC System (Agilent Technologies, Inc. Wilmington, DE, USA) with an Agilent 5973 Mass Selective Detector (electron impact ionization mode; ionization energy, 70 eV). The volatiles were separated on a DB-WAXetr column (0.25 mm i.d., 0.5-µm film thickness, 30 m, J&W Scientific), the carrier gas was 99.9999 % helium and the gas flow was 1.5 mL/min. The temperature program was as following: 30 °C for 10 min, increasing by 1°C min^{-1} to 40 °C, by 3 °C min^{-1} to 70 °C, and by 6.5 °C min^{-1} to 230 °C, with a hold time of 5 min. Integration of peaks and identification of compounds were performed by using HP Chemstation software (G1701CA version C.00.00, Agilent Technologies), the Wiley 130K Mass Spectral Database (HP 61030A MS Chemstation, John Wiley and Sons Inc., Agilent Technologies), and NIST98 Mass Spectral Library (version 1.6d, US Secretary of Commerce, Gaithersburg, MD) and NIST/EPA/NHI Mass Spectral Library (NIST05) with NIST Mass Spectral Search Program for Microsoft Windows (version 2.0d, US Secretary of Commerce). Blanks and standard samples was run before, during, and after the sample series. The relative concentrations of individual terpenes were calculated based on the internal standard ethyl heptanoate and expressed as ng per g fat.

2.5. Sensorial evaluations

The sensorial quality of each milk sample was evaluated by three accredited milk graders in the TINE dairy company, according to a hedonic scale from 1 to 5, with 5 as best score (quality scoring). Grading with 3 or lower should be followed by a comment according to the nomenclature given by International Dairy Federation (1987, 1997).

The cultured cream was evaluated by 11 assessors. The overall quality of the samples was graded by quality scoring as mentioned above. Descriptive sensory analyses was used for the evaluation of the flavour attributes “aromatic”, “oxidized”, “rancid” and “off flavour”. A scale from 1 to 7 with increasing intensity of the attribute was used. The experimental cultured cream was compared with a commercial sample of cultured cream with the same fat content from TINE dairy company, bought in a nearby retail shop the day before each evaluation. The expiring day for the commercial samples were always about 14 days later. If the attribute was graded similar in strength to the commercial sample the sample obtained the score 4.

2.6. Statistical analyses

The following model $y_{ijk} = \mu + \alpha_i + \lambda_j + e_{ijk}$ has been used for the statistical analyses presented in Tables 1-3. Here, μ is the overall mean content of a terpene, α_i is the effect of year *i* and λ_j is the effect of farm *j*. The measured content of a terpene in sample *k* with a feeding regime, year *i* and farm *j* is y_{ijk} and e_{ijk} is the error term related to this measurement. A Bonferroni test with a 5 % level of significance has been used to find differences between farms. The same model has also been used to compare the amount of various terpenes in the milk and in the cultured cream, but there λ_j is the effect of day *j*, 5 different days. The effect of milk is α_1 and the effect of cultured cream is α_2. It has been tested by the F-test if there are differences between the mean terpene content in milk and cultured cream. In Table 4 the model $y_{ij} = \mu + \alpha_i + e_{ij}$ has been fitted. Here α_i is the effect of feeding period *i (i* = 1 = in-door, *i* = 2 = early alpine rangeland and *i* = 3 = late alpine rangeland. It has been tested by the Bonferroni method at a 5% level of significance if cultured cream that is produced in different feeding periods has different mean terpene content.

3. Results and discussion

3.1. Terpenes in milk

The sum of terpenes in milk sampled from the in-door feeding period, early alpine rangeland feeding period and late alpine rangeland feeding period from the seven different Norwegian mountain farms in three years, is shown in **Figure 1.** The amounts of the individual terpenes from the same three feeding periods are shown in **Tables 1-3,** respectively.

The terpene profile of milk from the in-door and alpine rangeland feeding periods, showed the presence of several terpenes, and the main terpenes identified and semi-quantified were the monoterpenes β-pinene ($C_{10}H_{16}$), α-pinene ($C_{10}H_{16}$) α-thujene ($C_{10}H_{16}$), camphene ($C_{10}H_{16}$), sabinene ($C_{10}H_{16}$), δ-3-carene ($C_{10}H_{16}$), limonene ($C_{10}H_{16}$), γ-terpinene ($C_{10}H_{16}$), camphor ($C_{10}H_{16}O$), β-citronellene (syn:3,7-dimethyl-1,6-octadiene or dihydromyrcene) ($C_{10}H_{18}$), and the sesquiterpene β-caryophyllene ($C_{15}H_{24}$). Four of the 11 terpenes were detected only in the alpine rangeland milk, that is, sabinene, γ-terpinene, β-caryophyllene, and β-citronellene, indicating that the alpine pasture contributed to the presence of these compounds. The various terpenes identified are known compounds reported in earlier studies of milk and milk products (Abilleira et al., 2010; Di Cagno et al., 2007; Fernandez, Astier, Rock, Coulon, & Berdagué, 2003; Panseri et al., 2008).

The average of the mean terpene content from the in-door feeding period in the milk from the seven farms was 269 ng g^{-1} milk fat. The average content of total terpenes in the milk in this period varied between 52.6 and 177 ng g^{-1} milk fat in milk samples from six of the seven farms, while one of the farms (farm 3) delivered milk with a higher total content of terpenes, 1206 ng g^{-1} milk fat (**Table 1**). However, results on terpenes were available from only one in-door sampling day (day 67 in year 1) for this farm. The lowest value for terpenes observed in any milk sample from this feeding period was 11.8 ng g^{-1} milk fat. Since the amount of α-pinene dominated the total amount of terpenes in the samples, the differences in the total amount of terpenes were highly influenced by the amount of α-pinene present in the samples. Significantly higher amounts of four of the seven measured terpenes were present in the milk sample from farm 3 compared to the milk samples from the six other farms. No significant differences in the content of the individual terpenes in any of the milk samples from the six other farms were observed. Three of the seven terpenes (α-pinene, β-pinene and δ-3-carene) were detected in milk samples from all seven farms collected during the in-door feeding period. D-Limonene was detected in milk from six of the seven farms, while three of the terpenes, camphor, camphene and α-thujene, were detected in milk from five, four and two of the seven farms, respectively (Table 1).

The results presented in Tables 1-3 revealed that the average content of terpenes increased three times from the in-door feeding period (269 ng g^{-1}) to the early alpine rangeland feeding period when the average total content of terpenes in the milk from all the seven farms was 815 ng g^{-1} milk fat. The average terpene content increased further during the alpine rangeland feeding period, and was five times the level of the in-door period in the late alpine rangeland feeding period, with an average value of 1311 ng g^{-1} milk fat.

In the early alpine rangeland feeding period the average of the mean terpene content in the milk ranged from 444 to 1413 ng g^{-1} milk fat in the samples from the seven farms (**Table 2**). In addition to the terpenes registered in milk from the in-door feeding period, four more terpenes were detected in milk from the early alpine rangeland feeding period, namely; sabinene, γ-terpinene, β-citronellene, and β-cariophyllene. Camphor was observed in relatively minor amounts in samples from only two farms. **Table 2** shows the differences in the content of some terpenes among milk samples from various farms. Farm 3 delivered milk with significantly higher amount of α-pinene, α-thujene and camphene than farm 1, while farm 4 had milk with significantly higher amount of β-cariophyllene than farm 1. For the other terpenes analysed, no significant differences among milk samples from the seven farms from the early alpine rangeland feeding period were obtained. Again it is obvious that the significant differences in total amount of terpenes between the samples from the seven farms are dominated by the differences obtained for the content of α-pinene.

The average of the mean terpene content in the milk from the late alpine rangeland feeding period ranged from 820 to 2303 ng g^{-1} milk fat in the samples from the seven farms (**Table 3**). Also in milk from this feeding period the dominating terpene was α-pinene, which therefore also influenced the statistical differences in the total amount of terpenes between milk samples from the various farms. The same terpenes were detected in milk samples collected from the late alpine rangeland feeding period as in the samples from early alpine rangeland feeding period. In the late alpine rangeland feeding period camphor was observed in minor amounts only in samples from farms 4, 6 and 7, while this terpene was observed only in milk from farms 2 and 5 in milk samples from the early alpine rangeland feeding period. Farm 3 produced milk with significantly higher content of α-pinene and camphene than farms 1, 5 and 6. The milk collected from farm 6 had however lower content of camphene than the milk collected from farm 7. The milk samples from farm 3 had significantly higher amount of α-thujene than the milk delivered from farm 6. A comparison of the values in **Tables 2** and **3** and in **Figure 1** shows a very significant increase in the amount of the various terpenes from the in-door feeding period to the late alpine rangeland feeding period.

Differences in the amount of terpenes among milk samples from various farms and a significant increase in the amount of the various terpenes from early alpine rangeland feeding period to the late alpine rangeland feeding period is of particular interest. This indicates that the herds from different farms had access to different plant material when grazing in the alpine rangeland and that the availability or use of plant species high in terpenes was higher at the end of the season than in the beginning of the alpine rangeland feeding season. The particularly high amount of terpenes in the milk from farm 3 may be, at least partly, attributed to the fact that this herd was grazing wild alpine rangeland also during the night, contrary to the herd of the six other farms which had grazed, during the nights, in enclosure fields surrounding the summer farms. Several research groups have shown that diversified pasture forage including dicotyledons is rich in terpenes and that the content of terpenes will vary according to the maturity stage of the plants and environmental conditions. It has also been concluded that the presence of various terpenes in the milk can be expected to be directly linked to the terpenes available in the plants. The transfer of the terpenes from the fodder to the milk is also known to be very fast and may therefore influence the content of terpenes in the milk less than 24 hours after intake (Bugaud et al., 2000; Cornu et al., 2001; Lejonklev et al., 2013; Mariaca et al., 1997; Morand-Fehr et al., 2007; Tornambe et al., 2006).

3.2 Terpenes in cultured cream

Results from the analysis of various terpenes in the cultured cream manufactured from cream from the in-door feeding period, the early alpine rangeland feeding period and the late alpine rangeland feeding period are presented in **Table 4.**

Only three of the terpenes; α-pinene, δ-3-carene and D-limonene, could be found in recordable amounts in the cultured cream from the in-door feeding period. The content of α-pinene was significantly lower in cultured cream made from milk from the in-door feeding period than from the alpine rangeland feeding period. Opposite results were obtained for D-limonene which appeared in significantly higher amounts in cultured creams made from milk produced during the in-door feeding period than in products made from milk produced during the two alpine rangeland feeding periods. β-Cariophyllene could not be detected in products from the early alpine rangeland period, while all 10 terpenes analysed were present in cultured cream made from milk collected in the late part of the alpine rangeland feeding period. Camphor, present in the milk samples, was not present in any of the cultured cream samples. The content of α-pinene, α-thujene, β-pinene, sabinene, δ-3-carene, γ-terpinene, camphene and β-citronellene, were significantly higher in cultured cream made from milk produced in the late part of the alpine rangeland feeding period than in products from the early part of the alpine rangeland feeding period.

No statistical differences between the amount of terpenes between fresh cultured creams and stored cultured creams were observed. Therefore the results from both fresh and stored cultured cream samples are included in the averages given in **Table 4**. Expected differences in the total amount of terpenes were observed between cultured cream samples from all three feeding periods.

The fact that α-thujene, sabinene, γ-terpinene and β-citronellene were only detected in milk and cultured cream from the alpine rangeland feeding periods, leads to the possibility that these four terpenes could be used as indicators in order to prove that both milk and cultured cream containing these terpenes actually descend from the alpine rangeland feeding period. It might also be possible to use the total amount of terpenes as an indication of the feeding regime used for the milk production. Possible differentiation of the geographical origin of dairy products based on the presence of certain terpenes in both cows, ewes and goats milk cheese has been postulated by others (Chion et al., 2010; Cornu et al., 2001, Favaro et al., 2005; Fernandez et al., 2003; Viallon et al., 1999, 2000). Mohrand-Fehr et al. (2007) conclude however that it is not easy to use terpenes as a proof of the use of various diets for sheep and goats.

A comparison of the amount (ng g^{-1} fat) of various terpenes in the milk and in the cultured cream was done. For four of the terpenes analysed (α-pinene, α-thujene, sabinene and δ-3-

carene) the content was significantly higher in the cultured cream samples than in the milk with the ratios of 1.485, 1.208, 1.425 and 1.364, respectively. No reports seem to be published about the terpenes in cultured cream, and no clear explanation of such increase in the amount of these four terpenes can be given. Some reports indicate however that terpenes may be formed by microorganism in the milk or in milk based products (Fernández-García et al., 2002; Larsen (1998); Martin et al. (2001). None of these reports has however been studying possible production of terpenes by the mesophilic lactic acid bacteria used in the fermentation of cultured cream. It would therefore be of a certain interest to investigate if these varieties of lactic acid bacteria, or if lactic bacteria in general, are able to produce terpenes during their growth in milk or dairy products. To our knowledge such information is not available at present.

3.3. Sensorial evaluation of milk and fresh and stored cultured cream

The results from sensorial evaluation of all milk samples collected from the seven farmers during three years at various collecting days during the in-door feeding period and the two alpine rangeland feeding periods are presented in **Table 5.** The milk collected was in general of very high sensorial quality. Only a few samples had an off-flavour which could be characterised by the milk graders. Of the 67 milk samples only 4 obtained scores of 3 or lower. In these cases the graders characterised the samples as either "oxidized" or "bitter/sharp", comments not clearly related to the presence of terpenes.

The results from the sensorial evaluation of various product properties of the cultured cream; overall quality, aroma, oxidized and off-flavour for both fresh and stored cultured cream products are shown in **Table 6**. As described in section 2.5. "Sensorial evaluation", the various quality attributes graded were compared to the same attributes in a commercial sample of cultured cream. If an attribute was graded with similar score as the commercial sample, the attribute of the experimental sample obtained the score 4 on a scale from 1-7. This implies that if a sample obtained higher score than 4 for an evaluated attribute, the experimental sample had a stronger taste of the relevant attribute, or a better overall quality. The cultured cream manufactured from milk produced on day 67 (early in-door feeding period) had significantly better overall quality than the cultured cream that was manufactured from milk produced on day 191 and 248 (out-door feeding period). Storage of the cultured cream for three weeks at 4°C gave however the products a significantly less pronounced aroma and a somewhat stronger oxidized flavour. The storage of the samples gave no statistical significant increase in the intensity of rancid flavour or off-flavour.

A correlation matrix of sensory scores of the milk quality versus the content of the individual terpenes revealed that in no cases had the content of the analysed terpenes a significant influence on the score given for the milk quality. A similar calculation of the possible correlation of the sensory scores of the various product properties evaluated in cultured cream, and the amount of the individual terpenes analysed in these products gave the same result as for the milk. It can therefore be concluded that even the highest amounts of individual terpenes observed did not reach the threshold value for these terpenes either in milk or in cultured cream, in spite the fact that a number of the terpenes are known to be associated with specific flavours like for instance “green/grassy”, “mint/chlorophyll”, “thyme/oregano” and ”citrus-like” flavour in dairy products (Fedele et al., 2005; Nogueira et al., 2005; Tornambé et al., 2008; Urbach, 1990). However, the threshold values for the various terpenes identified seem to be unknown in milk and cultured cream. Since the amount of the individual terpenes was semi-quantified as ng per g fat, the amount of the terpenes in cultured cream was in fact almost 10 times higher than in the milk, based on the fact that the fat content in the milk will be approximately 4 % and the fat content in the cultured cream was adjusted to

38%. It is therefore of interest to observe that even these concentrations of various terpenes in cultured cream could be identified as neither positive nor negative by the graders. The lack of correlation between flavour and odour of dairy products and the amount of various terpenes in milk or dairy products is however in agreement with the findings of Coulon et al. (2004), Nogueira et al. (2005) and Viallon et al. (1999).

4. Conclusions

This study reports for the first time, terpene content in Norwegian milk sampled during alpine rangeland feeding period. Average from seven farmers in a three year period showed a 3 fold increase in the total terpene content during the early alpine rangeland feeding period and a 5 fold increase in the late alpine rangeland feeding period, compared to the in-door feeding period. The sensorial score of the milk collected was in general very high, indicating that the level of terpenes did not influence the milk quality negatively. The content of terpenes in cultured cream manufactured from the milk followed the same tendency as the milk, with increasing terpene content during the alpine rangeland feeding period. However, D-Limonene content was highest in the in-door feeding period, and D-limonene content was similar and ten-fold lower in the cultured cream from early and late alpine rangeland feeding periods. Fresh cultured creams and three weeks stored cultured creams had the same amount of terpenes. Some of the terpenes could be used as indicators for alpine rangeland milk and cultured cream from this region of Norway.

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Figure captions

Figure 1. Total amount of analysed terpenes in milk samples from in-door feeding (black column), early alpine rangeland feeding (grey column) and late alpine rangeland feeding (white column) from seven different farms in three years. The amounts of terpenes are given as ng g^{-1} fat. Error bars are shown as standard error of mean.

*Table 1-6

TABLES

Table 1
Terpene content in milk (ng g^{-1} fat) sampled from in-door feeding period (day no. 67, 84 and 87) from seven different farms for two years.

Compound	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7
α-Pinene	65.5[a] (60.4[b]) B[c]	53.8 (46.8)B	786 (-[d])A	18 (13.8)B	11.8 (6.38)B	19.2 (9.98)B	52.5 (40.4)B
δ-3-Carene	27.1 (26.4)B	28.4 (14.2)B	193 (-)A	28.3 (4.94)B	16.5 (8.64)B	5.98 (5.98)B	24.1 (21.4)B
β-Pinene	17.3 (8.98)B	10.9 (5.47)B	71.5 (-)A	6.22 (4.05)B	12.0 (6)B	0.04 (0.04)B	11.0 (11.0)B
D-Limonene	41.3 (24.1)	69.2 (47.8)	79.2 (-)	45.8 (26.4)	32.2 (16.2)	21.4 (13)	n.d[e]
Camphor	6.5 (6.52)	6.3 (4.40)	18.2 (-)	6.35 (4.35)	n.d	6.02 (5.17)	n.d
Camphene	11.0 (5.58)	8.02 (8.02)	18.9 (-)	n.d	2.76 (2.76)	n.d	n.d
α-Thujene	n.d	n.d	39.1 (-)A	8.67 (8.67)B	n.d	n.d	n.d
Total	169 (100)B	177 (111)B	1206 (-)A	113 (26.0)B	75.3 (30.8)B	52.6 (30.1)B	87.6 (72.8)B

Footnotes:
[a] The amounts of terpenes are given as ng g^{-1} milk fat based on the internal standard ethyl heptanoate, and the reported values are the means of three sampling days (calendar day 64 and 87 in year 1 and day 84 in year 3).
[b] Standard error of mean.
[c] Different letters indicate significant differences ($P < 0.05$), calculated by Bonferroni test.
[d] Farmer 3 had only one sampling from the in-door feeding period (day 67 in year 1).
[e] n.d, not detected.

Table 2

Terpene content in milk (ng g^{-1} fat) sampled from early alpine rangeland feeding period (day no. 191, 197 and 198) from seven different farms for three successive years.

Compound	Farm 1	2	3	4	5	6	7
α-Pinene	281 [a] (42.4 [b])B [c]	702 (49.5)AB	953(205)A	361 (112)AB	364 (56.6)AB	367 (14.3)AB	704 (226)AB
α-Thujene	17.9 (6.90)B	35.1 (14.4)AB	72.2 (31.3)A	24.0 (11.5)AB	31.4 (13.6)AB	30.2 (13.3)AB	51.9 (24.2)AB
Camphene	2.37 (1.57)B	8.86 (1.56)AB	27.0 (6.75)A	21.3 (7.58)AB	6.15 (1.51)AB	9.73 (3.93)AB	10.8 (5.11)AB
β-Pinene	16.3 (2.46)	38.0 (6.20)	41.4 (10.4)	30.7 (12.0)	17.3 (4.38)	18.6 (3.88)	34.4 (11.8)
Sabinene	20.0 (4.66)	43.3 (19.7)	86.4 (39.4)	26.3 (9.13)	32.1 (12.4)	22.9 (7.99)	69.6 (30.2)
δ-3-Carene	38.8 (21.4)	28.3 (3.90)	49.4 (20.8)	21.7 (10.9)	19.7 (9.53)	22.7 (11.1)	41.4 (8.86)
D-Limonene	30.4 (7.36)	49.5 (19.0)	82.2 (17.7)	53.7 (16.3)	52 (22.9)	27.4 (13.9)	34.4 (0.97)
γ-Terpinene	4.36 (2.25)	8.76 (2.54)	29.0 (6.53)	5.37 (2.68)	9.80 (1.19)	12.9 (8.80)	16.7 (5.51)
β-Citronellene	16.7 (8.83)	51.5 (0.36)	45.4 (22.9)	10.8 (10.8)	18.1 (9.40)	11.6 (6.49)	45.1 (22.9)
β-Cariophyllene	16.9 (7.70)B	26.4 (6.48)AB	26.5 (1.81)AB	73.7 (25.9)A	45.1 (11.5)AB	59.8 (12.4)AB	37.0 (2.01)AB
Camphor	n.d [d]	2.82 (2.82)	n.d	n.d	1.04 (1.04)	n.d	n.d
Total	444 (55.4)B	995 (33.8)AB	1413 (315)A	628 (117)AB	596 (81.7)AB	583 (20.0)AB	1045 (313)AB

Footnotes:

[a] The amounts of terpenes are given as ng g^{-1} milk fat based on the internal standard ethyl heptanoate, and the reported values are the means of three sampling days (calendar day 191 in year 1, day 197 in year 2 and day 197 in year 3).

[b] Standard error of mean.

[c] Different letters indicate significant differences ($P < 0.05$), calculated by Bonferroni test.

[d] n.d, not detected

Table 3
Terpene content in milk (ng g^{-1} fat) sampled late alpine rangeland feeding period (day no. 220, 231, 233 and 248) from seven different farms for three successive years.

Compound	Farm						
	1	2	3	4	5	6	7
α-Pinene	533[a] (103[b])B[c]	1020 (94.5)AB	1579 (246)A	927 (129)AB	473 (138)B	537 (155)B	979 (356)AB
α-Thujene	50.0 (20.8)AB	75.2 (27.2)AB	139.8 (44.5)A	86.1 (26.7)AB	53.6 (23.6)AB	41.6 (20.9)B	88.0 (34.5)AB
Camphene	7.28 (4.22)BC	17.2 (5.59)ABC	29.6 (4.69)A	16.0 (3.01)ABC	9.15 (5.43)BC	5.13 (3.96)C	21.0 (7.27)AB
β-Pinene	23.3 (6.20)B	52.8 (8.22)AB	70.0 (14.8)A	44.0 (6.37)AB	20.2 (7.56)B	25.4 (8.51)B	49.8 (18.6)AB
Sabinene	66.0 (29.7)	101 (41.8)	177 (49.0)	126 (45.1)	56.9 (24.6)	62.4 (37.8)	133 (55.5)
δ-3-Carene	30.2 (12.6)	38.1 (12.8)	64.5 (8.37)	31.9 (10.9)	36.2 (10.4)	29.8 (5.53)	42.9 (16.7)
D-Limonene	55.5 (24.5)	74.1 (26.2)	80.0 (15.9)	48.8 (18.6)	32.3 (12.6)	27.7 (6.58)	32.3 (11.6)
γ-Terpinene	16.6 (3.04)AB	33.4 (1.64)AB	46.0 (5.25)A	24.5 (7.25)AB	12.6 (2.93)AB	9.36 (4.71)B	18.5 (11.6)AB
β-Citronellene	33.3 (19.0)	64.8 (2.04)	81.5 (13.5)	47.1 (20.0)	41.4 (22.1)	17.1 (17.1)	55.0 (20.8)
β-Cariophyllene	30.1 (3.68)	40.5 (25.6)	35.3 (7.96)	71.6 (7.45)	84.5 (10.1)	65.2 (14.1)	28.1 (9.91)
Camphor	n.d[d]	n.d	n.d	0.39 (0.39)	n.d	0.63 (0.63)	0.76 (0.76)
Total	846 (205)B	1517 (161)AB	2303 (355)A	1424 (195)AB	820 (230)B	821 (257)B	1448 (537)AB

Footnotes:
[a] The amounts of terpenes are given as ng g^{-1} milk fat based on the internal standard ethyl heptanoate, and the reported values are the means of four sampling days (calendar days 220 and 248 in year 1, day 232 in year 2 and day 232 in year 3).
[b] Standard error of mean.
[c] Different letters indicate significant differences ($P < 0.05$), calculated by Bonferroni test.
[d] n.d, not detected

Table 4

The amount of terpenes (ng g^{-1} fat) in cultured cream manufactured from milk produced during in-door feeding period, early alpine rangeland feeding period and late alpine rangeland feeding period, and the significant effects of feeding period on the amount of terpenes in cultured cream from the various feeding periods.

Compound	Feeding period		
	In-door	Early alpine rangeland	Late alpine rangeland
α-Pinene	61.8[a] (2.57[b])C[c]	671 (19.7)B	1411 (50.2)A
α-Thujene	n.d C	47.9 (2.38)B	94.9 (2.98)A
Camphene	n.d C	14.0 (0.69)B	23.8 (1.79)A
β-Pinene	n.d C	42.8 (1.94)B	73.1 (3.61)A
Sabinene	n.d C	89.2 (5.61)B	196 (10.3)A
δ-3-Carene	12.0 (4.10)C	30.4 (3.26)B	61.7 (4.07)A
D-Limonene	300 (44.1)A	42.1 (6.02)B	44.4 (2.42)B
γ-Terpinene	n.d B	7.40 (4.28)B	36.3 (2.49)A
β-Citronellene	n.d C	49.2 (3.62)B	69.6 (3.33)A
β-Cariophyllene	n.d	n.d	29.4 (18.7)
Total	374 (40.2)C	994 (22.7)B	2040 (68.3)A

Footnotes:

[a] The amounts of terpenes are given as ng g^{-1} milk fat based on the internal standard ethyl heptanoate.

[b] Standard error of mean.

[c] Different letters indicate significant differences ($P < 0.05$), calculated by Bonferroni test.

[d] n.d, not detected.

Table 5

Sensorial scores for milk from seven different farms. Milk collected at various days during the in-door feeding period (day 67, 87 and 84) and during alpine rangeland feeding period (day 191, 197, 198, 220, 231, 233 and 248) for three successive years. Superscripts give information of given comments by the graders.

Year	Day of collection (day number in the year)	Farms 1	2	3	4	5	6	7
1	67	5	5	5	4[1)]	5	5	5
1	87	4	5	-	4	5	5	5
1	191	5	5	5	4[1)]	5	5	5
1	220	5	5	5	5	5	5	5
1	248	5	-	5	5	-	4[1)]	3[2)]
2	198	5	5	5	5	5	5	5
2	233	5	5	5	4[3)]	5	5	2[3)]
3	84	3[2)]	4[1)]	-	5	5	5	5
3	197	5	5	5	5	5	5	5
3	231	5	5	5	3[3)]	5	5	5

[1)] Fodder

[2)] Oxidized

[3)] Bitter/sharp

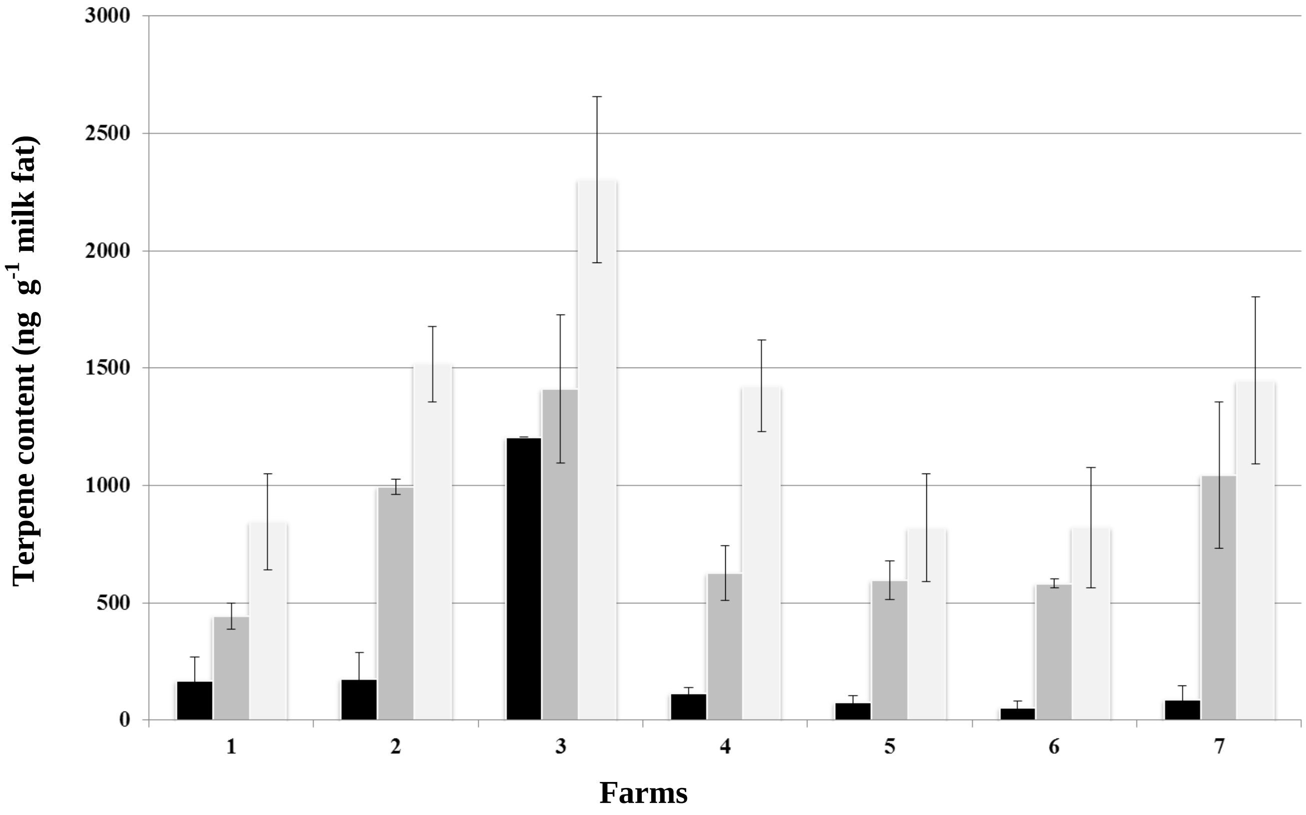
Table 6

Sensorial score for various properties of fresh and stored (3 weeks) cultured cream made from milk collected at various days during the in-door feeding period (day 67 and 87) and during alpine rangeland feeding period (day 191, 220 and 248).

Day of milk collection (day number in the year)	Overall quality		Aroma		Oxidized		Rancid		Off-flavour	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
67	4.40	4.38	5.00	5.00	3.17	3.54	3.50	3.83	3.33	2.50
87	4.33	4.18	5.13	4.27	3.40	4.18	4.07	4.09	2.60	4.27
191	4.06	3.85	4.04	3.95	3.80	3.70	4.00	3.95	4.28	4.20
220	4.30	3.75	5.23	4.13	4.07	4.13	4.10	4.19	3.90	3.75
248	3.83	4.00	4.29	4.05	4.04	4.25	3.96	4.05	4.29	4.00
Total mean	4.18	4.03	4.74A	4.28B	3.70B	3.96A	3.93	4.02	3.68	3.74

Figure1

Figure 1



Highlights

- Terpene content in milk sampled during alpine rangeland feeding period in Norway is reported
- The terpene content in milk increased five times during the alpine rangeland feeding period
- The terpenes did not influence the sensorial quality of the milk or the cultured cream