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Effect of scalding temperature on sensory and biochemical properties in a hard goat milk cheese

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ABSTRACT

A procedure to produce a hard cheese made from goat milk set to approach Manchego cheese was developed. The effects on cheese characteristics of two scalding temperatures, 38 °C and 40 °C, during cheesemaking were evaluated in an experimental design with three replicate blocks. The cheese was analysed for volatiles, organic acids, proteolysis (as measured by capillary electrophoresis) and sensory properties. After 18 weeks of ripening, sensory descriptive analysis showed that the cheeses had a high total flavour and odour intensity, and a low degree of oxidised flavour. A consumer acceptability test showed that the cheese was liked. Cheese scalded at 40 °C obtained higher concentrations of volatiles and was described as more mature, umami, sweet and sour compared with cheese scalded at 38 °C, which had the highest concentration of organic acids and was described to be slightly more oxidised, bitter, and acidic.

1. Introduction

The Norwegian dairy goat is a traditional livestock that thrives on rangeland pastures and can exploit unused resources in the Norwegian landscape. Traditionally Norwegian goat milk has been used for brown whey cheese, but due to substantial improvements in the goat milk quality, organoleptic properties, and cheese-making qualities, new opportunities have opened up for the use of Norwegian goat milk (Inglingstad et al., 2016; Skeie, 2014.).

Norwegian consumers have obtained increased attention towards aged goat milk cheeses. Therefore, developing a procedure for an aged hard cheese variety based on Norwegian goat milk would be desirable. Manchego, which is a hard cheese made from ewes' milk, has in later years become a well-known cheese worldwide. Manchego is known among chefs as a Gateway Cheese™, which means that Manchego is often used as a first introduction to semi-hard and hard ewes and goat milk cheeses. Most of the characteristic flavour in a Manchego cheese comes from the decomposition of free fatty acids (FFAs), and these are especially derived from short-chain fatty acids (FAs) (Seseña, Poveda, Cabezas, & Palop, 2013). These short-chain FAs are also prominent in goat milk fat (Inglingstad et al., 2016), which indicates that a Manchego-type cheese might be a promising model for a new matured hard cheese based on goat milk. Since the gross composition of goat milk and its cheese-making properties differ from those of ewes' milk, the technology to meet the desired cheese properties such as size, moisture content, pH as well as flavour and texture must be adapted.

Manchego cheese production differs depending on the scale of production; industrial or artisanal. Industrial cheeses are often made from pasteurised milk using a commercial starter culture, whereas artisanal production traditionally relies on bacteria present in unpasteurised milk (Gómez-Ruiz, Ballesteros, Viñas, Cabezas, & Martínez-Castro, 2002). However, some artisanal producers are adding an additional starter to better control the pH development and product quality. In traditional Manchego manufacturing a mesophilic culture and a scalding temperature in the range of 36–40 °C is most often used (Gomez, Rodriguez, Gaya, Nuñez, & Medina, 1999). However, small changes within this temperature range influence the state of the mesophilic lactic acid bacteria, their survival, and their autolysis.

Autolysis will release intracellular enzymes from the lactic acid bacteria into the cheese and the rate of autolysis will thereby influence the biochemical activity and the further ripening of the cheese.

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It has been shown, both by growth in broth and in cheese, that a temperature of 39 °C is a critical threshold for the autolysis of different mesophilic lactic acid bacteria (LAB) (Chapot-Chartier, Deniel, Rousseau, Vassal, & Gripon, 1994; Chen, Shen, Hellgren, Jensen, & Solem, 2015; Sheehan, O'Loughlin, O'Cuinn, FitzGerald, & Wilkinson, 2005). Therefore, the scalding temperature during cheese-making regulates the enzyme content present in the cheese and the further activity during cheese ripening (Guinee & Wilkinson, 1992; Sousa, Ardö, & McSweeney, 2001). However, although most of the mesophilic bacteria most probably autolyse at a scalding temperature above 39 °C, thermophilic bacteria will thrive around 40 °C (Sheehan, Fenelon, Wilkinson, & McSweeney, 2007). Therefore, a difference in scalding temperature of only 2 °C most probably will lead to sensory differences between the cheeses.

The objective of this study was to develop a production technology for a matured hard cheese from goat milk adapting the technology used for Manchego cheese. In addition, to investigate how a slight difference in scalding temperature affects the chemical and sensory properties of the cheeses during the ripening process.

2. Materials and methods

2.1. Experimental design

The cheese was made by applying two different scalding temperatures (38 °C and 40 °C) in three replicate blocks (A, B and C). At each replicate block, two vats of cheese were made, and a new batch of goat milk was used every time. A total of six cheese vats were made. The cheeses are further referred to by the replicate block and the scalding temperature, e.g., A-38° refers to replicate block A, scalding temperature 38 °C.

2.2. Milk sampling and cheese manufacture process

For each replicate block, goat milk from the Norwegian University of Life Sciences (NMBU) (Ås gård research farm, SHF) was collected over a period of three days in June. At the point of milk collection, the goats were in lactation month 4/5, and they had been grazing for approximately 1 month at outdoor pasture, with some additional concentrate feed.

A flowchart of the total cheese-making process is shown in Fig. 1. The raw milk (~3.8% fat) was separated into skim milk and cream at 55 °C, pasteurised at 72 °C for 15 s and cooled to 32 °C. The fat content was standardised in the vat to 3.5% with goat cream. A DVS starter culture (FD-DVS RSF-736, Chr. Hansen A/S, Hørsholm, Denmark) with mesophilic [*Lactococcus* (*Lc.*) *lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris*) and thermophilic (*Streptococcus* (*Str.*) *thermophilus* and *Lactobacillus* (*Lb.*) *helveticus*] lactic acid starter bacteria was used. The DVS culture was activated at 32 °C for 1.5 h in UHT milk (1.5% fat) (TINE, Oslo, Norway) prior to the addition of 5 U 100 L^{-1} to the cheese milk. At pH 6.5, 25 mL rennet 100 L^{-1} milk was added (rennet, CHYMAX, (200 IMCU mL^{-1}), Chr. Hansen A/S). At clear cut (~23 ± 2 min), as evaluated by an experienced cheesemaker, the coagulum was cut into ~5 mm cubes and stirred

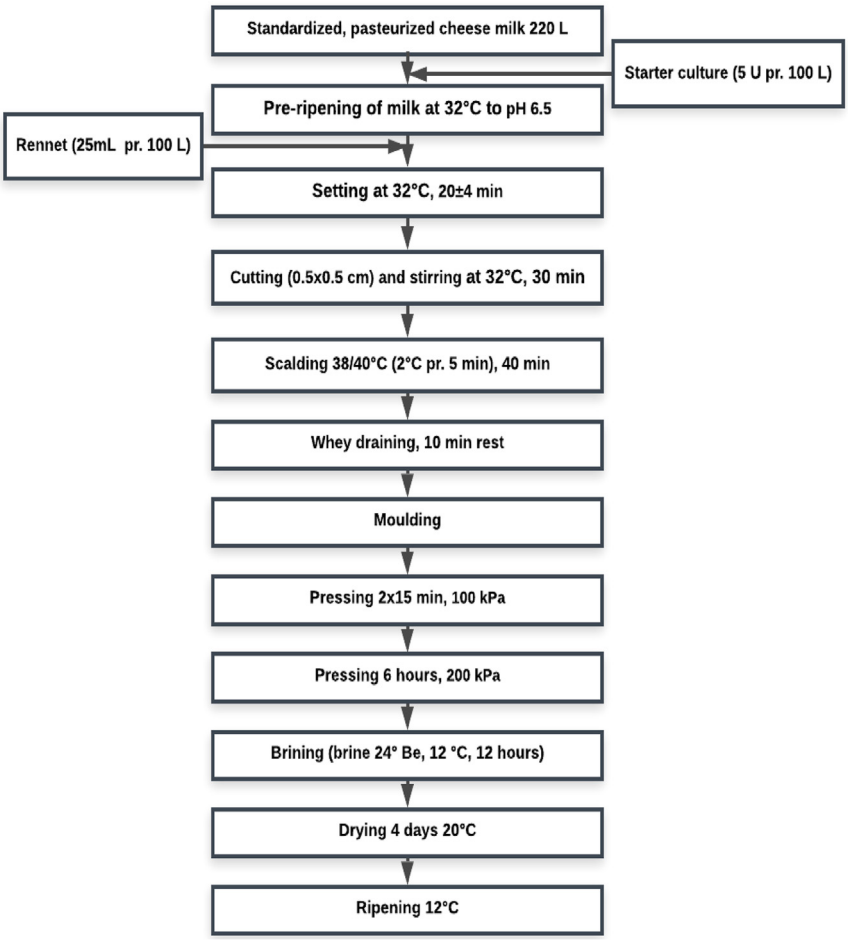

Fig. 1. Flowchart of the cheese-making protocol of a hard goat milk cheese.

at 32 °C for 30 min. The curds were then slowly heated by an increase of 2 °C every 5 min until the target scalding temperatures of 38 °C or 40 °C (±0.23 °C) were achieved, then the curd was stirred for a total of 40 min (unfortunately, sample A-40° had at one point in the scalding process a temperature of 40.7 °C for approximately 10 min). The cheese curds were transferred to a pre-pressing vat and the whey was drained off when the transfer from the cheese vat was complete. The curd then rested in the pre-pressing vat for an additional 10 min before being transferred to moulds (d = 20 cm and h = 11 cm). The cheese was pre-pressed in the moulds for 2 × 15 min at 100 kPa and then further pressed for 6 h at 200 kPa, turned, and trimmed between pre-pressing and final pressing. The cheese was salted in saturated brine (24° Be, 12 °C, pH 5.0–5.3) for 12 h. The cheese was dried at 20 °C for 4 days at 65% RH and turned twice a day, before vacuum packing in plastic cheese bags (44 microns, moisture vapour transmission rate of RH 90% at 38 °C) (OSB3000, CRYOVAC®, Charlotte, NC, USA). The cheese was ripened at 12 °C for 18 weeks and further stored at 12 °C. The cheese was sampled for analysis at 0, 6, 12 and 18 weeks of ripening.

2.3. Sensory analysis

Descriptive sensory profiling was performed based on the Generic Descriptive Analysis methodology as described by Lawless and Heymann (2010). The analysis was made in accordance with ISO standard 13299:2016 (ISO, 2016). All tests were performed at the sensory laboratory at NOFIMA (Norwegian Institute of Food, Fisheries, and Aquaculture, Ås, Norway), and designed following the ISO standard 8589:2007 (ISO, 2007). The Nofima sensory panel consists of 10 highly trained assessors, selected by their sensory abilities, and trained following ISO standard 8586:2012 (ISO, 2012). The panel undergoes regular training, testing, and calibration prior to every project.

For the evaluation of these specific goat milk cheeses, a pre-trial session was conducted to determine the relevant descriptive terminology. Samples A-40° and C-38°, which were expected to be most different, were used to develop a relevant vocabulary. The terminology agreed upon consisted of 5 taste attributes (sweet, acidic, salt, bitter and umami), 5 odour attributes (total intensity, acidic, lactic acid, goaty and oxidised) 7 flavour attributes (total intensity, sour, dairy, mature, goaty, bitter and oxidised) and 5 texture attributes (hard, dry, sticky, crumbly and astringent). The full description of all sensory attributes is shown in Supplementary material Table S1.

There was a total of three pre-trial sessions to fully calibrate the assessors in the use of vocabulary and rating scales. The cheese was tempered to 18 °C (±1 °C) and served in 2 × 2 × 1 cm cubes in inert metal containers with a lid and marked with a three-digit code containing two pieces of each of the experimental samples of cheese. Samples were served following a balanced experimental design to account for carry-over effects and sample position (William's Latin square) (Saurav, Varghese, Varghese, & Jaggi, 2017). The assessors evaluated the samples individually at their own pace, having a small break midway. Hot and cold water and unsalted crackers were used to cleanse the palates between the cheese samples. The intensity of the sensory attributes was measured on a scale from low intensity = 1, to high intensity = 9, shown as a non-numeric 15 cm line increasing in intensity from left to right. The response from the direct recording was transformed into numbers using EyeQuestion (Logic8 BV, Utrecht, Nederland) and checked using PanelCheck V.1.4.2, (Nofima AS, Ås, Norge).

A consumer hedonic test of the cheese scalded at 40 °C was performed at a food festival (Smak Ås 2019, Campus Ås, Norway) which was open and free for the public. As such, the consumer pool was a convenience sample, without purposive recruitment or screening, and participation was based on willingness and being a cheese acceptor. Acceptability was rated and a check all that apply question (CATA) was answered using Google Forms. A QR Code (QR Code Generator, 2019) and a shortened URL (TinyURL.Com — Shorten That Long URL into a Tiny URL; Tiny URL, 2019) were created for easy digital access to the survey. It was also possible to respond on paper. When asked about liking of the cheese, a hedonic scale (Peryam & Pilgrim, 1957) from 1 to 9 was used, where 1 was "do not like" and 9 was "like very much". The remaining questions were designed to map demographics, preferences, and willingness to buy the hard goat milk cheese. A total of 225 voluntary participants volunteered. The participants had a gender distribution consisting of 59% females and 41% males, and an age composition where 53% were under the age of 39, while the remaining 47% were 40 years old or older.

Before the test, the cheese was cut into equally sized triangle-shaped pieces, tempered to 20 °C (±1 °C) and served in generic plastic dishes. The cheese samples were kept in a Styrofoam box during the execution, keeping the samples at a stable temperature. To minimise the potential for bias, two individuals who were not associated with the study were responsible for conducting the survey. The volunteering participants were provided with a place to sit down and answer the survey.

2.4. Chemical analysis of milk and cheese

Fourier-transform infrared spectroscopy (FTIR) (MilkoScan™ FT1 FTIR, Foss, Hillerød, Denmark) was used to analyse the composition of the cheese milk. pH was measured throughout the experiment on both milk and cheese (Thermo Scientific OrionStar A211 with a BL21 pHT BNC-N C164919 005 electrode, Thermo Fisher Scientific, Waltham, MA, USA). The cheeses were sampled and analysed after 18 weeks of ripening. Sampling for chemical analyses was made according to IDF-standard 50C (IDF, 1995). At sampling, pH, and dry matter (DM) were analysed immediately, for all other analysis, samples were frozen in glass jars and stored at −20 °C prior to analysis. Cheese pH was measured according to Skeie, Lindberg, and Narvhus (2001). Total solids (TS%) was determined according to IDF standard 4A (IDF, 1982) with a slight modification as the samples were pre-dried at room temperature for 20 h prior to drying in the oven instead of using sand.

After 18 weeks of ripening, the cheeses were analysed for organic acids using high-performance liquid chromatography (HPLC) (Skeie, Inglingstad, Brunborg, & Eknæs, 2014).

Volatile compounds were analysed using automated thermal desorber gas chromatography mass spectrometry (ATD-GCMS). Five grams of sample were weighed into a 50 mL aluminium beaker (Sigma Aldrich, MO, USA), and 20 μL of internal standard (30 ng μL^{-1} H_2O, 4-methyl-1-pentanol, Sigma Aldrich, MO, USA) was added. The beaker was placed into a Micro-Chamber/Thermal Extractor M-CTE250 (Markes International Ltd., Bridgend, UK), where the volatiles were swept directly onto a sorbent tube (Tenax TA/Carbograph 1TD, Markes International Ltd., Bridgend, UK) at 45 °C for 20 min, with an N_2-flow of 50 mL min^{-1}. The sorbent tube was subsequently analysed in a Thermal Desorber TD-100 (Markes International Ltd.) combined with a 7890B GC system (Agilent Technologies, Santa Clara, CA, USA). Primary desorption was conducted by heating the tube to 280 °C with an N_2- flow of 50 mL min^{-1} for 10 min. The stripped volatiles were trapped in a cold trap (U-T2GPH-2S, Markes International Ltd.) at 25 °C, which was subsequently heated at 280 °C for 3 min (secondary desorption). The volatile compounds were separated by a DB-WAXETR GC column (Agilent

Technologies), 30 m × 0.25 mm i.d. with a film thickness of 0.5 μm. The oven temperature was programmed to 35 °C for 5 min; then using a heating rate of 10 °C min^{-1}, the temperature was increased to 230 °C and held for 10 min. The carrier gas was helium (Aga, Oslo, Norway) at a flow of 1 mL min^{-1}. The GC column was connected to an ion source (temperature 230 °C) of an Agilent 5975 inert XL Mass Selective Detector (Agilent Technologies), with an interface line at 250 °C. The mass spectrometer was operated at scan mode within a mass range of *m/z* 33 to 400 at 1 scan s^{-1}. Ionisation was done by electronic impact at 70 eV, calibration was done by autotuning. The software program was Masshunter GC/MS Acquisition B.07.00.1413 (Agilent Technologies). Compounds were tentatively identified by computer-matching of mass spectra with those of the NIST 17 Mass Spectral Library (Agilent Technologies).

The protein profile was analysed by capillary electrophoresis (CE) according to (Jørgensen et al., 2016). Protein was analysed in accordance with IDF standard 20b (IDF, 1993), and 45 mL of the citrate soluble fraction was used further for the CE analysis. The citrate soluble solution was transferred to a 50 mL Falcon centrifuge tube and centrifuged (Thermo Fisher Scientific, Waltham, MA, USA) at 3400 × *g* for 30 min at 4 °C. The fat layer on top was removed with a cotton swab and the remains were thoroughly mixed before 600 μL was transferred to an Eppendorf tube. Nine hundred microlitres of "CE sample buffer" consisting of 0.0393 g DL-dithiothreitol and a sample buffer (6 m urea, 0.83 mg mL^{-1} HPMC, 42 mm MOPS, 167 mm Tris and 67 mL EDTA with pH 8.6 ± 0.1) were added. The sample was stirred on a shaking table (PSU-20i Biosan, Riga, Latvia) for 1 h at room temperature and centrifuged in an Eppendorf 5415D microcentrifuge (Eppendorf, Hamburg, Germany) for 3 min at 13,000 rpm. To avoid the fat layer formed on top of the sample after centrifugation, a syringe with a needle was used for the withdrawal of the sample. The sample was then filtered through a 0.2 μL cellulose acetate membrane filter (VWR, Radnor, PE, USA) to a new Eppendorf tube, before 100 μL sample solution was transferred to a CE tube and added 700 μL run buffer (6 m urea, 0.83 mg mL^{-1} HPMC, 20 mm sodium nitrate, 0.19 m citric acid, pH 3.0 ± 0.1), and run on an Agilent G1600 AX (Agilent Technologies) capillary electrophoresis instrument.

2.5. Microbiological analyses

Microbial enumerations were made 24 h after starter addition (fresh cheese), and during maturation (at 6, 12, and 18 weeks) according to (Porcellato et al., 2013). Microbial enumerations were performed using M17 agar (Merck, Darmstadt, Germany) and selective agar (LBS agar; Difco, Detroit, USA) plating according to agar plates were incubated at 30 and 42 °C.

2.6. Statistical treatment of data

An ANOVA was performed using the scalding temperature as a fixed effect and the production day (block) as a random effect using R-Studio© (Pinheiro J, Bates D, R Core Team (2022). _nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–160, <https://CRAN.R-project.org/package=nlme>.) Differences were considered significant when $p < 0.05$.

The organic acids, volatile compounds and sensory results of the cheese were analysed using Principal Component analysis (PCA) in Unscrambler® (Unscrambler®, 11.0.1086.22328, CAMO Software AS, Oslo, Norge). The chemical data set was weighted by dividing each response variable by the standard deviation of that variable. All data were validated using full cross-validation.

3. Results

3.1. Gross composition of cheese milk and cheese

The cheese milk was of good quality with an average of 3.0% protein [2.31% casein (CN)], 3.5% fat, 0.7 mmol L^{-1} free fatty acids and a pH of 6.6. An overview of the goat milk composition is given in Table 1. The pH in cheese 24 h after starter addition was on average 4.99, which increased to an average of 5.04 during the 18 weeks of maturation. No significant difference between the two scalding temperatures was found in dry matter (DM) neither in cheese 24 h after starter addition (mean DM 57.1%) nor after 18 weeks of ripening (mean DM 63.6%). The salt content of the cheese was on average 1.3%.

Capillary electrophoresis revealed no clear differences in the primary proteolytic profile between the two scaling temperatures, 38 and 40 °C with similar degradation profiles for β-CN, α_{S1}-CN and α_{S2}-CN as shown by the electropherograms in Fig. 2 (results shown only for block C, all cheeses with the same treatment showed the same pattern).

After 12 weeks of ripening the cheese produced with a scalding temperature of 40 °C obtained the highest numbers enumerated on M17 incubated at 30 °C ($p < 0.01$). However, no further significant differences were observed between the two scalding temperatures with regard to the number of lactic acid bacterias in the cheese.

3.2. Organic acids

Principal component analysis of the organic acids in the 24 h cheese showed clustering of the cheeses according to scalding temperature and the two first PCs explained 49% and 32% of the variation respectively (Fig. 3). The cheese scalded at 38 °C clustered together in the upper left quadrant and these cheeses had a higher concentration of formic-, lactic- and α-ketoglutaric acid. The cheese scalded at 40 °C did not create a clear cluster and showed a more scattered distribution by the PCA. After 18 weeks of ripening, the distribution of the organic acids in the cheeses as shown by PCA still explained 80% of the variation by the first two

Table 1
Gross composition of cheese milk, 24-h cheese and cheese ripened for 18 weeks.[a]

Component	Concentration	Scalding temperature	
		38 °C	40 °C
Cheese milk			
Fat (%)	3.54 ± 0.04		
Protein (%)	2.98 ± 0.07		
Casein (%)	2.31 ± 0.06		
Lactose (%)	4.50 ± 0.08		
Casein/protein (%)	77.59 ± 0.29		
pH	6.60 ± 0.05		
Free fatty acids (mmol L^{-1})	0.68 ± 0.05		
Cheese 24 h after starter addition			
pH		4.98 ± 0.01	5.00 ± 0
Dry matter (%)		56.76 ± 0.58	57.35 ± 0.07
Cheese after 18 weeks of ripening			
pH		5.03 ± 0.01	5.05 ± 0.03
Dry matter (%)		63.5 ± 0.35	63.8 ± 0.67
NaCl (%)		1.31 ± 0.22	1.32 ± 0.13

[a] Values are presented as a mean ± standard deviation (n = 3), for each scalding temperature.

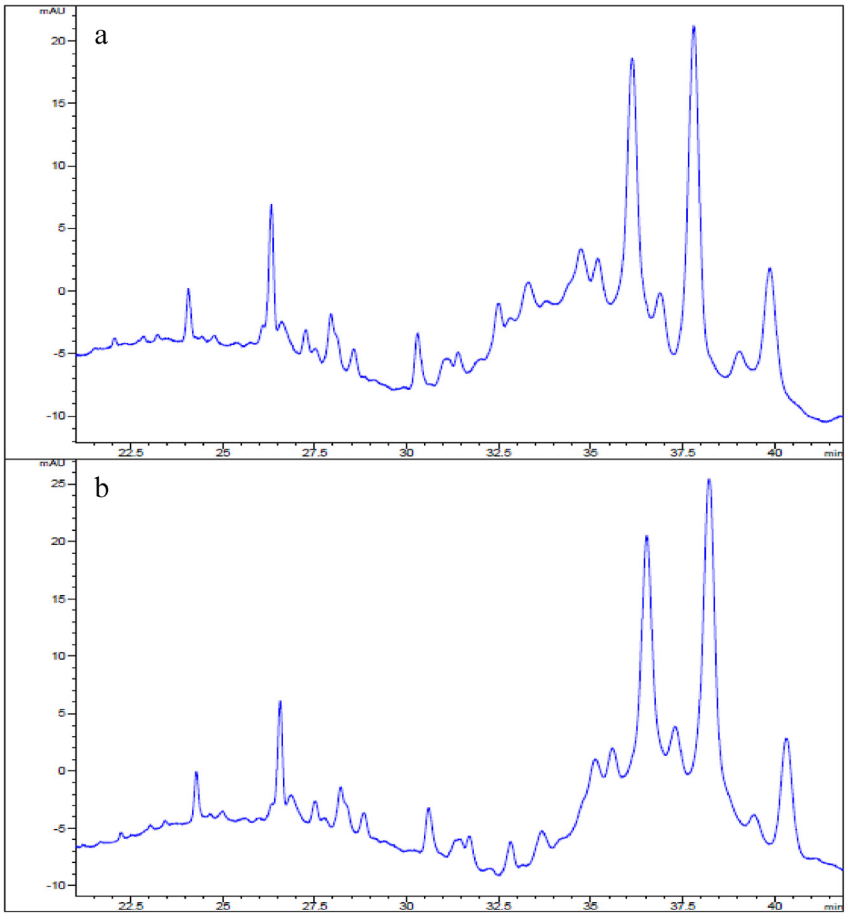

Fig. 2. Electropherograms from capillary electrophoresis showing the protein profile of the hard goat milk cheese scalded at (a) 40 °C and (b) 38 °C and ripened for 18 weeks. The cheeses were made from the same batch of milk (production day C).

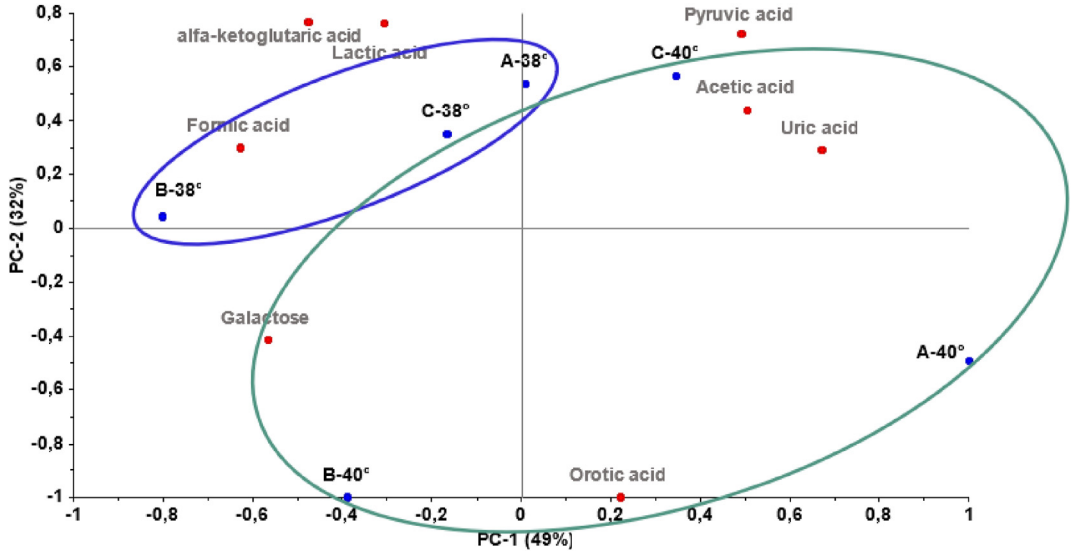

Fig. 3. Principal component analysis (PCA) of the organic acids in the cheeses 24 h after starter addition. The first letter (A, B or C) refers to the production day of the cheese samples, and the two last digits represent the different scalding temperatures used (38 °C, blue ring; 40 °C, green ring). Principal components 1 and 2 explain 49% and 32%, respectively, of the variation. Citrate was equally distributed among the fresh cheeses and was removed from the PCA.

components (Fig. 4) and the ripened cheeses were divided into two clear clusters by the two scalding temperatures. The cheese scalded at 38 °C were all clustered together showing higher concentrations of almost all organic acids, and mainly had the highest concentrations of the organic acids, α-ketoglutaric-, formic-, pyruvic- and orotic acid compared with the cheese scalded at 40 °C.

3.3. Volatile compounds

By analysing the cheeses matured for 18 weeks using automated thermal desorber gas chromatography–mass spectrometry (ATD-GCMS), 81 volatile compounds were identified (Supplementary material Table S2). Principal component analysis showed clustering based on scalding temperature, and 46% and 36% of the variation in 61 of the volatile compounds could be explained by PC-1 and PC-2, respectively. The cheese made with a scalding temperature of 38 °C (Fig. 5) clustered together and was characterised by having a generally lower content of most volatile compounds.

Cheese made with a scalding temperature of 40 °C had higher ($p < 0.05$) levels of alcohols and ketones as well as octanoic and decanoic acid than cheese made with a scalding temperature of 38 °C. Additionally, there was a notable trend towards higher levels of hexanoic acid in the cheese made with a scalding temperature of 40 °C than 38 °C, although the difference was borderline significant

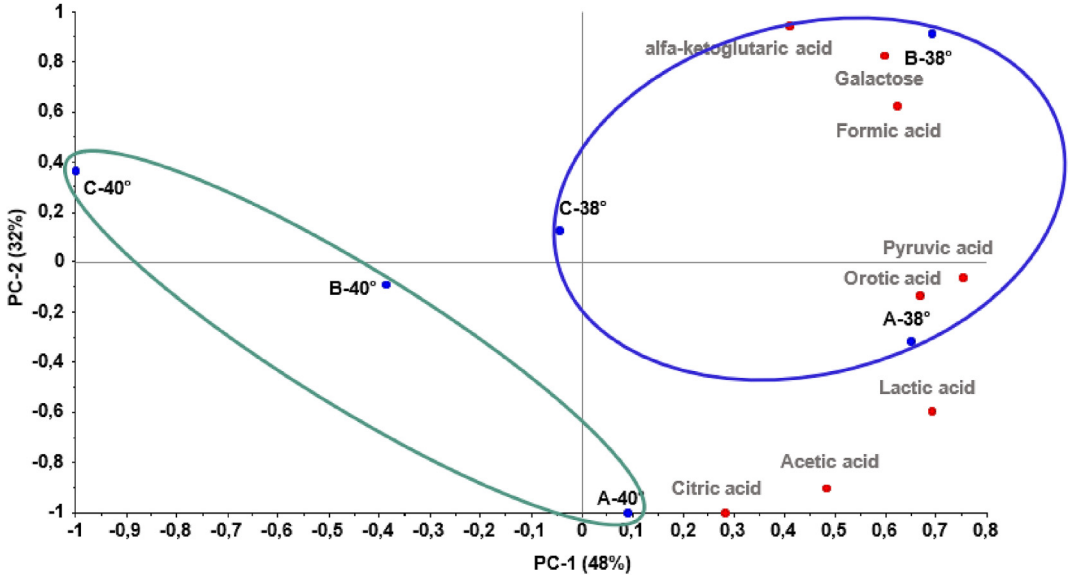

Fig. 4. Principal component analysis (PCA) for the organic acids in the cheeses ripened for 18 weeks. The first letter (A, B, or C) refers to the production day of the cheese samples, and the last two digits represent the different scalding temperatures used (38 °C, blue ring; 40 °C, green ring). Principal components 1 and 2 explain 48% and 32%, respectively, of the total variation.

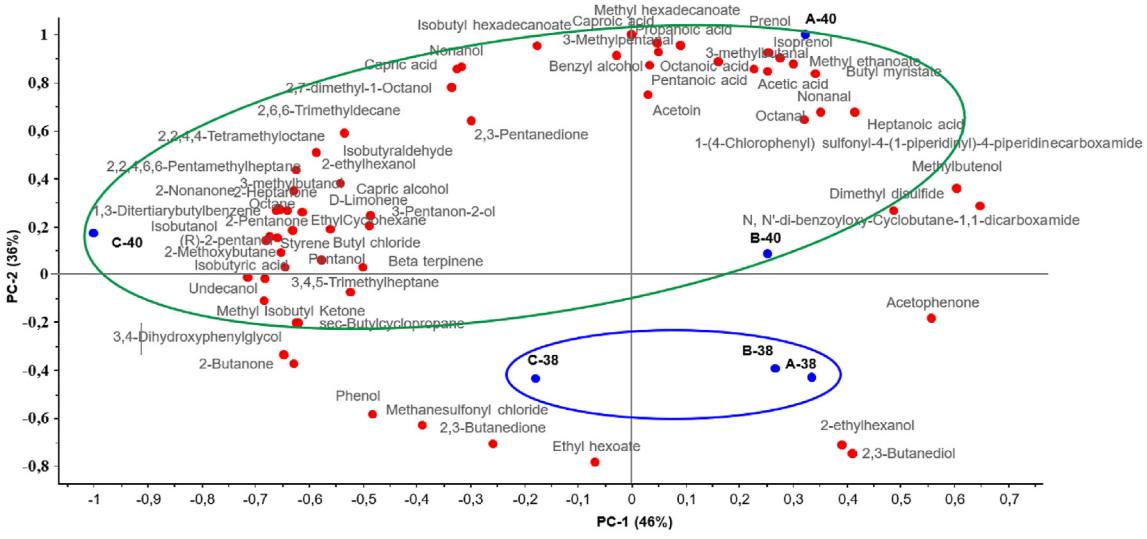

Fig. 5. Principal component analysis (PCA) of the volatile compounds in the cheeses ripened for 18 weeks. The first letter (A, B or C) refers to the production day of the cheese samples, and the two last digits represent the different scalding temperatures used (38 °C, blue ring; 40 °C, green ring). Principal components 1 and 2 explain 46% and 36%, respectively, of the total variation.

Table 2
Sensory scores using Quantitative Descriptive Analysis (QDA®) of the cheese ripened for 18 weeks.[a]

Attribute	Scalding temperature	
	38 °C	40 °C
Odour		
Intensity	6.1 ± 0.2	5.9 ± 0.3
Sour	3.5 ± 0.2	4.3 ± 0.6
Sour milk	4.7 ± 0.2	4.7 ± 0.2
Goat	4.6 ± 0.1	4.4 ± 0.4
Oxidised	2.6 ± 0.2	2.0 ± 0.3
Taste		
Sweet	2.9 ± 0.1	3.4 ± 0.1
Acidic	4.5 ± 0.0	4.5 ± 0.0
Salty	4.3 ± 0.3	4.4 ± 0.0
Bitter	5.2 ± 0.1	4.9 ± 0.1
Umami	3.7 ± 0.3	4.5 ± 0.2
Flavour		
Dairy	3.9 ± 0.1	3.9 ± 0.2
Mature	3.6 ± 0.4	4.6 ± 0.1
Goat	4.9 ± 0.1	4.8 ± 0.3
Bitter	4.6 ± 0.2	4.3 ± 0.1
Oxidised	2.4 ± 0.6	2.0 ± 0.1
Intensity	6.1 ± 0.1	6.1 ± 0.1
Sour	3.5 ± 0.6	4.3 ± 0.4
Texture		
Hardness	4.6 ± 0.1	4.5 ± 0.4
Dryness	6.0 ± 0.3	5.8 ± 0.3
Stickiness	5.5 ± 0.2	5.4 ± 0.3
Granularity	5.5 ± 0.2	5.3 ± 0.4
Astringency	5.8 ± 0.3	5.5 ± 0.2

[a] Sensory attributes were measured on a scale from 1 to 9 with low intensity = 1, high intensity = 9. Values are presented as a mean ± standard deviation (n = 3) for each scalding temperature.

($p < 0.1$). Notable but not significant ($p > 0.05$), cheese made with a scalding temperature of 40 °C displayed a higher total concentration in all the other categorised functional groups (aldehydes, organic acids, esters, benzenic compounds, and miscellaneous compounds) compared with cheese made with a scalding temperature of 38 °C.

3.4. Sensory analysis

3.4.1. QDA

The sensory characterisation of the cheeses using QDA® showed that all the cheeses, regardless of the scalding temperature, were characterised by a high intensity score for the attributes "total odour intensity" and "flavour intensity" (Table 2), while the intensities of "oxidised flavour" and "oxidised odour" were low. By analysing all the sensory attributes using PCA, the cheeses were divided into two clusters according to the scalding temperature (Fig. 6), with PC-1 and PC-2 explaining 71% and 15% of the variation, respectively. The cheeses in the right quadrants of PC-1, all scalded at 40 °C, were characterised by a high degree of sour odour and sour taste, but they were also described by having a higher mature and umami flavour as well as having a sweet taste. The cheese scalded at 38 °C clustered in the left quadrants of PC-1 indicating a higher degree of oxidised flavour and odour, bitter and acidic taste than the cheese scalded at 40 °C.

3.4.2. Consumer acceptability

The distribution of the liking scores given by the prospective consumers is shown in Fig. 7. The overall liking of the cheese was highly positive among the 225 participants. Most of the responses (94.2%) fell within the higher end of the scale (5 or higher), with approximately 74% of the answers falling within the top three scores (7–9). The median score of 8 indicate that the participants had a strong affinity for the cheese.

Participants described the cheese as mature, sour, and with a goaty flavour, accompanied by a crumbly texture. When considering the preferred context for serving the cheese, most participants expressed a preference for incorporating it into a cheese platter (82.1%), serving it alongside tapas (68.3%), integrating it into pasta dishes and salads (54.9%), or consuming it as a snack (48.7%). However, the cheese was considered less suitable for consumption as a dessert or in gratin dishes (both 17.9%). In terms of purchasing intent, a significant majority of potential consumers (82%)

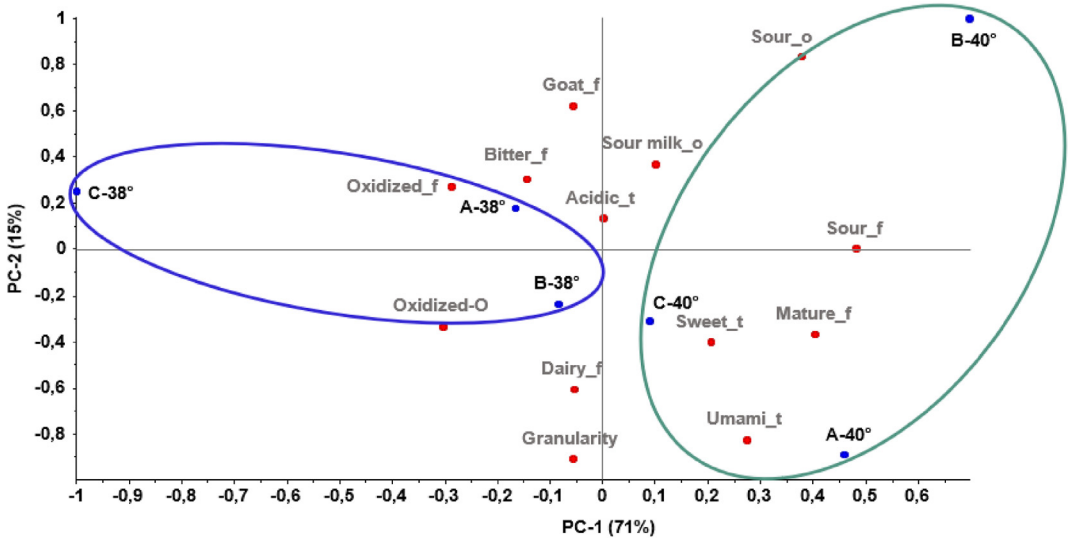

Fig. 6. Principal component analysis (PCA) of the sensory attributes as found by Quantitative Descriptive Analysis (QDA®) of the cheeses ripened for 18 weeks. The first letter (A, B or C) refers to the production day of the cheese samples, and the two last digits represent the different scalding temperatures used (38 °C, blue ring; 40 °C, green ring). Principal components 1 and 2 explain 71% and 15%, respectively, of the variation.

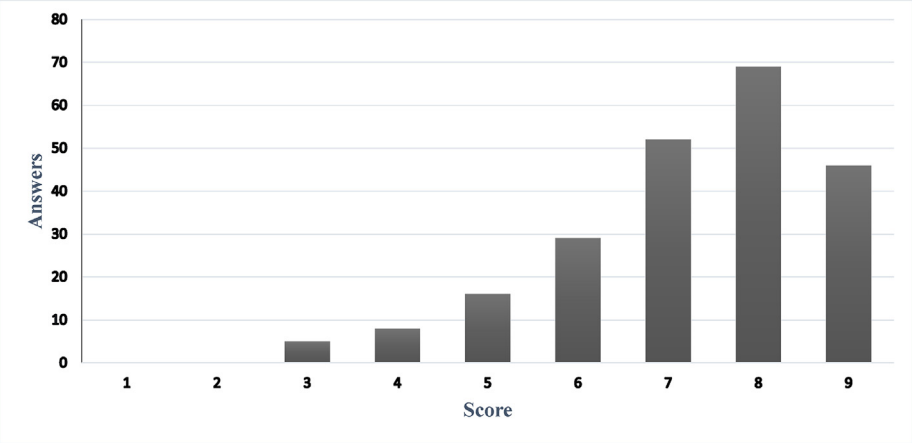

Fig. 7. The distribution of the subjective liking score from 225 Norwegian consumers answered at Smak Ås, a food festival. A hedonic scale (Peryam & Pilgrim, 1957) from 1 to 9 was used, where 1 was "do not like" and 9 was "like very much".

expressed a positive willingness to purchase the cheese if it were commercially available.

4. Discussion

The primary objective of this study was to develop a production technology for a long-ripening hard goat milk cheese, based on the Manchego cheese-making process, but specifically optimised for goat milk. The development of such a protocol is crucial to utilise goat milk in an innovative manner. It should be noted that Manchego cheese is traditionally made from the milk of Manchega sheep, which is characterised by its high protein and fat content. Adapting the traditional technology for Norwegian goat milk, which possesses lower fat and protein contents [4.52% and 3.39% respectively (TINE, 2022)] than the ewes' milk normally used in Manchego, necessitated modifications to the process. The compositional and protein profile differences between ewe and goat milk require milk standardisation and a more gentle treatment of the cheese coagulum. The goat milk coagulum exhibits increased brittleness compared with that of ewe milk. Consequently, adjustments were made, including larger curd size and longer duration of the stirring and heating phase to attain the desired dry matter content without excessive casein loss to the whey.

Traditionally, Manchego cheese curds are hooped into moulds and pressed to achieve the desired open structure. However, for the goat milk curd, it was found that a better and more gentle treatment of the curds was to rest under whey in a cheese pressing vat without pressing for 2 min, before draining off the whey. After whey drainage, the cheese curds were left in the pressing vat exposed to air for 10 min to fuse the cheese curds into a cohesive block with the desired piped structure. The fused block was then cut into smaller pieces of approximately 2 kg before moulding and pressing. To ensure that the ripening conditions did not differentially affect the cheeses, all cheeses were packaged in plastic cheese bags. This practice was supported by findings from Nunez, Gaya, Medina, Rodríguez-Marín, and Garcia-Aser (1986), who demonstrated that the flavour of Manchego cheese was not significantly influenced by vacuum packaging. By employing this consistent packaging method, a more equitable comparison among the cheeses was facilitated, thereby minimising potential confounding factors related to ripening conditions.

Given the similarity in gross composition, starter culture, primary proteolysis, and pH of the cheeses produced, the observed sensory and chemical differences are likely attributed to the variations in the scalding temperature used. The cheese produced at the higher scalding temperature (40 °C) displayed slightly more pronounced flavours of maturity, sweetness, sourness, and umami, which are often considered desirable cheese flavour profiles (Lawlor & Delahunty, 2000). Conversely, the cheese scalded at 38 °C was perceived by the sensory panel as exhibiting slightly more oxidised, bitter, and acidic attributes, which are typically less favoured cheese flavours (Cadwallader & Singh, 2009). Consequently, the cheese scalded at 38 °C was not included in the subjective consumer liking test, due to its slightly less favourable sensory profile. The consumer acceptability test further confirmed that the cheese tested exhibited a desirable flavour profile, leading to a high level of consumer acceptance and willingness to buy. This outcome is of high importance, particularly if there are plans to produce this hard goat milk cheese commercially using the newly developed protocol.

Bacteria must autolyse or be lysed to release intracellular enzymes into the cheeses, resulting in a different enzymatic activity in the cheese, which further may influence the degradation of proteins, peptides, amino acids and FFAs into volatiles. The release of intercellular enzymes from autolysis may also be less specific in their transformation of the different components during ripening than a controlled metabolic enzymatic pathway reaction inside LAB (Ardö, 2021).

Chen et al. (2015) studied the acid production and growth rate of the "thermo-tolerant" *Lc. lactis* ssp. *lactis* TM29 and the well-characterised laboratory strain *Lc. lactis* ssp. *cremoris* MG1363 incubated in broth at 38 °C and 40 °C, and showed that even the "thermo-tolerant" TM29 strain was strongly affected by a temperature of 40 °C. Both strains had a high metabolic production of lactate, formate, and acetate at 38 °C. However, phase-contrast imaging revealed swelling of *Lc. lactis* ssp. *cremoris* MG1363, indicating stress of this bacteria strain even at 38 °C. When increasing the temperature by only one degree from 38 °C to 39 °C, the heat-tolerant TM29 were the only *Lc. lactis* able to grow at this temperature, but at a reduced capacity.

Chen et al. (2015) emphasised that temperatures exceeding 39 °C represent a critical growth threshold for numerous *Lc. lactis* strains. The findings of this study provide a plausible explanation for the observed variations in volatile compounds and organic acids in cheeses produced using scalding temperatures slightly below and above the assumed stress threshold of most mesophilic *Lc. lactis* strains. In instances where the scalding temperature surpasses the temperature threshold, it is likely that certain

mesophilic starter bacteria undergo autolysis or cell lysis, thereby releasing essential intercellular enzymes such as peptidases, esterases, and phosphatases, which play an essential role in the cheese ripening process (Ardö, 2021). The starter used in this study was a DVS culture, while the content of milk-derived enzymes such as plasmin, lipoprotein lipase, alkaline phosphatase, and lactoperoxidase (Moatsou, 2010) remained consistent within each experimental block but potentially varied across different blocks due to different milk batches. The starter culture used contains both mesophilic (*Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris*) and thermophilic (*Str. thermophilus* and *Lb. helveticus*) bacteria. The two scalding temperatures used during the production of this cheese, therefore, most probably represent the range of a crucial threshold for survival and autolysis of the mesophilic starter bacteria during cheese ripening. This threshold will further determine the enzymatic activity in the cheeses during ripening, and most probably explains the differences observed.

The higher content of lactate, formate, and α-ketoglutarate 24 h after starter addition in the cheese scalded at 38 °C might indicate a higher metabolic activity in these cheeses by the starter LAB. The mesophilic starter culture is an active acid producer, especially the more heat-sensitive *Lc. lactis* ssp. *cremoris* (Lee & Collins, 1976). The higher content of galactose and α-ketoglutarate in cheese scalded at 38 °C also indicate a higher activity of the thermophilic *Str. thermophilus* (Tanous, Kieronczyk, Helinck, Chambellon, & Yvon, 2002) than in cheese scalded at 40 °C. In addition, in ripened cheese, the cheese scalded at 38 °C also had a higher concentration of diacetyl (2,3-butanedione) and 2,3-butandiol, of which the latter is the oxidised and reduced version of acetoin. The starter bacteria in this experiment are Cit^- and could not produce acetoin from citrate, but all strains of *Lc. lactis* can convert Asp to acetoin through the same metabolic pathway (Ardö, 2006). As Asp normally accumulates in cheese during ripening (Skeie, Kieronczyk, Næss, & Østlie, 2008), these results indicate the possibility of a higher metabolic activity of the survived mesophilic starter in the cheese scalded at 38 °C during ripening.

The CE analysis revealed that the primary proteolysis of casein in the cheeses was similar, thereby confirming that the observed variations in volatiles are unlikely to originate from differences in the primary proteolysis. This finding suggests that other factors or mechanisms than the initial proteolysis are responsible for the composition of volatile compounds observed in the cheeses. Each of the identified functional groups of volatile compounds is known to contribute to the specific sensory characteristics that make the overall flavour profile of cheese. Notably, the cheese scalded at 40 °C exhibited a significantly higher total content of alcohols and ketones compared with the cheese scalded at 38 °C.

The reducing conditions in the cheese create an environment that favours the formation of alcohols through the conversion of aldehydes and ketones. Despite their relatively high levels, alcohols themselves play a limited role in shaping the aroma of cheese. However, they play a crucial role in the production of esters which are characterised by their floral and fruity notes, contributing to the overall aroma profile of cheese by mitigating the sharpness and bitterness that can arise from fatty acids and amines (Ferreira, Pinho, & Sampaio, 2009). Notably, a study by Carbonell, Nuñez, and Fernández-García (2002) found that ewe cheeses of superior quality exhibit higher concentrations of esters and free fatty acids, further underscoring the significance of ester formation in relation to cheese aroma.

By extending the ripening period of the newly developed cheese beyond the 18 weeks used in this study, there may be a potential for increased esterification, which can contribute to the development of desirable flavours. The increased levels of octanoic, decanoic and, hexanoic acid in the cheese scalded at 40 °C (also denoted as caproic, caprylic, and capric acid), are well-documented fatty acids commonly found in higher amounts in goat milk compared with ewes' and cows' milk (Markiewicz-Keszycka, Czyzak-Runowska, Lipinska, & Wójtowski, 2013). As demonstrated by Gan, Yan, Linforth, and Fisk (2016) hexanoic acid is characterised by a sweaty, pungent, and rancid nature, and octanoic acid contributes to a goaty and waxy flavour, while decanoic acid is responsible for imparting fatty and citrusy odours. A study by Shiota, Iwasawa, Suzuki-Iwashima, and Iida (2015) identified ethanol, ethyl acetate, hexanoic acid, and octanoic acid as the metabolites exhibiting the highest sensory scores for sweetness, fruity aroma, and sulphurous when mapping factors of importance for the preference of cheese flavours by Japanese consumers. These two studies (Gan et al., 2016; Shiota et al., 2015) demonstrate the challenges associated with the sensory characterisation of individual compounds, thereby highlighting the intricate nature of the collaboration between different volatile compounds and their perception.

Oxidised flavour in cheese is often related to light-induced oxidation of FFAs (Mortensen, Bertelsen, Mortensen, & Stapelfeldt, 2004; Wold, Jørgensen, & Lundby, 2002). The cheese in this experiment was vacuumed and stored in a dark maturation room during the 18 weeks of cheese ripening, making light-induced oxidation a less probable cause of the oxidised flavour observed in the cheese scalded at 38 °C. Sample C-38° was perceived as the cheese with the most oxidised odour and flavour, but also being the sample showing the lowest content of both organic acids and volatiles. Therefore, the absence of other flavour compounds in the cheese scalded at 38 °C might have resulted in a higher perception of oxidised odour and flavour compared with the cheese scalded at 40 °C. However, in general, a low score for these attributes (oxidised odour and flavour) was found in all cheese samples.

The richness of the volatile compounds found in the cheese scalded at 40 °C was most probably of importance for the attributes sweet and umami taste as well as mature flavour in the goat milk cheese developed in this study, this needs further investigations.

The results of this study indicate that the ideal scalding temperature for this type of cheese should be equal to or higher than 40 °C to increase the autolysis of the mesophilic bacteria, releasing more intracellular enzymes that will enhance the development of volatile compounds in the cheese, and might lower the perception of the less desirable attributes like oxidised flavours. The possibility of a more uncontrolled enzymatic activity in the cheese scalded at 40 °C may explain why they were more diverged in their sensory properties, and why the sensory properties of the cheese scalded at 38 °C were more consistent. The scattered result of the 40 °C cheese might also show the importance of holding a steady temperature during the scalding stage, since one vat at one point was 0.7 °C higher, which might have started other enzymatic reactions or caused an even higher degree of autolysis of the starter bacteria. Although this was only one sample, this might indicate that a scalding temperature closer to 41 °C could be desirable for this type of cheese and should be further investigated.

The influence of the lactation stage of the milk was not investigated in this experiment, however, as the production of goat milk is, to a high degree, season based. The influence of lactation/season most probably needs to be revealed before this cheese can be put into production. The cheese developed in this study could open a new avenue for the utilisation of Norwegian goat milk, which normally has been used for brown whey cheese or fresh cheeses. In this study, the cheese was ripened for 18 weeks but could most probably be suitable for longer ripening times. This cheese variety has, therefore, most probably a potential to be sold at varying maturity levels depending on the preferences by the consumers, which should be tackled in future studies.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2023.105767.

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