International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.12675

ORIGINAL RESEARCH

Salt reduction in film-ripened, semihard Edam cheese

WOLFGANG HOFFMANN,[1] GIUSEPPINA LUZZI,[2] MARCO STEFFENS,[1] INGRID CLAWIN-RÄDECKER,[1] CHARLES M A P FRANZ[2] and JAN FRITSCHE*[1]

[1]*Department of Safety and Quality of Milk and Fish Products* , and [2]*Department of Microbiology and Biotechnology, Max Rubner-Institut, Hermann-Weigmann-Str. 1, 24103 Kiel, Germany*

As a potential measure to improve public health, this study aimed to reduce the sodium (Na) content of film-ripened, semihard Edam cheese to ≤0.4 g Na/100 g (≤1 g NaCl/100 g), while retaining typical quality and safety characteristics. For this, mineral salt substitutions containing potassium (K) were compared with simple NaCl reduction in brine, alongside an adjustment of starter cultures in an effort to enhance taste. Desired Na and K values were achieved, and microbial quality was not compromised in Na-reduced Edam after six weeks of ripening. However, all Na-reduced cheeses tasted bitter and were therefore organoleptically unsatisfactory.

Keywords Cheese, Ripening, Dairy processing, Organoleptic properties, Chemical composition, Cheese microbiology.

INTRODUCTION

High intake of the mineral sodium (Na), which forms an ionic compound with chloride (NaCl), can increase blood pressure and is therefore an important risk factor for cardiovascular and kidney disease. While the World Health Organization (WHO 2012) recommends ≤5 g NaCl as the daily sodium intake for adults, the German Nutrition Society (DGE) recommends ≤6 g NaCl/day (Strohm *et al.* 2016). However, in Germany 70% of women and 80% of men consume more salt than recommended. Indeed, an intake of >10 g NaCl/day is reached by 39% of German women and 50% of German men (Strohm *et al.* 2016). Therefore, the German Federal Ministry of Food and Agriculture promotes the 'reformulation' of prepacked food with less salt, but also with less fat and sugar, and has initiated a comprehensive innovation research programme to support the national reformulation strategy (Federal Ministry of Food and Agriculture 2018).

Dairy products, including cheese, contribute to 10–11% of NaCl consumption in the nutrition of German women and men (Max Rubner-Institut 2008). Improvements in cheese technology, as well as hygienic progress, theoretically allow for a reduction in the salt content. However, a typical semihard cheese such as Edam still contains about 0.6–0.8 g Na/100 g (1.5–2.0 g NaCl/100 g) in Germany.

NaCl has a complex function in the manufacturing process of cheese and affects the microbiological, physicochemical and biochemical, rheological and sensory properties (International Dairy Federation 2014). Therefore, a reduction of NaCl in cheese has to consider many different aspects impacting cheesemaking and product characteristics. The effects of sodium reduction on functionality, sensory properties and public health were reviewed by Cruz *et al.* (2011), emphasising the need for further knowledge regarding acceptable salt levels in Na-reduced cheese.

Much scientific work focusing on the reduction or substitution of NaCl in ripened cheese was published during the last three decades. The aim of studies carried out by Barth *et al.* (1989) and Prokopek *et al.* (1990) was to manufacture an organoleptically acceptable, naturally ripened Edam cheese with ≤0.45 g Na/100 g. The resulting cheeses met the requirements for a standard variety during a shelf life of 12 weeks. However, partial substitution of Na with potassium (K) or calcium (Ca) was not successful, as the resulting cheeses exhibited a bitter taste.

More than 40 studies between the years of 1982–2012 dealing with the substitution of NaCl

*Author for correspondence. E-mail: jan.fritsche@mri.bund.de

by potassium chloride (KCl) were analysed by Hoffmann in 2014. Flavour defects, in particular bitterness and loss of salty flavour, remained a problem. Further studies have reported on the reduction of sodium in a variety of cheeses including dry-salted Cheddar (e.g. Murtaza *et al.* 2014), Gouda (Ruyssen *et al.* 2013), Danish Samsoe (Sondergaard *et al.* 2015) and Tybo (Sihufe *et al.* 2018). In the production of Na-reduced Gouda (Ruyssen *et al.* 2013), one-third of NaCl in the brine was substituted by KCl. In addition to the usual starter cultures, *Lactobacillus* (*Lb.*) *helveticus* und *Lb. paracasei* were implemented as adjunct strains in an effort to improve taste. There were no significant differences in the chemometric results, but a trained taste panel determined significant differences in saltiness, bitterness, texture and preference between the reference NaCl-brined cheeses and the NaCl/KCl-brined cheeses.

In further studies, mixtures of NaCl/KCl and flavour enhancers (L-arginine, yeast and oregano extract) were used in typical Brazilian semihard probiotic Prato cheese (Silva *et al.* 2018a,b). Extensive analyses including quantitative descriptive analysis and temporal dominance of sensations (TDS) for sensory profiling were applied. Sodium reduction and the use of probiotic cultures may be an effective alternative for the production of a potentially functional cheese. Sodium reduction, addition of xylooligosaccharides, yeast extract and arginine were also explored by Ferrão *et al.* (2018) in requeijão cremoso processed cheese, another typical Brazilian cheese. These authors demonstrated that it was possible to manufacture a potentially prebiotic cheese with 50% Na reduction and 80% fat reduction that showed comparable physicochemical, rheological and sensory characteristics to the full-fat, regular-salt product. Coalho, another popular Brazilian cheese with a mild aroma and a firm but soft texture, is traditionally manufactured with at least 2% NaCl to maintain its texture during heating. Costa *et al.* (2018) studied the effect of partial substitution of NaCl by KCl on the characteristics of Coalho and in a consumer test demonstrated that partial replacement of up to 50% NaCl by KCl may be a feasible alternative. Furthermore, São João cheese from Pico Island (Portuguese Azores) is a regionally highly consumed cheese with a high salt content and a moderate to intense aroma. Considering sensory, physicochemical and microbiological results, Soares *et al.* (2015) demonstrated that a 25% salt reduction in this cheese is feasible on an industrial scale as it was not detected by the regular consumer.

The ambitious aim of the present study was to reduce the Na content of film-ripened, semihard Edam cheese to ≤0.4 g Na/100 g (≤1 g NaCl/100 g), while retaining the typical quality and microbiological safety of this cheese. This was carried out by applying simple NaCl reduction and mineral salt substitution mixtures containing Na and K to reduce overall sodium content, as well as by implementing specific starter and adjunct cultures in an effort to improve taste.

MATERIALS AND METHODS

Materials

Raw bovine milk for cheese production was obtained from the experimental farm (Schaedtbek, Germany) at the Max Rubner-Institut (MRI). The starter cultures implemented were obtained from Chr. Hansen (Nienburg, Germany). The species descriptions, as provided by the manufacturer, are outlined below. Further species clarification was not obtainable due to the trade secret of their recipes. The F-ES Easy-Set® FLORA™ C-1060 culture contained *Lactococcus* (*Lac.*) *lactis* subsp. *cremoris*, *Lac. lactis* subsp. *lactis* biovar diacetylactis, *Lac. lactis* subsp. *lactis* and an unspecified *Leuconostoc* species. F-DVS CR-550 was a mixed culture of *Lactobacillus* (*Lb.*) species and *Lac. lactis* subspecies, while F-DVS LH-32 contained only *Lb. helveticus*. Finally, F-DVS CR-BUTTERY01 contained *Lb. paracasei*, *Lb. rhamnosus* and *Lac. lactis* subsp. *lactis*.

Different mineral salts and salt mixtures were used in the brine: NaCl (food salt, AkzoNobel, Hengelo, The Netherlands), KCl (KaliSel, K + S Kali-Chemie GmbH, Kassel, Germany), sub4salt® with NaCl, KCl and Na-gluconate (24 g Na/100 g, 11 g K/100 g, 45 g Cl/100 g; Jungbunzlauer Ladenburg GmbH, Ladenburg, Germany), LomaSalt® 2.0 with 21.8 g Na/100 g and 17.5 g K/100 g (Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), Salona™ containing natural mineral salts from the Dead Sea with 2 g Na/100 g, 12 g K/100 g, 39 g Cl/100 g and 8 g Mg/100 g (ICL Food Specialties, Ladenburg, Germany) and Lactosalt Optitaste, a dairy mineral salt with 8 g Na/100 g, 30 g K/100 g, 40 g Cl/100 g and 3.6 g lactose/100 g (Armor Proteines, France, distributed by Draco Ingredients GmbH, Hamburg).

Cheese production

Cheese was manufactured in a pilot plant at the MRI cheese laboratory. Time lapse, temperature settings, pH values and addition of ingredients are presented in Table 1. The raw milk was skimmed using a disc centrifuge (GEA Westfalia Separator Group, Oelde, Germany). A calculated amount of the separated cream was added to the skimmed milk to adjust the fat content (2.45–2.70 g/100 g) according to the protein content (3.30–3.55 g/100 g). The adjusted milk was pasteurised using an APV Rosista milk heater (300 L/h; Unna, Germany) and cooled. Further manufacturing was performed in a cheese vat. During warming of the milk, 0.02 g $CaCl_2$/100 g and 0.015 g KNO_3/100 g were added. The freeze-dried starter cultures were thawed in warm water (30 °C) for 1 h prior to addition to the milk. For inoculation of the cheese milk with starter cultures, 14 units of F-ES Easy-Set® Flora™ C-1060, 35 units of F-DVS CR-550, 4.5 units of F-DVS LH-32 and 15 units of F-DVS CR-BUTTERY01 were added. For curd gelation, 9 mL/180 kg of microbial rennet (Hannilase® XP 750 NB, 100%

Table 1 Production process for film-ripened Edam cheese.

Time lapse	*Manufacturing step*	*pH value*
Pre-processing	Centrifugation (48 °C) and standardisation of fat content in raw milk. Pasteurisation (73 °C/18 s) and cooling (8 °C) until next day	
0 min	Slow warming of cheese milk (180 kg) to 32 °C, addition of $CaCl_2$ and KNO_3	6.69–6.72
1 h	Addition of starter cultures	
1 h 30 min	Addition of microbial rennet	6.55–6.58
2 h 15 min	Cutting of the gel (with 5–7 mm cube edge length), stirring (32 °C)	6.48–6.52
2 h 45 min	First whey drainage (40% of cheese milk weight), stirring	
2 h 47 min	Begin scalding and addition of water (45–47 °C), stirring	
3 h 15 min	End scalding (water addition: 30% of cheese milk weight), curd/whey mixture at 38.5 °C, stirring with increased speed	
3 h 45 min	End of stirring	
3 h 55 min	Transfer of curd into 16 cheese moulds, pressing in whey (100 kPa)	6.36–6.40
4 h 10 min	Turning of loaves and pressing (200 kPa) for 30 min at room temperature (23–24 °C)	
4 h 45 min	Turning of loaves and pressing (300 kPa) for 1 h	
6 h	Transfer of loaves to brine (pH 5.30, 13–14 °C, 0.13 g Ca/100 g), brining for 45 min to 4 h with constant brine movement, drying of the surface for 16 h at 14 °C/80% relative humidity	5.40–5.45
22 h	Packaging into cheese ripening film, vacuuming and sealing, shrinking the film in hot water (90 °C) for 2–3 s, begin ripening	5.32–5.40
6 weeks	End of ripening at 13 °C	5.46–5.56

mucorpepsin, produced by submerged fermentation with *Rhizomucor miehei*; Chr. Hansen, Nienburg, Germany) was used. After cutting and curd/whey treatment in the cheese vat, the curd was transferred into 16 cylindrical plastic cheese moulds with individual press covers (diameter and height 14 cm; MilkySky GmbH, Lauben, Germany). After three pressing cycles with increasing pressure (Table 1), the resulting loaves were individually weighed and marked. Subsequently, the loaves were distributed equally into two brine solutions of different composition per experiment. The addition of 0.13 g Ca/100 g brine (as $CaCl_2 \times 2\ H_2O$) was recommended by a commercial dairy as the typical content in brine. One brine solution always contained 17 g NaCl/100 g brine and served as the classically salted Edam control cheese, whereas the second brine solution was adapted to the specific experiment, for example through the implementation of sub4salt® (Table 2). The loaves lay between two perforated sheets of stainless steel so that they were continually covered with the brine. After varying brine retention times, the loaves were transferred to wooden boards and allowed to dry until the next morning. They were then packed into plastic film shrink bags for cheese (300 × 400 mm; IP Ingredients, Suederluegum, Germany), vacuum packed and sealed (PTHW-KV-410-T-1, Allpax, Lauben, Germany). Before ripening, the packed cheeses were immersed briefly in hot water to shrink the plastic film.

In addition to alterations in brine composition and retention times, an experiment in which the milk used for the production of sodium-reduced cheese was deliberately inoculated with 1×10^5 cfu/mL of *Listeria* (*L.*) *innocua,* to simulate contamination with the related pathogen

Table 2 Effect of brine composition and retention time on the Na and K contents of Edam during preliminary tests (n = 2 brines each, 2 cheeses per brine).

	Na and K contents in ripened cheese (g/100 g)	
Mineral salts in brine, retention time	*Na (Mean ± SD)*	*K (Mean ± SD)*
17% NaCl, 45 min	0.33 ± 0.00	0.07 ± 0.00
17% NaCl, 1 h	0.50 ± 0.01	0.08 ± 0.00
17% NaCl, 2 h	0.58 ± 0.04	0.08 ± 0.00
17% NaCl, 3 h	0.67 ± 0.03	0.06 ± 0.00
17% NaCl, 4 h	0.77 ± 0.06	0.05 ± 0.00
10% NaCl + 8% KCl, 3 h	0.38 ± 0.02	0.46 ± 0.01
10% NaCl + 8% KCl, 4 h	0.43 ± 0.02	0.53 ± 0.02
9% NaCl + 9% Salona™, 1 h	0.33 ± 0.01	0.16 ± 0.00
9% NaCl + 9% Salona™, 2 h	0.40 ± 0.04	0.18 ± 0.01
9% NaCl + 9% Salona™, 3 h	0.42 ± 0.01	0.17 ± 0.01
9% NaCl + 9% Salona™, 4 h	0.43 ± 0.02	0.17 ± 0.01
19% LomaSalt® 2.0 + 1.5% NaCl, 1 h	0.34 ± 0.00	0.34 ± 0.00
19% LomaSalt® 2.0 + 1.5% NaCl, 2 h	0.37 ± 0.00	0.39 ± 0.01
19% LomaSalt® 2.0 + 1.5% NaCl, 3 h	0.40 ± 0.01	0.45 ± 0.01
19% LomaSalt® 2.0 + 1.5% NaCl, 4 h	0.42 ± 0.02	0.47 ± 0.02
14% NaCl + 4% KCl, 1 h	0.41 ± 0.02	0.21 ± 0.01
14% NaCl + 4% KCl, 2 h	0.49 ± 0.00	0.26 ± 0.01
20% sub4salt®, 1 h	0.34 ± 0.02	0.20 ± 0.02
20% sub4salt®, 2 h	0.42 ± 0.03	0.23 ± 0.03
12% NaCl + 5% Lactosalt Optitaste, 1 h	0.24 ± 0.00	0.17 ± 0.01

SD, standard deviation; % = g/100 g brine.

L. monocytogenes during manufacturing, was performed in biological triplicates. *Listeria innocua* is recommended by the 'European Union Reference Laboratory for *L. monocytogenes*' for implementation as a surrogate for the pathogen *L. monocytogenes* in pilot plant studies based on comparable growth conditions (EURL 2019). All other technical parameters remained the same as indicated above.

Finally, selected cheese variations were manufactured in the laboratory of a commercial dairy under conditions simulating industrial manufacturing. The starter cultures and microbial rennet were the same as before. Manufacturing conditions were adapted to the larger film-ripened loaves with a weight of about 15 kg. In particular, the retention period of the cheese loaves in the brine had to be extended considerably. Varying retention times in the brine resulted in low salt (short retention time) and regular salt (long retention time) variants for both a NaCl brine and a mineral salt brine using sub4salt®. The exact brine retention times of these 15 kg loaves were not revealed by the commercial dairy laboratory.

Chemical and physical analyses

The development of pH was monitored during cheese manufacture and ripening by using an insertion pH electrode (SenTix® Sp, WTW, Weilheim, Germany). For analysis of the ripened cheese, 3.5 cm thick slices were cut from the middle of two cheeses of each batch. Approximately 1 cm of the outer cheese surface (rind) was removed and the remaining core was grated prior to analysis. After six weeks of ripening, the pH and the content of dry matter, fat, protein, as well as NaCl in cheese were determined according to German standard methods (VDLUFA 2003). Technical triplicates of the grated core of two loaves from the same brining batch were analysed.

Na and K were analysed using ion-sensitive electrodes according to Rabe (1983) in a Konelab 20i (Thermo Fisher Scientific, Waltham, MA, USA). Homogenisation of the cheese samples using Tris buffer (pH 7.8; Tris and HCl from Merck, Darmstadt, Germany) was carried out in a Stomacher blender (Stomacher Mix 1, Kleinfeld Labortechnik, Gehrden, Germany) for 6 min at maximum speed and subsequently tempered at 80 °C in a shaking water bath. Technical quadruplicates of cheese samples from two loaves of the same brining batch were analysed. Before analysing the cheese loaves of each experiment, calibration curves were constructed considering the measuring range of the electrodes. The calibration curve for Na (nine measured values) ranged from 20 to 200 mmol/L (≈0.46 g Na/100 g), and the curve for K (seven measured values) ranged from 2 to 20 mmol/L (≈0.078 g K/100 g). The calibration solutions used were NaCl or KCl, dissolved in Tris buffer. Additionally, recovery tests of each analysed cheese were performed. Before homogenisation of the cheese using Tris buffer in the Stomacher blender, adequate amounts of NaCl and KCl were added and their recovery was checked. These tests confirmed the applicability and consistency of the method. The content of Na in cheeses containing only NaCl was also determined according to German standard methods (VDLUFA 2003), and the Na results were confirmed using an ion-selective electrode.

Analysis of peptides

For liquid chromatography–mass spectrometry (LC-MS) analysis of peptides in cheese, 2.5 g of cheese was homogenised in 50 mL of 0.1 M sodium phosphate buffer (pH 6.7) in a Stomacher blender (Stomacher Mix 1, Kleinfeld Labortechnik, Gehrden, Germany) for 6 min at maximum speed and room temperature. The cheese extract was adjusted to pH 4.6 with 4 M HCl and centrifuged at 4000 *g* at 4 °C for 30 min. A volume of 0.5 mL was removed from the supernatant and purified using a 200 mg solid phase extraction column (Strata-X Polymeric SPE, Phenomenex®, Aschaffenburg, Germany). Each cheese sample was extracted in duplicate, and each extract was analysed with LC-MS in triplicate and compared to a sample of commercial Edam cheese used as a control sample.

The determination of peptide profiles was performed with LC-MS using an HPLC system (UltiMate™ 3000 RSLCnano system, Thermo Fisher Scientific, Bremen, Germany) and an LTQ XL™ linear ion trap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). For chromatographic separation, a PepSwift™ monolithic capillary LC column (200 µm × 25 cm nanoViper™ column; Thermo Fisher Scientific, Bremen, Germany) with a PepSwift™ monolithic guard column (200 µm × 5 mm nanoViper™ column, Thermo Fisher Scientific, Bremen, Germany) was used at a flow rate of 1 µL/min and a column temperature of 40 °C. The mobile phases consisted of bi-distilled water with 0.1 mL/100 mL formic acid, and 80 mL/100 mL acetonitrile with 0.1 mL/100 mL formic acid, respectively, and applied in a linear gradient between 2 and 50% acetonitrile for 40 min. Samples were kept at 5 °C until injected, and the injection volume was 1 µL. The MS experiments were performed in the positive ion mode using nanospray ionisation with a spray voltage of 2 kV and a capillary temperature of 200 °C. A data-dependent scan with fragmentation of the three most intense ions (activation type = CID [35 eV]) and an isolation width of 2 Da was performed. MS data interpretation was performed with a Proteome Discoverer 1.4 (Thermo Fisher Scientific, Bremen, Germany) using the search algorithms SEQUEST and MASCOT without enzyme specification. The UniProtKB database (The UniProt Consortium 2019) restricted to *Bos taurus* was used for identifying peptides according to their MS values (Li *et al.* 2019), and the peptides identified by the algorithm were manually verified. Briefly, the identified peptide sequences were verified by comparing the measured fragment spectra of the peptides to the theoretical peptide

fragments. The spectra have to match at least five y-, b- or a-ions of the theoretical peptide fragments. Furthermore, all major fragment masses of the spectra with intensities greater than 10–20% of the maximum intensity in the MS/MS must match theoretical peptide fragments. The peak area of the selected precursor ion of each peptide was calculated relative to the peak area of the same peptide in a commercial Edam cheese sample.

Microbiological analyses

For microbiological testing, samples were taken at five time points during manufacturing in the MRI cheese laboratory: after inoculation of the milk with starter cultures, from the curd directly before pressing, and after one, three and six weeks of ripening. Biological triplicates of each cheese production were analysed, and each bacterial count was measured in technical triplicates.

For testing, 10 g of cheese were placed in a BagFilter® 400 P (Interscience for Microbiology, Saint Nom, France) laboratory blender bag with a <250 μm lateral filter, to which 90 mL of 2 g/100 mL sodium citrate solution (pre-warmed to approx. 30 °C) was added. The bag was placed in a BagMixer® (Interscience for Microbiology, Saint Nom, France) laboratory blender for 2 min at maximum speed. One mL of the filtered supernatant was diluted in a tenfold dilution series. Volumes of 100 μL of appropriate dilutions were spread-plated onto M17 agar (Terzaghi and Sandine 1975) to determine the bacterial count (cfu/g = colony-forming units per gram) of lactococci and onto MRS agar (VWR International GmbH, Darmstadt, Germany) to determine the total number of lactobacilli (MRS pH 5.7) or leuconostocs (MRS pH 5.4). For *Listeria* counts, the samples were spread-plated onto ALOA agar (Ottaviani *et al.* 1997).

In addition, all samples taken after six weeks of ripening were tested for possible contamination with either enterobacteria by using Violet Red Bile Dextrose medium (VRBD, Merck, Darmstadt, Germany), enterococci by using Kanamycin Esculin Azide Agar (KAA, Merck, Darmstadt, Germany), yeast or moulds using Yeast Glucose Chloramphenicol Agar (YGC, VWR International GmbH, Darmstadt, Germany) and pseudomonads using Cetrimide Agar (CFC medium as described by Merck (2010) with the addition of 1 g/100 mL Delvocid® Instant [DSM Food Specialties, Delft, Netherlands] and 10 mL/100 mL glycerine [Carl Roth, Karlsruhe, Germany]). The plates were incubated aerobically at 25 °C for 48 h (M17, MRS pH 5.4, YCG, CFC), 43 °C for 48 h (MRS pH 5.7), 30 °C for 24 h (VRBD) and 37 °C for 24 h (ALOA) or for 48 h (KAA).

Water activity (a_w) was measured at all of the above-mentioned time points using a HygroLab C1 bench-top indicator with digital a_w humidity–temperature probes (Rotronic Measurement Solutions, Bassersdorf, Switzerland).

Sensory analyses

A pool of 40 panellists (25 female/15 male) was trained at the MRI in Kiel for the perception of cheese odour and taste. At least 20 panel members participated in each sensory test. Descriptive sensory analysis (profile testing) according to DIN EN ISO 13299:2016-09 (2016) was performed. Odour was tested for intensity, sour and off-odour, while taste included intensity as well as bitter, salty, sour, metallic and off-taste. The perception of each of these attributes was scored between 0 (none) and 4 (very strong).

Additionally, a consumer test according to DIN EN ISO 11136:2017-10 (2017) with 64 participants (employees of the MRI in Kiel and Hamburg) was carried out on the cheeses produced in the cheese laboratory of the dairy under simulated industrial conditions. These cheeses were assessed by 35 women and 29 men, of which 26 were ≤40 years old while 38 were >40 years old. Here, the only criterion tested was 'preference' (measured using a nine-point hedonic scale ranging from 'dislike extremely' to 'like extremely').

Study replications

Following preliminary experiments, three biological replicates for Na-reduced cheese production with either a NaCl or a mineral salt brine (sub4salt®) were analysed. Three independent experiments were performed for cheese with and without *L. innocua* co-inoculation. The arithmetic mean was calculated for each experiment, and error calculations were indicated as standard deviation or standard error of the mean in microbiological analyses. Further details are indicated in the table and figure legends.

RESULTS AND DISCUSSION

Chemical and physical properties

Initially, film-ripened Edam cheese was produced in the MRI cheese laboratory using a range of mineral salts in the brine with different retention periods of the pressed loaves (Table 2). The goal of these preliminary tests was to obtain benchmarks of Na and K content in the resulting cheeses. All starter cultures with the exception of F-DVS BUTTERY01 were implemented in these initial cheese production experiments. The cylindrical loaves of Edam cheese had an average mass of 1 kg, with their dry matter ranging between 52.1 and 54.7 g/100 g. An average pH value of 5.40 ± 0.08 after cheese ripening was measured. The results of Na and K contents analysed by ion-sensitive electrodes are presented in Table 2. The content of Na was between 0.24 and 0.77 g/100 g in cheese. Nine different salt/time combinations in the brine resulted in cheeses with ≤0.4 g Na/100 g, corresponding to the aim of this study. Fourteen combinations had ≤0.3 g K/100 g and, as sensory tests showed that K content exceeding this value in ripened cheese presented a bitter taste (results not shown), brine/

retention time combinations resulting in >0.3 g K/100 g were not suitable for Na reduction in Edam cheese and were disregarded for further experiments. Cheeses that were salted in pure NaCl brine contained 0.05–0.08 g K/100 g. These data correspond with the average value of 0.067 g K/100 g in semihard cheese stated in the Federal Ministry of Food and Agriculture (2017).

Evaluation of the preliminary experiments allowed for the selection of seven variants of brine/retention combinations, six with mineral salt combinations and one simply with reduced NaCl (Table 3). The chosen combinations of NaCl and the commercial mineral salts in the brine are based on their Na and K contents. Our aim was to produce cheeses with low Na content and a K content of ≤0.3 g/100 g. Sub4salt® was applied in the brine without additional NaCl, as the proportion of Na and K in this product resulted in the desired content of these minerals in the resulting cheeses (Table 2). Each of the mineral salt combinations was tested against a pure NaCl cheese, always immersing eight of the pressed loaves in the control brine (17 g NaCl/100 g brine, 45 min) and eight loaves in the brine with the different mineral salts. To increase aroma development, the starter cultures were supplemented with F-DVS BUTTERY01 (recommended by Chr. Hansen, Nienburg, Germany). The results outlining Na, K and dry matter in the cheese are presented in Table 3. The average dry matter for all combinations was between 53.68 and 55.83 g/100 g. Therefore, the minimum dry matter of 53% for German standard Edam cheese with 40% fat in dry matter was always exceeded. This desired effect was achieved by a more intensive curd treatment, which resulted in higher whey drainage during cheese manufacture compared to the preliminary experiments. An effect of the higher dry matter was that the Na content was continuously lower than 0.4 g/100 g. The average pH value of all cheeses analysed 24 h after the addition of starter cultures was 5.35 ± 0.05, which was 0.03 higher than in the preliminary tests. After ripening, the pH had increased to 5.51 ± 0.03, which was 0.12 higher than in the preliminary tests. This increase in pH value may be explained by proteolysis influenced by the additional adjunct starter culture F-DVS BUTTERY01.

The Na content in the cheeses that were immersed in the pure NaCl brine was 0.24–0.34% (Table 3), a result of the slightly higher dry matter, as mentioned above. For example, a dry matter of 55.6 g/100 g resulted in only 0.24–0.27 g Na/100 g and a K content of 0.06–0.07 g/100 g, confirming preliminary results. The cheeses salted in brine containing mineral salts had 0.20–0.27 g Na/100 g and 0.13–0.20 g K/100 g. The aim for <0.4 g Na/100 g was also achieved for the mineral salt cheeses.

Of the seven variants of brine/retention combinations, one of the mineral salt variants (sub4salt®; brine retention time of 1.5 h), the simple NaCl reduction variant (short brine retention time of 1 h = low salt, extended by 15 min for a slightly higher Na content) and a reference using the NaCl brine without shortened retention time (long brine retention time of 4 h = regular salt) were chosen mainly for detailed microbiological analyses in a further set of cheese production experiments (Table 4). These experiments used identical processing conditions as before. The sub4salt® mineral salt mixture was chosen because its composition was suitable to achieve the Na content aims of this project. The preliminary tests suggested sub4salt® could be applied without the need for additional NaCl in the brine, contrary to all other substitution salts tested. However, clear sensory advantages between any of the mineral salt products tested

Table 3 Effect of brine composition and retention time on the Na, K and dry matter contents of seven selected Edam variants (n = 1 brine each, 2 cheeses per brine).

	Na, K and DM contents (g/100 g)		
Mineral salts in brine, retention time	*Na (Mean ± SD)*	*K (Mean ± SD)*	*DM (Mean ± SD)*
17% NaCl, 45 min	0.33 ± 0.02	0.06 ± 0.00	54.07 ± 0.13
6% NaCl + 12% LomaSalt® 2.0, 1 h	0.24 ± 0.02	0.20 ± 0.00	54.45 ± 0.27
17% NaCl, 45 min	0.34 ± 0.03	0.07 ± 0.00	53.68 ± 0.13
9% NaCl + 9% Salona™, 80 min	0.27 ± 0.02	0.15 ± 0.00	53.91 ± 0.25
17% NaCl, 45 min	0.30 ± 0.01	0.07 ± 0.00	54.28 ± 0.22
14% NaCl + 4% KCl + 10% Trehalose, 1 h	0.24 ± 0.03	0.16 ± 0.00	55.13 ± 0.12
17% NaCl, 45 min	0.29 ± 0.02	0.06 ± 0.00	54.97 ± 0.06
20% sub4salt®, 1.5 h	0.26 ± 0.01	0.17 ± 0.00	55.41 ± 0.12
17% NaCl, 45 min	0.27 ± 0.02	0.06 ± 0.00	55.64 ± 0.13
20% sub4salt®, 1 h	0.20 ± 0.02	0.17 ± 0.00	55.64 ± 0.33
17% NaCl, 45 min	0.24 ± 0.02	0.06 ± 0.00	55.62 ± 0.08
13% NaCl + 4% Lactosalt Optitaste, 1 h	0.20 ± 0.02	0.13 ± 0.00	55.83 ± 0.25

SD, standard deviation; DM, dry matter; % = g/100 g brine.

Table 4 Chemical and physical properties of the brine composition and retention times for Na-reduced Edam produced in the MRI pilot plant for detailed microbiological analyses (n = 3) and in the research laboratory of a commercial dairy (n = 1).

	Na, K and DM contents (g/100 g)		
Mineral salts in brine, retention time	*Na (Mean ± SD)*	*K (Mean ± SD)*	*DM (Mean ± SD)*
Pilot plant			
17% NaCl, short retention time (low salt)	0.29 ± 0.05	0.06 ± 0.00	55.43 ± 0.19
17% NaCl, long retention time (regular salt)	0.43 ± 0.04	0.06 ± 0.00	55.77 ± 0.47
20% sub4salt®, short retention time (low salt)	0.21 ± 0.01	0.16 ± 0.00	56.06 ± 0.23
Commercial dairy laboratory			
17% NaCl, short retention time (low salt)	0.33	0.08	55.79
17% NaCl, long retention time (regular salt)	0.56	0.07	57.56
20% sub4salt®, short retention time (low salt)	0.29	0.19	54.16
20% sub4salt®, long retention time (regular salt)	0.44	0.27	54.46

SD, standard deviation; DM, dry matter; % = g/100 g brine; brining times for the commercial dairy laboratory samples were up-scaled to achieve similar Na and K values as in the MRI pilot plant cheeses.

in preliminary experiments were not noticed. The dry matter of these manufactured cheese loaves was between 55.43 and 56.06 g/100 g as a result of the protein content (≥3.51 g/100 g) and corresponding adjusted fat content (≥2.68 g/100 g) being at the upper limit of the milk used for cheese production. The NaCl cheeses with short and long retention times contained 0.29 and 0.43 g Na/100 g, respectively, and both contained 0.06 g K/100 g on average. The cheese with sub4salt® had 0.21 g Na/100 g and 0.16 g K/100 g on average.

Cheeses produced in the laboratory of the commercial dairy had pH values between 5.46 and 5.53. As mentioned previously, the cheese loaves had a weight of about 15 kg and required a considerably prolonged brining time in contrast to the 1 kg loaves manufactured in the MRI laboratory. Nevertheless, the terms 'short' and 'long' retention times, resulting in 'low' and 'regular' salt content, were retained as the resulting cheeses were comparable to those manufactured in the MRI pilot plant (Table 4). The two variants immersed in the NaCl brine had an average dry matter of 55.79 g/100 g (short retention time = low salt) and 57.56 g/100 g (long retention time = regular salt), respectively, while the cheeses immersed in sub4salt® brine had average dry matter of 54.16 g/100 g (low salt) and 54.46 g/100 g (regular salt) (Table 4). The additional variant with extended retention in the sub4salt® brine (regular salt) was produced to get additional information on resulting Na and K contents with minimum effort. The NaCl-brined cheeses of the low and regular salt variations had a Na content of 0.33 and 0.56 g/100 g and a K content of 0.08 and 0.07 g/100 g, respectively. The sub4salt® brined cheese variants with low and regular salt contained 0.29 and 0.44 g Na/100 g, and 0.19 and 0.27 g K/100 g, respectively. Both the mineral salt substitution method and the reduced NaCl method could be applied successfully to production in the larger 15 kg format used in the commercial dairy research laboratory. However, the Na and K values measured in the cheese experiments performed in the MRI pilot plant were minimally lower than those measured in the samples produced in the commercial cheese dairy laboratory. This discrepancy could be due to slightly variable diffusion rates based on the different loaf weight (1 kg at the MRI and 15 kg in the commercial dairy).

Effect of salt reduction on proteolysis

Analysis of the peptides in Na-reduced Edam by LC-MS revealed a complex peptide pattern depending on the maturation stage of cheese samples, but showed no effect of brine composition or brining time. Bitterness in cheese is commonly associated with the accumulation of hydrophobic peptides formed through the hydrolysis of α_{s1}- and β-caseins (Baptista *et al.* 2017). Two large, hydrophobic peptides released from β-casein (β-CN) could be identified by their fragmentation patterns of the MS^2-spectra. The peptides β-CN f193-209 and β-CN f194-209, the latter of which has been shown to be extremely bitter (Visser *et al.* 1983; Ardö *et al.* 2017), could be quantified relative to a commercial Edam cheese sample (Figure 1). The highest amounts of both peptides were found after one week of cheese maturation, declining during further cheese ripening and this was independent of brine composition. No effect of salt reduction on the generation of these bitter peptides was found, but further analysis of the influence of salt reduction on proteolysis and the role of taste enhancing peptides (Harth *et al.* 2018) should be done in further studies.

Growth of starter cultures

Chemical and physical analyses were complemented by microbiological studies to determine the effect of different brine composition/retention times (simple NaCl reduction

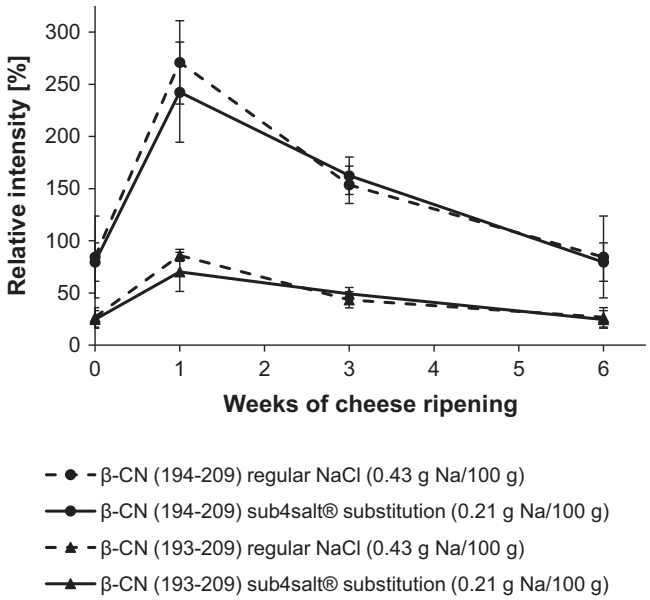

Figure 1 Relative intensity [%] of the peptides β-CN f194-209 (mass-to-charge-ratio [m/z] 859, MH^+ 1718 Da) and β-CN f193-209 (m/z 941, MH^+ 1881 Da) during cheese ripening. The peak area of the selected precursor ion of each peptide was calculated relative to that of a commercial Edam cheese sample. Depiction: arithmetic mean (n = 3) ± standard deviation.

and sub4salt® substitution) on the growth of starter cultures during manufacturing and ripening of Edam cheese in the MRI cheese laboratory. The bacterial count of *Lactococcus* species increased rapidly from approx. 5×10^7 cfu/mL at inoculation to 5×10^8 cfu/g in the curd, further increasing to 1×10^9 cfu/g after one week, before decreasing to approx. 5×10^8 cfu/g and 3×10^7 cfu/g at three and six weeks, respectively (Figure 2a). The number of *Leuconostoc* species increased slightly from 1×10^5 cfu/mL at inoculation to approx. 3×10^5 cfu/g in the curd, before rising to and remaining between 5×10^6 cfu/g and 1×10^7 cfu/g for the remainder of the ripening time (Figure 2a). Finally, *Lactobacillus* species counts developed from 6×10^6 cfu/mL at inoculation to approx. 2×10^7 cfu/g in the curd before reaching, and remaining at, 1×10^8 cfu/g from one to six weeks of ripening (Figure 2a). Both the sub4salt® and the low NaCl as well as the regular NaCl cheeses showed similar bacterial growth in cheese. The progression of microbial growth of the starter and adjunct cultures during fermentation and ripening in Na-reduced Edam followed a typical development for Edam as documented in previous studies (Wachowska 2011; Ruyssen *et al.* 2013; Porcellato and Skeie 2016). As Edam cheese is mainly manufactured from pasteurised milk, nonstarter lactic acid bacteria (NSLAB) originating from the raw milk are of little significance. The interaction of starter lactic acid bacteria and NSLAB was reviewed by Blaya *et al.* (2018), who emphasised that the main source of NSLAB in cheese is the raw milk.

In addition, bacterial counts for *Lactococcus*, *Lactobacillus* and *Leuconostoc* species showed a similar progression both with and without co-inoculation with *L. innocua* (Figure 2b). The *Listeria* counts showed approx. 3×10^5 cfu/mL at inoculation, rising to 8×10^5 cfu/g in the curd, reaching a maximum at approx. 1×10^6 cfu/g after one and three weeks, before decreasing slightly to 7×10^5 cfu/g after six weeks. The presence of *L. innocua* did not affect starter LAB growth, and the reduced sodium conditions did not lead to any substantial change in bacterial growth patterns of *L. innocua*. As *L. innocua* was implemented as a surrogate for *L. monocytogenes* based on its comparable growth conditions, it can be inferred that the growth of the pathogen *L. monocytogenes* would not be affected by the reduced Na conditions tested in this study.

After six weeks of ripening, selective agar tests for enterobacteria, enterococci, yeasts, moulds and pseudomonads showed bacterial counts of $<1 \times 10^2$ cfu/g (limit of detection), regardless of the brining conditions of the cheese samples, suggesting that the microbial quality of the Na-reduced Edam produced in this study was not compromised by these spoilage and potentially opportunistic pathogens. In addition, the a_w values of sodium-reduced Edam (both mineral salt substitution and simple NaCl reduction) were similar to standard sodium cheeses after six weeks of ripening (Table 5). The a_w values always exceeded 0.97, and the sodium content in the aqueous cheese phase was between 0.48 and 0.98 g/100 g water in cheese. As the preservative effect of NaCl lies in its ability to reduce a_w (Guinee and Fox 2017), our findings suggest that the NaCl content of the sodium-reduced Edam produced both with and without co-inoculation with the *L. monocytogenes* surrogate *L. innocua* did not affect preservation of these cheeses. However, further testing for additional pathogens is necessary.

Sensory characteristics

Following preliminary experiments, the cheeses produced in seven variants with different brine/retention combinations (Table 3) were evaluated by the MRI sensory panel considering the defined criteria. Additionally, commercial Edam cheese with 53.95 g/100 g dry matter, 40% fat in dry matter, 0.82 g Na/100 g and a pH of 5.32 was also organoleptically tested. This Edam originated from the same commercial dairy where the cheeses simulating industrial manufacturing were produced. The loaf was pressed in a bread form, had a weight of about 3 kg and was ripened for six weeks. The Na content corresponding to more than 2 g NaCl/100 g approximately represents the upper limit of Na in commercial German Edam cheese. The commercial Edam used for comparison was saltier and less bitter, but also sourer than all sodium-reduced cheeses produced (data not shown). All other variants showed no differences in any

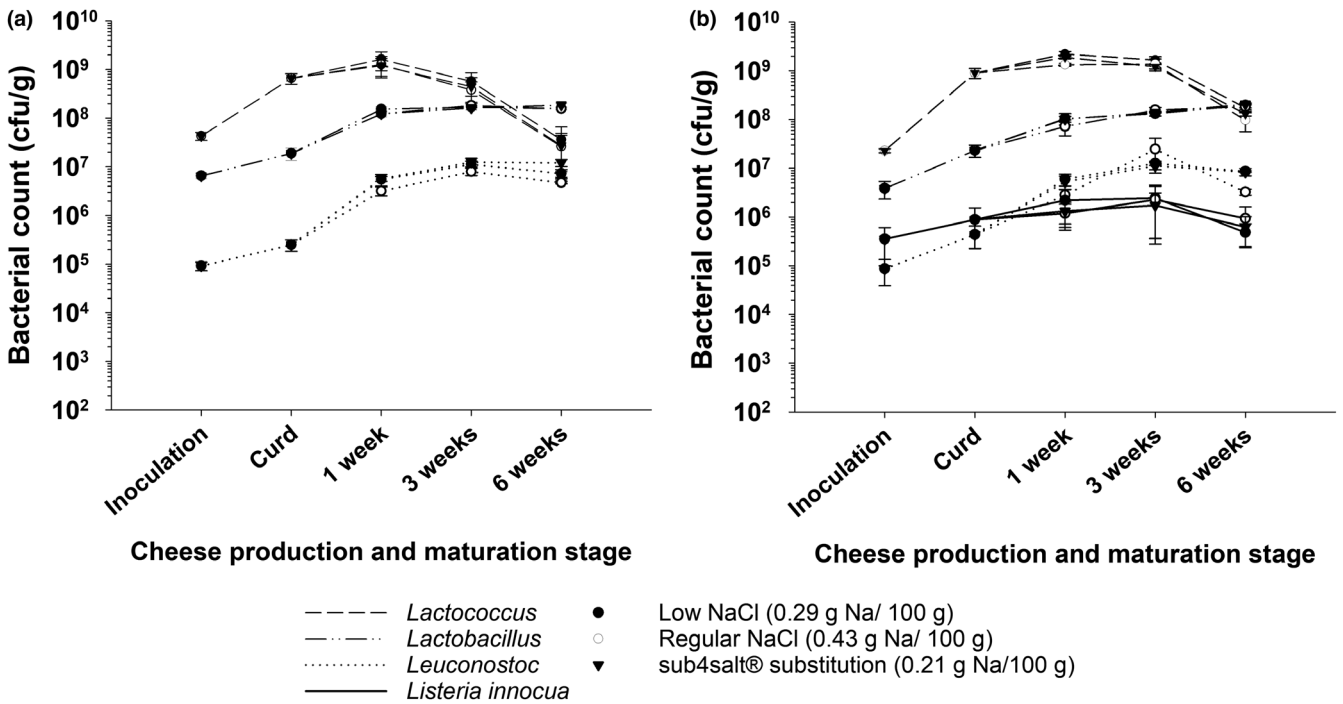

Figure 2 Lactic acid bacterial counts of *Lactococcus*, *Leuconostoc* and *Lactobacillus* species during (a) standard cheese production and ripening (n = 3), and (b) during cheese production co-inoculated with 1 × 10^5 cfu/mL *Listeria innocua* (n = 3). Samples were taken directly after inoculating the pasteurised milk with starter cultures, from the curd immediately prior to pressing and from cheese samples after one, three and six weeks of ripening at 13 °C. Depiction: arithmetic mean (n = 3) ± standard error of the mean.

criterion. This trend was also observed in the simple NaCl reduction cheeses, supporting the notion that salt masks bitter taste occurring in cheeses of standard sodium content. Visser *et al.* (1983) found that the β-CN f193-209 peptide resulted in a very bitter taste and a study by Møller *et al.* (2013) in Cheddar cheese reported that the release of this peptide was Na-sensitive. However, our results showed no difference in the release of this peptide in relation to reduced Na content (Figure 1). Additional bitter peptides and other causes must therefore be investigated in further studies. Based on the preliminary results, the sub4salt® and the low NaCl cheeses were further organoleptically tested and showed similar trends to the preliminary experiments (data not shown).

To simulate industrial processing, cheese blocks of about 15 kg passed through a NaCl brine or through a sub4salt® brine for two different retention times, producing cheeses with low and regular salt content (Table 4). The Na and K contents and the sensory profile analysis after cheese ripening are presented in Figure 3. The intensity of salty taste reflected the analysed Na content of these cheeses, which ranged from 0.29 to 0.56 g/100 g. From these results, it is evident that the intensity of salty taste corresponds to the total taste intensity. Off-odour was only perceived in cheeses that passed through the sub4salt® brine and these cheeses also presented a more bitter taste. In contrast, a difference in the intensity of metallic taste was not detected in all four cheeses (Figure 3). The bitter taste possibly overlapped other forms of off-taste so that they were not perceived by the

Table 5 Water activity of Na-reduced Edam produced with and without *Listeria innocua* co-inoculation (n = 3, mean values).

Sampling time point	*Cheese variation*	*Edam*	*Edam with* Listeria innocua
Processing	Curd	0.999	0.997
Week 1	Low NaCl	0.988	0.987
	Regular NaCl	0.981	0.984
	sub4salt® substitution	0.989	0.987
Week 3	Low NaCl	0.977	0.978
	Regular NaCl	0.975	0.977
	sub4salt® substitution	0.983	0.981
Week 6	Low NaCl	0.977	0.979
	Regular NaCl	0.977	0.976
	sub4salt® substitution	0.981	0.982

The standard error of the mean for all samples was ≤0.004; low NaCl = 0.29 g Na/100 g; regular NaCl = 0.43 g Na/100 g; sub4salt® substitution = 0.21 g Na/100 g.

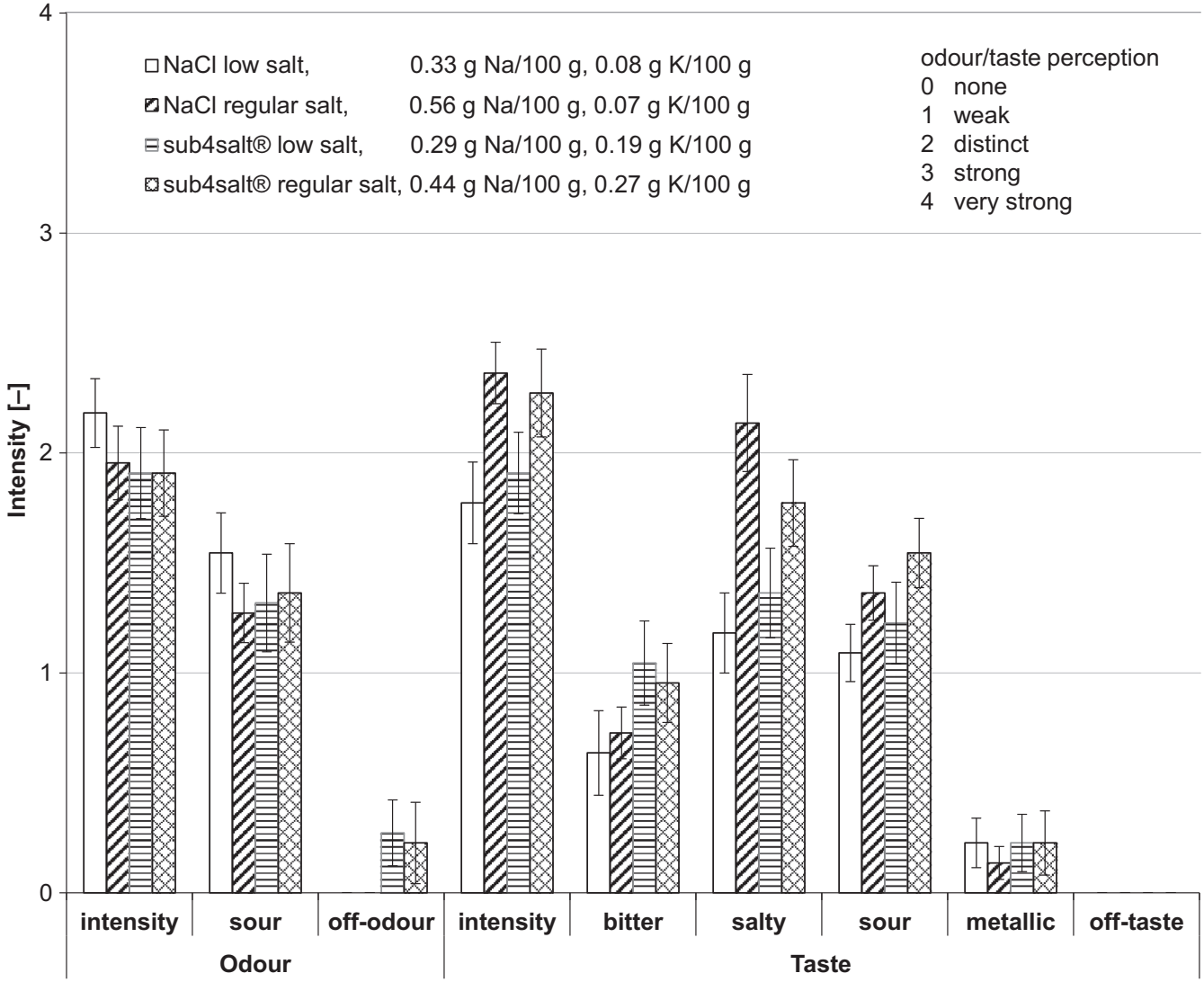

Figure 3 Sensory profile of ripened Edam cheese produced in 15 kg loaves at the research laboratory of a commercial dairy, displaying different brine composition (NaCl and sub4salt®) and retention times (low salt and regular salt) of the cheese loaves. Depiction: arithmetic mean (n = 22) ± standard error of the mean.

panel. As off-flavour is an interaction of off-odour and off-taste, it can be noted that the cheeses passing through the sub4salt® brine developed such a flavour.

These findings agree with the bitterness and off-flavours originating from the addition of potassium that have been reported in a previous study focussing on partial substitution of NaCl with KCl in the related, Dutch-type cheese Gouda (Ruyssen *et al.* 2013). Many studies on salt reduction in various cheese types, as well as other foods (e.g. fish products), have shown that the replacement of NaCl with KCl is possible up to about 30% (Hoffmann 2014; Giese *et al.* 2019). The mineral salt substitution product chosen for further analyses in this study (sub4salt®) claims to allow for a 35% sodium reduction compared to conventional salt (Scholten 2007).

The same cheese variants were also tested for preference by a consumer panel without sensory testing expertise (Figure 4). The results of the preference test were graded between 'like slightly' and 'like very much' for all Na-reduced and regular samples. Although there was quite some discrepancy among individual evaluators within the consumer panel, these findings give an indication of consumer preference of the Na-reduced Edam samples among the general population. We are aware that the tests with the MRI sensory panel and with the consumers were only a starting point for more detailed experiments and analyses. Da Silva *et al.* (2014) determined the potency and equivalence of seven different salt substitutes in cream cheese, using magnitude estimation and TDS. The reference cream cheese for salty taste contained 1.0% NaCl. This corresponded to 1.2%

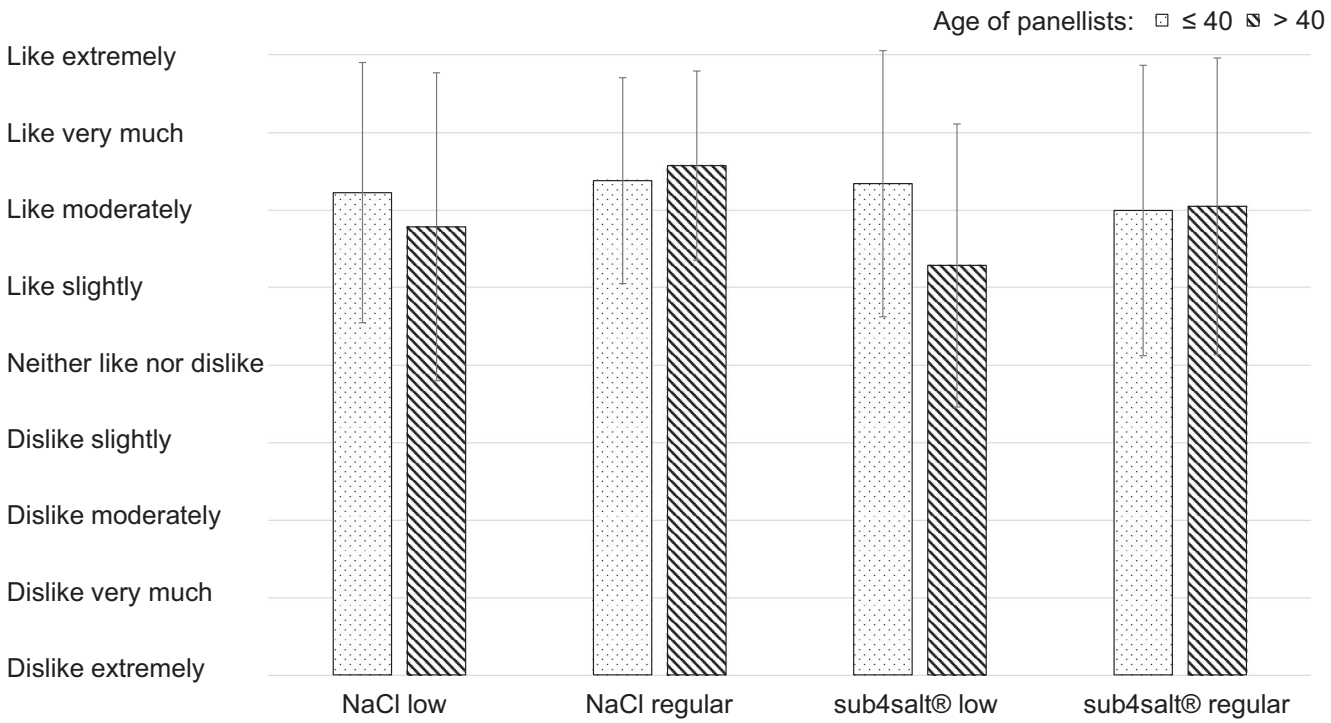

Figure 4 Preference of Na-reduced Edam, displaying different brine composition (NaCl and sub4salt®) and retention times (resulting in low salt and regular salt cheese) and tested by a consumer panel (n = 64). Depiction: arithmetic mean (n = 26 for ≤40 years old and n = 38 for >40 years old) ± standard deviation.

KCl, 2.5% $MgCl_2$ or 2.98% potassium phosphate. The TDS analysis revealed that the seven salt substitutes resulted in other tastes in addition to salty taste, including significant sour and bitter tastes. Therefore, additional TDS experiments with Na-reduced Edam cheese are necessary, especially since ripened cheese is a more complex matrix than cream cheese.

CONCLUSIONS

The present study showed that through the change of brine composition and brining times, combined with adjustment of the implemented starter cultures, production of a sodium-reduced Edam cheese with <0.4 g Na/100 g was possible. Analyses during production and after six weeks of ripening showed that the desired Na and K values in sodium-reduced Edam cheese were achievable without compromising the microbial quality of the cheeses and that there was no effect of salt reduction on the generation of the analysed bitter peptides. However, despite a considerable reduction of Na being technologically possible, the mineral salt substitution cheeses displayed a bitter taste typical for potassium. The addition of adjunct cultures for additional aroma production through increased proteolysis and the generation of volatile compounds did not suffice to prevent the detection of off-flavours. Simple NaCl reduction also resulted in Edam cheese that was bitterer than a commercial cheese with 0.8 g Na/100 g, in comparison with which it was also less salty and less sour. Both the mineral salt substitution method (sub4salt®) and the simple NaCl reduction method could be applied successfully to production in the larger 15 kg format used in the laboratory of a commercial dairy. In general, Na reduction to <0.4 g/100 g is possible in Edam cheese; however, further work needs to be done to reduce off-flavours in Na-reduced cheese.

ACKNOWLEDGEMENTS

This project was supported by funds from the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany under the innovation support programme managed by the Federal Office for Agriculture and Food (BLE). The authors thank Norbert Johannsen and Sandra Kleiner for the manufacture of the cheeses, Angelika Thoß and Cornelia Voß for the chemical and physical analysis of products (Department of Safety and Quality of Milk and Fish Products), and Jennifer Grundmann (Department of Microbiology and Biotechnology) for microbiological analyses. Further thanks to the DMK Deutsches Milchkontor GmbH (Edewecht, Germany) and Chr. Hansen GmbH (Nienburg, Germany) for their technical support.

REFERENCES

Ardö Y, McSweeney P L H, Magboul A A A, Upadhyay V K and Fox P F (2017)Biochemistry of cheese ripening: proteolysis. In *Cheese: Chemistry, Physics and Microbiology*, pp 445–482. McSweeney P L H, Fox P F, Cotter P D and Everett D W, eds. London, UK: Elsevier Ltd.

Baptista D P, da Silva Araujo F D, Eberlin M N and Gigante M L (2017) Reduction of 25% salt in Prato cheese does not affect proteolysis and sensory acceptance. *International Dairy Journal* **75** 101–110.

Barth C, Krusch U, Meisel H, Prokopek D, Schlimme E and De Vrese M (1989) Possibilities and limits of reducing the salt content in semi-hard cheese. *Kieler Milchwirtschaftliche Forschungsberichte* **41** 105–136.

Blaya J, Barzideh Z and LaPointe G (2018) Symposium review: interaction of starter cultures and nonstarter lactic acid bacteria in the cheese environment. *Journal of Dairy Science* **101** 3611–3629.

Costa R G B, Alves R C, da Cruz A G, Sobral D, Teodoro V A M, Junior L C G C, de Paula J C J, Landin T B and Miguel E M (2018) Manufacture of reduced-sodium Coalho cheese by partial replacement of NaCl with KCl. *International Dairy Journal* **87** 37–43.

Cruz A G, Faria J A F, Pollonio M A R, Bolini H M A, Celeghini R M S, Granato D and Shah N P (2011) Cheeses with reduced sodium content: effects on functionality, public health benefits and sensory properties. *Trends in Food Science & Technology* **23** 276–291.

DIN EN ISO 11136:2017-10 (2017) *Sensory analysis - Methodology - General guidance for conducting hedonic tests with consumers in a controlled area*. Berlin, Germany: Beuth Verlag GmbH. https://doi.org/10.31030/2679538

DIN EN ISO 13299:2016-09 (2016) *Sensory analysis - Methodology - General guidance for establishing a sensory profile*. Berlin, Germany: Beuth Verlag GmbH. https://doi.org/10.31030/2496417

European Union Reference Laboratory (EURL) for Listeria monocytogenes (2019). EURL Lm technical guidance document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods [Internet document] URL https://eurllisteria.anses.fr/en/system/files/LIS-Cr-201909D2.pdf. Accessed 25/09/2019.

Federal Ministry of Food and Agriculture (2018) Nationale Reduktions- und Innovationsstrategie: Weniger Zucker, Fette und Salz in Fertigprodukten [Internet document] URL https://www.bmel.de/DE/Ernaehrung/_Texte/ReduktionsstrategieZuckerSalzFette.html. Accessed 14/08/2019.

Ferrão L L, Ferreira M C S, Cavalcanti R N *et al*. (2018) The xylooligosaccharide addition and sodium reduction in requeijão cremoso processed cheese. *Food Research International* **107** 137–147.

Federal Ministry of Food and Agriculture (2017) German Nutrient Database. Bonn, Germany: Federal Ministry of Food and Agriculture.

Giese E, Meyer C, Ostermeyer U, Lehmann I and Fritsche J (2019) Sodium reduction in selected fish products by means of salt substitutes. *European Food Research and Technology* **245** 1651–1664.

Guinee T P and Fox P F (2017) Salt in cheese: physical, chemical and biological aspects. In *Cheese: Chemistry, Physics and Microbiology*, pp 317–375. McSweeney P L H, Fox P F, Cotter P D and Everett D W, eds. London, UK: Elsevier Ltd.

Harth L, Krah U, Linke D, Dunkel A, Hofmann T and Berger R G (2018) Salt taste enhancing L-arginyl dipeptides from casein and lysozyme released by peptidases of basidiomycota. *Journal of Agricultural and Food Chemistry* **66** 2344–2353.

Hoffmann W (2014) Partial substitution of sodium chloride by potassium chloride in natural cheeses. In *Special Issue 1401: The Importance of Salt in the Manufacture and Ripening of Cheese*, pp 74–83. International Dairy Federation, ed. Brussels, Belgium: International Dairy Federation.

International Dairy Federation (2014) *IDF Special Issue 1401: The Importance of Salt in the Manufacture and Ripening of Cheese*. Brussels, Belgium: International Dairy Federation.

Li B, Habermann D, Kliche T *et al*. (2019) Soluble *Lactobacillus delbrueckii* subsp. *bulgaricus* 92059 PrtB proteinase derivatives for production of bioactive peptide hydrolysates from casein. *Applied Microbiology and Biotechnology* **103** 2731–2743.

Max Rubner-Institut (2008) Nationale Verzehrsstudie II, Ergebnisbericht, Teil 2. [Internet document] URL https://www.bmel.de/SharedDocs/Downloads/Ernaehrung/NVS_ErgebnisberichtTeil2.pdf?__blob=publicationFile. Accessed 25/9/2018.

Merck (2010) Microbiology Manual 12th Edition, Merck KGaA, Darmstadt, Germany [Internet document] URL https://www.analytics-shop.com/media/Hersteller/Kataloge/merck-de/Merck_Microbiology_Manual_12th_edition.pdf. Accessed 7/3/2019.

Møller K, Rattray F P, Bredie W L P, Høier E and Ardö Y (2013) Physicochemical and sensory characterization of Cheddar cheese with variable NaCl levels and equal moisture content. *Journal of Dairy Science* **96** 1953–1971.

Murtaza M A, Huma N, Sameen A, Murtaza M S, Mahmood S, Mueen-ud-Din G and Meraj A (2014) Texture, flavor, and sensory quality of buffalo milk Cheddar cheese as influenced by reducing sodium salt content. *Journal of Dairy Science* **97** 6700–6707.

Ottaviani F, Ottaviani M and Agosti M (1997) Differential agar medium for *Listeria monocytogenes*. *Industrie Alimentari* **36** 888.

Porcellato D and Skeie S (2016) Bacterial dynamics and functional analysis of microbial metagenomes during ripening of Dutch-type cheese. *International Dairy Journal* **61** 182–188.

Prokopek D, Barth C A, Klobes H, Krusch U, Meisel H, Schlimme E, De Vrese M and Wotha H J (1990) Reduction of the salt content in Edam cheese in industrial production. *Kieler Milchwirtschaftliche Forschungsberichte* **42** 565–596.

Rabe E (1983) Zur Natrium- und Kaliumbestimmung mit ionensensitiven Elektroden. *Zeitschrift für Lebensmittel Untersuchung und Forschung* **176** 270–274.

Ruyssen T, Janssens M, Van Gasse B *et al*. (2013) Characterisation of Gouda cheeses based on sensory, analytical and high-field H-1 nuclear magnetic resonance spectroscopy determinations: effect of adjunct cultures and brine composition on sodium-reduced Gouda cheese. *International Dairy Journal* **33** 142–152.

Scholten C. (2007) *sub4salt® - Jungbunzlauer's Way to Reduce Sodium*. [Internet document] URL http://www.taejingns.com/s4s/Article%20-%20sub4salt%20-%20Jungbunzlauer%27s%20way%20to%20reduce%20sodium%20Nov07.pdf. Accessed 20/6/2019.

Sihufe G A, De Piante V D, Marino F, Ramos E L, Nieto I G, Karlen J G and Zorilla S E (2018) Effect of sodium chloride reduction on physicochemical, biochemical, rheological, structural and sensory characteristics of Tybo cheese. *International Dairy Journal* **82** 11–18.

da Silva T L T, de Souza V R, Pinheiro A C M, Nunes C A and Freire T V M (2014) Equivalence salting and temporal dominance of

sensations analysis for different sodium chloride substitutes in cream cheese. *International Journal of Dairy Technology* **67** 31–38.

Silva H L A, Balthazar C F, Silva R, Vieira A H, Costa R G B, Esmerino E A, Freitas M Q and Cruz A G (2018a) Sodium reduction and flavor enhancer in probiotic Prato cheese: contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. *Journal of Dairy Science* **101** 8837–8846.

Silva H L A, Balthazar C F, Esmerino E A *et al*. (2018b) Partial substitution of NaCl by KCl and addition of flavor enhancers on probiotic Prato cheese: a study covering manufacturing, ripening and storage time. *Food Chemistry* **248** 192–200.

Soares C, Fernando A L, Mendes B and Martins A P L (2015) The effect of lowering salt on the physicochemical, microbiological and sensory properties of São João cheese of Pico Island. *International Journal of Dairy Technology* **68** 409–419.

Sondergaard L, Ryssel M, Svendsen C, Hoier E, Andersen U, Hammershoj M, Moller J R, Arneborg A and Jespersen L (2015) Impact of NaCl reduction in Danish semi-hard Samsoe cheeses on proliferation and autolysis of DL-starter cultures. *International Journal of Food Microbiology* **213** 59–70.

Strohm D, Boeing H, Leschik-Bonnet E, Heseker H, Arens-Azevedo U, Bechthold A and Kroke A (2016) Salt intake in Germany, health consequences, and resulting recommendations for action. A scientific statement from the German Nutrition Society (DGE). *Ernährungs Umschau* **63** 62–70.

Terzaghi B E and Sandine W E (1975) Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology* **29** 807–813.

The UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research* **47** D506–D515.

VDLUFA (2003) *Methodenbuch VI Milch. In Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. VI*. Darmstadt, Germany: VDLUFA-Verlag.

Visser S, Hup K J and Stadhousers J (1983) Bitter flavour in cheese, 3: comparative gel-chromatographic analysis of hydrophobic peptide fractions from twelve Gouda-type cheeses and identification of bitter peptides isolated from a cheese made with *Streptococcus cremoris* strain HP. *Netherlands Milk and Dairy Journal* **37** 181–192.

Wachowska M (2011) Microbiological changes in Edam-type cheese, brined in a mixture of sodium and potassium chloride during the ripening process. *Milchwissenschaft Milk Science International* **66** 381–384.

World Health Organization (2012) *Guideline: Sodium Intake for Adults and Children*. Geneva, Switzerland: WHO Press.

