



# 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 indoors, and by 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  $\beta$ -pinene,  $\alpha$ -pinene,  $\alpha$ -thujene, camphene, sabinene,  $\delta$ -3-carene,  $\nu$ -limonene,  $\gamma$ -terpinene, camphor,  $\beta$ -citronellene, and the sesquiterpene  $\beta$ -caryophyllene. The average total terpene content increased five times during the alpine rangeland feeding period. The terpenes  $\alpha$ -thujene, sabinene,  $\gamma$ -terpinene and  $\beta$ -citronellene were only detected in milk and cultured cream from the alpine rangeland feeding period and not in samples from the indoors feeding period. These four terpenes could be used, as indicators, to show that milk and cultured cream originate from the alpine rangeland feeding period. The terpenes did not influence the sensorial quality of the milk or the cultured cream.

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## 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 it changes with change in environmental conditions (Bugaud et al., 2000; Cornu et al., 2001; Mariaca et al., 1997; Tornambé et al., 2006). Chion et al. (2010) concluded that milk produced on pasture had higher contents of terpenes than had milk from winter diets based on hay. The terpene molecules from plants, in general, appear in

the rumen within 24 h 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 milk or cheese originating from herds fed on any grazing system and those fed indoors (Cornu et al., 2001; Favaro, Magno, Boaretto, Bailoni, & Mantovani, 2005; Viallon et al., 1999, 2000). Morand-Fehr, Fedele, Decandia, and Le Frileux (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 objective of this work was to compare the presence of terpenes in cow's milk from seven different farms, in alpine rangeland in Norway, with the presence of terpenes in milk from the indoors 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 investigated whether the content of terpenes in milk and cultured cream from the alpine rangeland feeding period could be used to distinguish this milk and cultured cream from samples collected and produced during the indoors feeding period.

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## 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. Sickel, Bilger, and Ohlson (2012) investigated the wild grazing plant species in the same area in July and August 2009.

During the winter, the indoors feeding period, a standard feeding regime with conserved green fodder and concentrate, was practised at all farms included in the investigation. During summer-farming in the mountains, 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-farms (farm No. 3 in the results chapter), the herd was grazing wild alpine plants in the outlying fields also during the night.

### 2.2. Milk sampling

During the indoors feeding period, milk was sampled from each of the seven farms at calendar days 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 practised 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 practised. 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 °C) 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 prior to 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, on 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 indoors 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 at the beginning of July, at the beginning of August and at the beginning of September. Cream

from each day of milk delivery was divided into four batches. 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 of cream (38% fat) were homogenised at 60 °C and 30 bar in a Rannie Homogenizer (type 16.50) (APV, Oslo, Norway). The homogenised cream was further heated to 90 °C for 5 min, then cooled to the incubation temperature of 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 inocula in the cream the cream was dispensed into disinfected plastic cups (200 ml) with lids. The cups were incubated at 20 °C in a temperature-regulated water bath until contents reached pH 4.5–4.6 (approximately 18 h after inoculation) and transferred to the 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 the end of incubation (fresh product) and after approximately 3 weeks, which is the commercial shelf life of this type of product 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 were weighed into ultracentrifuge tubes and centrifuged at 28,700 rpm (100,000×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 13,000 rpm (16,000×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 prior to 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 of 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<sup>–1</sup>, 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<sup>–1</sup>) for 5 min in the opposite direction to that 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 ionisation mode; ionisation 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 programme was as follows: 30 °C for 10 min, increasing by 1 °C min<sup>–1</sup> to 40 °C, by 3 °C min<sup>–1</sup> to 70 °C, and by 6.5 °C min<sup>–1</sup> 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

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