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Effect of sodium chloride reduction or partial substitution with potassium chloride on the microbiological, biochemical and sensory characteristics of semi-hard and soft cheeses

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Abstract

Sodium reduction in the human diet is currently one of the main concerns for public health agencies and, consequently, has become a challenge for the food industries. In this study, the impact of reduced sodium chloride content (20%) or its partial substitution with potassium chloride in soft (“Camembert”-type) and semi-hard (“Reblochon”-type) cheeses was evaluated. Analyses included physicochemical and biochemical composition, microbial counts, 16S rRNA gene metabarcoding and metatranscriptomic analysis, volatile aroma compounds and sensory analysis. Regarding soft cheeses, the salt content of cheeses affected proteolysis at 21 days of ripening. RNA sequencing revealed that the relative activity of *G. candidum* increased, whereas that of *P. camemberti* decreased in reduced salt cheeses in comparison to the controls. Higher global intensity of odor and taste was observed in cheeses with reduced salt content, consistent

with higher levels of alcohol and ester components. Regarding semi-hard cheeses, modifications of salt content did not significantly affect either their biochemical parameters and sensory characteristics or their technological microbial composition at day 21 of ripening. Finally, no impact of salt content was observed on the growth of the spoiler *Yarrowia lipolytica* in soft cheeses. In contrast, reducing salt content increased spoiler growth in semi-hard cheeses, as highlighted by a greater development of *Pseudomonas* that led to an increase in cheese proteolysis and lipolysis.

In conclusion, the effect of reducing salt content is highly dependent on the cheese type. This factor should thus be taken into account by the dairy industry when the reduction of salt content is being considered. Moreover, the quality of raw products, in particular, the level of spoiler microorganisms, must be controlled before use during dairy processes.

Keywords

sodium chloride reduction, sodium chloride substitution, soft cheese, semi-hard cheese, biochemical composition, cheese microbiota, sensory characteristics, spoilage microorganisms.

1. Introduction

Excessive sodium intake is known to cause high blood pressure and to increase the risk of hypertension, cardiovascular disease and gastric cancer (Mohan & Campbell, 2009; Song et al., 2017). The World Health Organization has proposed to reduce sodium intake by 30% in order to obtain the WHO guideline of 2 g/day (i.e., 5 g of salt/day) by 2020 (WHO, 2012). In 2015, 75 countries had developed a national sodium reduction strategy (Trieu et al., 2015). This strategy is multifaceted and includes, for example, the establishment of sodium content targets for foods as well as consumer education. Since sodium reduction in the human diet is one of the main objectives for public health agencies, it has become a major challenge for the food industries that have to reformulate products. Indeed, processed foods, including cheese products, are estimated to contribute over 70% to dietary sodium intake (Appel & Anderson, 2010; EFSA, 2006). In France, mean daily salt intakes range from approximately 7 to 10 g of salt and are well in excess of dietary needs. Dietary guidelines recommend a 20% salt reduction in foods to obtain a target of 6.5 to 8 g of salt intake per day (ANSES, 2012).

Cheese is perceived as being a food source that is highly concentrated in sodium, with amounts that can vary depending on the type of cheese. Soft, semi-hard and hard cheeses contain between 0.5% and 2.5% sodium chloride (NaCl), whereas blue-type cheeses contain between 3% and 5% NaCl. Sodium chloride fulfills many important functions in cheese: it modifies the physical properties of the cheese curd and rind (especially maintaining a low *aw*), controls the growth of the cheese-ripening microbiota, limits the development of pathogens or spoilers, and acts as a flavor enhancer (Cruz et al., 2011; Guinee & Fox, 2004; Johnson, Kapoor, McMahon, McCoy, & Narasimmon, 2009). Thus, the sodium reduction strategy must take these parameters into account. This strategy could be achieved either by a reduction in the NaCl level or a partial replacement of NaCl with other salts such as KCl.

66 Several studies have been conducted to characterize the effect of lowering NaCl content on the
67 quality of Cheddar cheeses (Khetra, Kanawjia, & Puri, 2016; McCarthy, Kelly, Wilkinson, &
68 Guinee, 2017; McCarthy, Wilkinson, Kelly, & Guinee, 2015, 2016; Murtaza et al., 2014;
69 Porcellato et al., 2014; Rulikowska et al., 2013), hard or semi-hard cheeses (Baptista, Araújo,
70 Eberlin, & Gigante, 2017; Sihufe et al., 2018; Soares, Fernando, Alvarenga, & Martins, 2016;
71 Soares, Fernando, Mendes, & Martins, 2015; Søndergaard et al., 2015) and white brined
72 cheeses (Arboatti et al., 2014; Ayyash, Sherkat, Francis, Williams, & Shah, 2011; Faccia,
73 Mastromatteo, Conte, & Del Nobile, 2012; Felicio et al., 2016; Kamleh et al., 2012;
74 Thibaudeau, Roy, & St-Gelais, 2015). These studies focused on the sensory properties and
75 physicochemical and biochemical characteristics of cheeses. Moreover, it was shown that a
76 decrease in salt resulted in an increase in **starter and non-starter** lactic acid bacteria (LAB)
77 growth in Cheddar cheese (Rulikowska et al., 2013). However, there is still a lack of
78 information regarding the impact of salt reduction on the whole microbiota of surface-ripened
79 cheeses. It was previously demonstrated that reducing NaCl content in an experimental soft
80 smear-ripened cheese resulted in microbial disturbances such as a lesser development of
81 *Debaryomyces hansenii* and a greater development of the Gram-negative bacteria *Hafnia alvei*
82 (Dugat-Bony et al., 2016). This community disequilibrium was associated with modifications
83 of both the proteolytic kinetics and the volatile aroma profiles. Other studies also highlighted
84 the impact of salt composition on the growth of other ripening cultures such as
85 *Brevibacterium aurantiacum* and *Geotrichum candidum* (Marcellino, Beuvier, Grappin,
86 Guéguen, & Benson, 2001; Mounier, Rea, O'Connor, Fitzgerald, & Cogan, 2007).
87 Consequently, NaCl content represents a major technological factor controlling, at least
88 partially, the main biochemical processes linked with the activity of surface microorganisms,
89 including proteolysis, lipolysis and the production of aroma compounds. Moreover, the
90 microbiological quality of NaCl reduction in cheese has to be further evaluated due to its critical

role for controlling spoilage and pathogenic microorganism growth (Taormina, 2010). Indeed, Cheddar cheeses produced with a 50% reduction in salt content were characterized by higher counts of *L. monocytogenes* than the controls after 50 days at 4°C (Hystead, Diez-Gonzalez, & Schoenfuss, 2013), and salt was shown to inhibit *Pseudomonas spp.* growth in model cheeses, mimicking the surface of soft smear-ripened cheeses (E. Dugat-Bony et al., 2016).

Regarding sensory perception, sodium reduction has been reported to cause detrimental effects on consumer acceptability (Jaenke, Barzi, McMahon, Webster, & Brimblecombe, 2017). One possible approach to limit this pitfall consists of partially replacing sodium chloride with potassium chloride (KCl). Other approaches have been studied: use of flavor enhancers such as monosodium glutamate, hydrolyzed vegetable protein, yeast extract, disodium inosinate and use of bitter blockers (Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013; Khetra, Kanawjia & Puri, 2016). Potassium intake may, in fact, have a positive effect on consumer health (He & MacGregor, 2001), and some studies have reported that partial replacement of NaCl with KCl in soft and semi-hard cheeses did not affect the physicochemical characteristics and organoleptic properties of cheese if the substitution did not exceed a ratio of 30% (Costa et al., 2018, 2019; Grummer, Karalus, Zhang, Vickers, & Schoenfuss, 2012; Soares et al., 2016). Nevertheless, there is still a lack of information regarding the impact of salt substitution on bacteria, yeast and mold development and metabolic activity during cheese ripening.

Understanding how changes in technological parameters can affect microbial functions and, subsequently, the quality of fermented foods, represents a great challenge. Recently, high-throughput sequencing technologies have become very popular to investigate microbial ecology in dairy products (De Filippis, Parente, & Ercolini, 2018; Jonnala, McSweeney, Sheehan, & Cotter, 2018). When combined with other techniques such as other omics, it provides the opportunity to understand the mechanisms that can affect the microbial ecosystem and the final characteristics of the products (Ferrocino & Cocolin, 2017). Only a few examples of such a

global approach to the analysis of cheese products exist, but they still makes it possible to evaluate the effect of the ripening stages (Dugat-Bony et al., 2015) and of temperature and humidity (De Filippis, Genovese, Ferranti, Gilbert, & Ercolini, 2016).

The objective of this study was to investigate the effect of reduced salt content and partial substitution of NaCl with KCl on the quality of soft (“Camembert”-type) and semi-hard (“Reblochon”-type) cheeses using a combination of multi-omics, biochemical and sensory approaches.

2. MATERIALS & METHODS

2.1 Soft and semi-hard cheese production

Soft (“camembert”-type) and semi-hard (“reblochon”-type) cheeses were produced at the ACTALIA Cheese Processing Unit (La Roche-sur-Foron). Cheeses were prepared from pasteurized milk (85°C, 30 seconds) and inoculated with specific starters and spoilage microorganisms when appropriate. Different salt contents were taken into account: standard level, 1.5 g/100 g of cheese (C cheese); reduced level, 80% of NaCl level (R cheese); and substitution level, 80% of NaCl and 20% of KCl levels (S cheese).

For soft cheese, CaCl₂ (470 g/L) was added at 25 mL/100 L of pasteurized milk and mesophilic lactic acid starter (MM100 at 6 DCU/100 L of milk, Danisco) and *Streptococcus thermophilus* (TA052 at 25 DCU/100 L, Danisco), *Geotrichum candidum* (Geo17, Danisco, 1 dose /1000 L) and *Penicillium camemberti* (PC neige Lyo, Danisco, 2 doses/1000 L) were inoculated. When appropriate, *Yarrowia lipolytica* ITFF2145 (Actalia) was inoculated as a spoilage yeast at 10³ cfu/L of milk. This inoculation dose, chosen in partnership with professionals from the dairy sector, corresponded to representative concentrations of field cases. *Y. lipolytica* ITFF2145 was isolated from defective soft cheese in 2013 in France. When the milk reached pH 6.45 and a temperature of 37°C, the rennet (530 mg/L of chymosin, Berthelot) was added (55 mL/100 L).

Coagulation was followed by a 40-min hardening in order to trap intragranular water by achieving a long hardening period. It corresponds to 5 times the rennet clotting time (8 minutes). The cheese curd was then cut (cut size of cheese curd particles: 10 mm x 10 mm) and shaped in polyurethane molds (diameter: 10.5 cm). At the beginning of the molding step, pH was 6.20-6.25 and temperature was 33.5°C. Whey was eliminated and molds were inverted three times. After 20h, cheeses were removed from their mold at pH 4.9-5.10 with a temperature of 16-18°C (day 0). Dry salting then took place (day 1) in order to obtain a final level of 15 g of NaCl/kg of cheese in the control cheese, and 11.5 g of NaCl /kg in the reduced and substituted cheeses. The cheeses (approx. 250 g) were maintained at 13°C and 85% relative humidity (RH) in order to dry their surfaces for 4 hours and were then incubated in a ripening chamber with a relative humidity of 95% for 20 days at 11°C.

Semi-hard cheeses were inoculated with the following starters: *S. thermophilus* and *Lactobacillus bulgaricus* (wild strains, SIR-Flore, Thônes, France, at 1.3 L/100 L), *G. candidum* (Geo2, SIR-Flore, 2 doses/1000 L), *Debaryomyces hansenii* (DHlyo, Danisco, 2 doses/1000 L) and *Brevibacterium linens* (FR22, Danisco, 1 dose/1000 L). *Pseudomonas fluorescens* strains isolated from either raw dairy products, cheese or the dairy environment (ITFF3965, ITFF3966, ITFF3967, ITFF3969) were inoculated as spoilage bacteria at a final concentration of 10⁵ cfu/L of milk. This inoculation dose, chosen in partnership with professionals from the dairy sector, corresponded to representative concentrations of field.

When the pasteurized milk reached pH 6.50-6.55 (temperature of 34°C), the rennet (530 mg/L of chymosin, Berthelot) was added (55 mL/100 L). Then, 30 min after rennet addition, the curd was cut (cut size of cheese curd particles: 20 mm x 20 mm) and gently stirred to eliminate whey. The curd was molded (34°C, pH 6.30-6.35) in polyurethane molds (diameter: 13 cm) and pressed for 90 min. Molds were inverted one time. After 4h, cheeses were removed from their mold (pH 5.15-5.25 and temperature of 24°C). Then, the cheeses were pickled in sterile brine

(density: 1140) at 16°C for a variable length of time, depending on the NaCl level target: 14 g of NaCl/kg of cheese in the control cheese (brining time of 80 min), and 10.5 g/kg in the reduced and substituted cheeses. The brining time was 35 minutes for the reduced cheese. KCl was added by dry salting. This brining step is defined as day 0 of ripening. After incubation for 4 days at 19°C and 100% RH, the cheeses (approx. 500g) were transferred to the ripening room, which was maintained at 13°C and 95-100% RH until day 21. The cheeses were turned every two days.

Soft and semi-hard cheeses were sampled at days 1, 7, 14 and 21. At each sampling day, three cheeses for each salting condition were taken and cut into eight parts. The parts were used for microbiological, 16S rRNA gene metabarcoding, metatranscriptomic and biochemical analyses. Volatile aroma compound profile and sensory analysis were performed at day 21.

2.2. Microbiological analyses

The cheese samples (10 g) were homogenized in a stomacher for 2 min (AES Chemunex, Easy Mix) and diluted up to 10^{-8} by 10-fold serial dilutions. Total microflora were counted according to NF EN ISO 4833, coliforms with NF V 08-050, yeasts and molds with NF V 08-059, and thermophilic lactic acid bacteria with ISO27205. Lactococci were counted on Elliker medium (Difco), *B. linens* on SCB medium (1.5% tryptone, 0.5% soytone, 0.6% yeast extract, 1.2% sodium lactate, 0.25% K_2HPO_4 , 4% NaCl and 1.5% agar) and *Pseudomonas spp.* on GSP medium (Glutamate Starch Phenol red agar, VWR).

2.3. Bacterial community structure using 16S rRNA gene metabarcoding

The kinetic evolution of the bacterial community structure was analyzed using samples from days 7, 14 and 21. DNA extraction from the cheese surface, PCR amplification of the V3-V4 region of the 16S rRNA gene, library preparation and sequencing were performed as previously

described (Eric Dugat-Bony et al., 2016). Paired-end reads were merged using Flash (Magoč & Salzberg, 2011) and analyzed using FROGS (Escudié et al., 2018), according to the standard operating procedure. Briefly, operational taxonomic units (OTUs) were built using Swarm with an aggregation distance of 3 (Mahé, Rognes, Quince, de Vargas, & Dunthorn, 2014), and each OTU that accounted for less than 0.005% of the total set of sequences was discarded, as previously recommended (Bokulich et al., 2013). Lastly, the OTU's affiliation was checked using the EzBiocloud database (Yoon et al., 2017). Merged sequence data were deposited at the Sequence Read Archive of the National Center for Biotechnology Information under the BioProject accession number PRJNA523139.

2.4. Metatranscriptomic analysis

Metatranscriptomic analysis was conducted on samples from day 21. RNA extraction and rRNA depletion were performed as previously described (Monnet et al., 2016). Libraries were prepared with the ScriptSeq™ RNA-Seq Library Preparation Kit (Illumina) and sequenced on an Illumina NexSeq 500 instrument. Data analysis was performed according to the workflow presented in Figure S1. Briefly, raw sequences were preprocessed using Cutadapt v1.12 (Martin, 2011) and filtered with SortMeRNA v2.0 (Kopylova, Noé, & Touzet, 2012). Non-rRNA reads were mapped using TopHat2 v2.1.1 (Kim et al., 2013) against a reference database composed of coding DNA sequences (CDS) extracted from the genome sequences of strains belonging to the same species as the microorganisms that were inoculated into the cheese. Accession numbers of the genomes are available in Table S1 and S2 for soft and semi-hard cheeses, respectively. In order to access the transcriptome of non-inoculated species that develop in cheese as well, unmapped reads were *de novo* assembled using Trinity (Grabherr et al., 2011), and gene prediction was conducted on this assembly using FragGeneScan (Rho, Tang, & Ye, 2010). Unmapped reads were mapped back to these predicted CDS using TopHat2.

Each CDS of the *de novo* assembly was taxonomically annotated using Kaiju (Menzel, Ng, & Krogh, 2016). All CDS from both the reference database and the *de novo* assembly were submitted to the GhostKoala server (Kanehisa, Sato, & Morishima, 2016) for uniformed functional annotation based on KEGG Orthology. The count matrix was processed using custom scripts built under the statistical environment R (<http://www.r-project.org/>) and based on the following packages: Deseq2 (Love, Huber, & Anders, 2014) and gplots (<https://CRAN.R-project.org/package=gplots>). Raw sequence data were deposited at the Sequence Read Archive of the National Center for Biotechnology Information under the BioProject accession number PRJNA523735.

2.5 Physicochemical and biochemical analyses

Cheese pH was measured using a penetration pH electrode (Mettler Toledo LE427-S7). Water activity was measured using a water activity meter (Aqualab, series 4TE). Chloride content was measured using a chloride analyzer (Corning Chloride Analyzer 926), and sodium ion concentration was determined using an Orion Dual Star pH/ISE meter (Thermo Scientific, Villebon-sur-Yvette, France).

D- and L-lactate concentrations were assayed using a commercially available kit (Biosentec, Auzeville Tolosane, France) according to the manufacturer's instructions. The concentrations of different nitrogen fractions were determined to evaluate the influence of reducing NaCl content on proteolysis activity. Total nitrogen (TN) was measured with the Kjeldhal method according to the NF EN ISO 8968 standard, and the non-protein nitrogen (NPN) content and soluble nitrogen (SN) content were measured according to NF ISO 27871. Lipolysis was evaluated by measuring the acid degree value. The number of mg of KOH necessary to neutralize the free acids in 1 g of sample was determined by titration.

2.6 Volatile aroma compound profile

Volatile aroma compounds were quantified at the end of the ripening period (21 days) in three independent cheeses for each condition. The cheese surface was grated and transferred into a 10-mL glass bottle that was tightly sealed before storage at -80°C , prior to aroma compound analysis.

Before analysis, the cheese samples were thawed at 4°C , vigorously shaken and diluted (1/5 for semi-hard cheese and 1/10 for soft cheese) with a cold solution of 2,5-dimethylpyrazine ($546\text{ }\mu\text{g/L}$). This solution was used as the internal standard for the experiment. Moreover, NaCl was added to cheese samples with reduced levels of NaCl to have the same NaCl content in order to prevent a salting out effect on the volatile compounds studied. The mixture was stored at 4°C for one night before analysis. Five milliliters of the mixture were then analyzed using a dynamic headspace analyzer (purge-and-trap concentrator, Tekmar-Dohrmann 3100, Tekmar Inc., Cincinnati, OH, USA) coupled to a gas chromatograph (model 6890, Agilent Technologies, Palo Alto, CA, USA) and a mass spectrometer detector (MSD 5973 inert quadrupole mass spectrometer, Agilent Technologies), and fitted with a $60\text{ m} \times 320\text{ }\mu\text{m}$ i.d. DB-5 MS capillary column (film thickness: $1\text{ }\mu\text{m}$). The oven temperature was programmed as follows: 5°C held for 9 min and then heated to 230°C at a rate of $4^{\circ}\text{C min}^{-1}$, held for 1 min. Volatiles were identified by comparison of their mass spectra with those of NIST98 and Wiley libraries. Peak absolute areas (arbitrary units) were calculated from the total ion current (TIC).

2.7 Sensory analysis

The sensory evaluation was performed using a sensory profile that evaluates the aspect, aroma, texture and taste properties of cheeses after 21 days of ripening. Attribute generation was carried out by 16 trained panelists during two specific preliminary sessions. After the generation step, the panelists agreed on a list of attributes shown in Table 4. A sensory profile using the

descriptive analysis method was then performed according to the NF ISO 13 299 method. The intensity of 22 (for semi-hard cheese) and 24 (for soft cheese) attributes was assessed on 10-point structured intensity scales. Panelists had to successively evaluate the aspect, aroma, texture and taste of the cheeses. Three replicates per product and per panelist were performed in two sessions. Cheese samples were placed on white plates labeled with randomly selected three-digit numbers. Sensory analyses were carried out in an air-conditioned room (20°C) with a hygrometry between 50 and 70% according to the NF ISO 8589 method. Samples were at approximately 15°C when they were tested. Cheeses were evaluated in a monadic way. The presentation order of samples was randomized across panelists to make sure that the run order did not introduce a bias in the results. Panelists were provided with mineral water for rinsing and flatbread for palate cleaning. The data were directly collected and recorded with FIZZ software (Biosystèmes).

2.8 Data analysis

All results were reported as mean \pm standard deviation. All data were subjected to one-way or two-way analysis of variance (ANOVA), and differences were considered significant when $p < 0.05$. A multiple comparison test (Student-Newman-Keuls test) was then applied. Distinct letters (a, b, c) were assigned to significantly different groups, whereas a combination of the corresponding letters was attributed to those located between several groups. Statistical analysis was carried out with XLStat software (Version 2018.4, Addinsoft, France).

3. Results

3-1 Physicochemical and biochemical characteristics

The composition profile at day 21 of ripening of soft and semi-hard cheeses is illustrated in Table 1 and Table 2, respectively. For soft cheese, salting conditions had no impact on pH, **aw**

and lactate content at day 21 of ripening (Table 1, two-way ANOVA, $p > 0.05$). Moreover, dry matter and moisture on fat-free dry matter were not affected by the salting conditions (data not shown). However, salting conditions significantly affected both the SN:TN and the NPN:TN ratios (reflecting primary and secondary proteolysis, respectively), and the lipolysis index (two-way ANOVA, $p < 0.05$). For cheeses produced without *Y. lipolytica*, the SN:TN ratio reached 43.8% in the control cheese, and 55.4% and 50.5% in R and S cheeses, respectively (Table S3). The lipolysis index was higher in R (10.8) and S cheeses (10.7) than in controls (9.1). This result indicated that reducing NaCl content in soft cheese leads to an increase in both proteolytic and lipolytic activities. The lipolysis index was also significantly modified by the presence of *Y. lipolytica*, independently of the salting conditions (Table 1). For example, it was higher in control cheeses produced without *Y. lipolytica* than in spoiled cheeses (9.1 versus 7.7) (Table S3). Finally, the presence of *Y. lipolytica* also significantly affected the final pH detected in the cheese core (Table 1). For semi-hard cheese salting conditions had no impact on pH, aw, lactate content and proteolysis at day 21 of ripening (Table 2). Moreover, dry matter and moisture on fat-free dry matter were not affected by the salting conditions (data not shown). The lipolysis index was affected by the interaction of the salting condition and the presence of the spoilage microflora (two-way-ANOVA, $p < 0.05$). It was higher in the R cheeses than in the C and S cheeses and higher in the non-spoiled cheeses than in the spoiled cheeses. The presence of *Pseudomonas* spp., significantly affected the final pH detected in the cheese core, lactate content and proteolysis, considering both SN:TN and NPN:TN ratios. The final pH values obtained in the cheese core and proteolysis were lower in spoiled cheeses than in non-spoiled cheeses, whatever the salting condition (Table S4). On the opposite, the lactate content was higher in spoiled cheeses than in non-spoiled cheeses.

3-2 Microbial composition and transcriptome analyses of the cheese surface microbiota

316 Cheese samples were analyzed once a week using classical cultivation-based counts (Table 3).
317 In parallel, 16S rRNA gene metabarcoding was used to more deeply observe the composition
318 of the bacterial community (Figures 1A and 1B). Finally, RNA-Seq was used at the end of
319 ripening (day 21) in order to assess the effect of salt content modification on the overall activity
320 of the whole microbial community (Figures 1C and 1D).

321 Regarding soft cheeses, no significant difference was observed for the counts of inoculated
322 lactic acid starter, *P. camemberti* and *G. candidum* between C, R and S cheeses during ripening
323 (Table 3A). RNA sequencing revealed that the relative activity of *G. candidum* increased in R
324 cheeses – at the expense of other dominant microorganisms, namely *Penicillium*,
325 *Debaryomyces*, *Streptococcus* and *Lactobacillus* - in comparison to C cheeses (Figure 1C).
326 Partial substitution of NaCl with KCl (S cheeses) restored an RNA-seq profile highly similar
327 to C cheeses. Differential expression analysis did not reveal important changes in the expression
328 of genes for the major microorganisms present in soft cheeses (data not shown), suggesting a
329 limited impact of salting conditions on the functioning of the individual microbial species that
330 compose the ecosystem. Reducing the NaCl content in soft cheeses had no impact on the
331 development of the inoculated spoilage microorganism (Sp cheeses), i.e., *Y. lipolytica* (data not
332 shown). Moreover, no significant difference was observed for the counts of coliform in control,
333 R and S cheeses during ripening (Table 3A). However, 16S rRNA gene metabarcoding analysis
334 highlighted an increase in the relative abundance of an OTU affiliated with the non-inoculated
335 *Enterobacter* group *aerogenes* in both R and S cheeses at days 14 and 21 when compared to C
336 cheeses (Figure 1A).

337 For semi-hard cheese, the microbial counts of both R and S cheeses were similar to those of the
338 C cheeses (Table 3B). The composition of bacterial communities, as revealed by 16S rRNA
339 gene metabarcoding (Figure 1B) and RNA-Seq profiles (Figure 1D) were also similar for the
340 three salting conditions. Regarding cheeses deliberately inoculated with *Pseudomonas spp.* (Sp

cheeses), we observed significantly higher *Pseudomonas* counts in both R and S cheeses than in C cheeses at day 7 (Figure 2). From day 14, the relative proportion of the OTU affiliated with *Pseudomonas group gessardi* was also higher in R and S cheeses than in C cheeses (Figure 1B). Finally, the expression of *Pseudomonas* genes increased at day 21 in R cheeses and, to a lesser extent, in S cheeses (Figure 1D). Among them, those encoding proteases and peptidases were overexpressed in R cheeses when compared to C cheeses (Figure 3). This was partially corrected in S cheeses. Altogether, these results showed that reducing the NaCl content in semi-hard cheeses caused a higher development and activity of *Pseudomonas spp.*, and that partial substitution of NaCl with KCl partially counterbalanced this effect.

3-3 Sensory characteristics

Regarding soft cheeses, significant differences in flavor perception were highlighted by the panel between the control, R and S cheeses. Indeed, 13/24 attributes were perceived as being significantly different between cheeses (Table 4). Surprisingly, no significant salty taste difference was observed between the C cheeses and the R and S cheeses. Both R and S cheeses were perceived as being significantly more flowing, acidic and bitter, and having more color, a stronger ammonia flavor, animal/cowshed odor and global intensity of odor and taste than C cheeses. In contrast, mushroom flavor was perceived to be higher in C cheeses than in R and S cheeses. The total areas measured by gas chromatography after 21 days of ripening in C and R cheeses were significantly different (Figure 4). No significant difference was noticed between C and S cheeses. High levels of alcohols and esters were measured in R cheeses. For example, the area of ethanol, 2-propanol, 2-butanol and ethylhexanoate were three to seven times higher in R cheeses than in control cheeses (Table S5).

Regarding semi-hard cheeses, no significant differences were observed in texture perception, global aroma, taste intensity and salty perception (data not shown). Salt reduction only affected

bitterness, which was increasingly noticed in both R and S cheeses (medium values of the bitterness attribute were 1.28, 1.8 and 2.03 for C, R and S cheeses, respectively). There was no difference in the volatile compound levels in any of the semi-hard cheeses (data not shown).

4. Discussion

The current study investigated the effect of salt reduction (about 20%) and partial substitution of NaCl with KCl (about 20%) on all microbiological, biochemical and sensory facets of soft and semi-hard cheeses.

The sensory evaluation indicated that saltiness intensity was the same in C, R and S cheeses for both soft and semi-hard cheeses. This result is in contradiction with other studies in which the authors observed a decrease in the perception of the salty attribute with 15 to 30% salt content reduction in Edam (Czarnacka-Szymani & Jezewska-Zychowicz, 2015) and Tybo cheese (Sihufe et al., 2018). However, both studies concluded that cheeses produced with a reduced salt content had a sensory acceptance that was similar to that of the control cheeses. Similar studies conducted on Prato and Coalho cheeses produced with reduced salt content on the order of 25-30% led to the same conclusion (Baptista et al., 2017; Costa et al., 2018).

The ripening of soft cheeses with a bloomy rind, such as Camembert or Brie, is mainly due to the activity of *G. candidum* and *P. camemberti*, and these two fungal species are major contributors to the texture and flavor of this type of cheese (Lessard, Bélanger, St-Gelais, & Labrie, 2012; Marcellino et al., 2001). It is generally accepted that *G. candidum* and *P. camemberti* play a role in the white and bloomy aspect of the rind, the firmness and the thickness, and have typical sensory characteristics due to the lipolysis and proteolysis that occurred during ripening (Boutrou & Guéguen, 2005; Lessard, Viel, Boyle, St-Gelais, & Labrie, 2014). Nevertheless, before this study, no data was available on the consequence of salt reduction on the quality of soft cheeses with a bloomy rind and on the behavior of these species

in this context. We did not observe higher counts of *G. candidum* in both R and S cheeses than in controls during ripening. A previous study on Torta del Casar, a Spanish soft ewe cheese reported that yeast-molds growth was not affected by NaCl content at levels comprised between 0.48 and 2.79% (Tabla, Gómez, Rebollo, & Roa, 2015). However, the relative activity of *G. candidum* in our study- as determined by RNA-seq profiles - increased. Reducing salt content did not significantly affect the counts of *P. camemberti* but led to a decrease of its relative activity. In this type of cheese, reducing NaCl content may thus modify the balance between *G. candidum* and *P. camemberti*. Although not observed in our study, where the NaCl reduction was only about 20%, it has already been reported that *G. candidum* may outcompete *P. camemberti* in cheese produced with low NaCl content and form a surface defect known as ‘toad skin’ (Kindstedt, 2014).

Modification of several sensory attributes, as observed in our results on soft cheese, could be attributed to the change of the balance between the activity of *G. candidum* and *P. camemberti*. Bitterness and ammoniacal flavors are common to cheeses matured by molds and are generally associated with an intense proteolytic activity (Galli, Martin, da Silva, Porto, & Spoto, 2016). For example, the production of small peptides from the breakdown of caseins contributes to the bitterness of cheeses (Engel, Septier, Leconte, Salles, & Quere, 2001; Galli et al., 2016). Bitterness has already been associated with higher proteolysis in Cheddar cheese produced with reduced salt content (Rulikowska et al., 2013). However, the bitterness rating for Cheddar cheese produced with KCl was not significantly different from the full-sodium control (Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013). Because *G. candidum* is strongly proteolytic (Boutrou, Kerriou, & Gassi, 2006), its greater relative activity, as observed with RNA-sequencing, in soft cheese produced with reduced NaCl content or partial replacement of NaCl with KCl could thus have resulted in stronger proteolysis and, consequently, higher bitterness perception.

Sensory analysis also revealed that R soft cheeses and, to a lesser extent, S cheeses, exhibited a higher odor and taste intensity than control soft cheeses. For R cheeses, this stronger perception might be associated with the higher area of total aroma compounds detected by GC-MS and, more precisely, the higher levels of some alcohols and esters. *G. candidum* can produce such compounds, e.g., 2-methylpropanol, 2- and 3-methylbutanol and ethylhexanoate (Boutrou & Guéguen, 2005; Mdaini, Gargouri, Hammami, Monser, & Hamdi, 2006). The production of dimethyl disulfide and other volatile sulfur compounds as well by *G. candidum* again could generate the “animal/cowshed” attribute (Berger, Khan, Molimard, Martin, & Spinnler, 1999), which was also more perceived by panelists in both R and S cheeses.

In our study, a 20% reduction of NaCl content in soft cheeses had no impact on the development of the inoculated spoilage yeast *Y. lipolytica*. However, the relative abundance of the non-inoculated *Enterobacter group aerogenes* was higher. *Enterobacter* sp. has already been detected in cheeses and is generally considered as a contaminant (Coton et al., 2012; Tabla et al., 2016). Controlling the microbial quality of both milk and the dairy environment would thus be crucial when producing soft cheeses with reduced levels of salt.

Semi-hard cheeses with washed rinds such as Reblochon are usually made using lactic acid bacteria and aerobic ripening bacteria like *B. aurantiacum* and yeasts, principally *G. candidum* and *D. hansenii* (Cogan et al., 2014). Our results revealed that salt reduction or partial NaCl substitution with KCl did not affect the physicochemical, biochemical, microbiological and sensory characteristics of this type of cheese, which is in agreement with several other studies performed on hard or semi-hard cheeses (Baptista et al., 2017; Costa et al., 2018, 2019; Sihufe et al., 2018; Soares et al., 2015). Moreover, studies showed that overall acceptance was the same for semi-hard Coalho and Minas Padrão cheeses with 25% KCl and control cheeses (Costa et al., 2018, 2019). However, studies performed on Cheddar, a hard cheese, indicated that decreasing salt content can result in enhanced growth of non-starter lactic acid bacteria at the

beginning of ripening, in higher proteolysis, in lower hardness and in higher off-flavor intensity and, more particularly, bitterness and sulfur attributes (Murtaza et al., 2014; Rulikowska et al., 2013). Other authors showed that salt content did not significantly influence the overall bacterial community structure, except that lower salt levels resulted in enhanced starter survival (Porcellato et al., 2014).

In this study, we observed a greater development of *Pseudomonas spp.* in both R and S cheeses when the spoiler was deliberately inoculated, which confirms the results obtained in previous studies (Dugat-Bony et al., 2016; Tabla et al., 2015). In the presence of *Pseudomonas spp.*, primary and secondary proteolysis as well as lipolysis were higher than in non-spoiled cheeses. Moreover, the expression of several genes encoding for proteases and peptidases in *Pseudomonas spp.* were overexpressed in low NaCl conditions. It is known that these bacteria are able to produce extracellular, heat-resistant proteolytic and lipolytic enzymes (Baur et al., 2015; Meng et al., 2017) that could generate off-flavors, but genes encoding those enzymes remain to be identified. The expression data presented here could be used in future studies to select a reduced set of candidate genes potentially involved in these functions.

In our study, sensory analysis was not performed on semi-hard cheeses spoiled with *Pseudomonas spp.*, but other authors demonstrated that volatile profiles of cheeses made from milk inoculated with *Pseudomonas* strains exhibit high levels of alcohols, esters and sulfur compounds (Morales, Fernandez-Garcia, & Nunez, 2005). For these reasons, once again, the microbial quality of milk and the dairy environment need to be carefully controlled by cheese makers when producing semi-hard cheeses with reduced salt content or the partial substitution of NaCl with KCl.

5. Conclusions

In conclusion, impacts on the physicochemical criteria of cheeses have been observed more intensely in soft cheeses than in semi-hard cheeses. For soft cheeses, sensory analysis detected many more changes related to changes in salting conditions than for semi-hard cheeses. Little impact on the architecture and the quantitative and qualitative composition of the microbial species was observed for either technology when the salting conditions were modified. In addition, NaCl appears to represent a barrier against *Pseudomonas* growth in semi-hard cheeses but not against *Yarrowia* in soft cheeses, which appears to be weakly affected by salt levels. The impact of a reduction in the NaCl level or its substitution with KCl is then dependent on the cheese technology studied. The physicochemical and microbiological modifications related to the salting methods highlighted in this study must be taken into account in order to adapt cheese production practices to the desired organoleptic qualities of the finished products. In addition, the elimination, reduction or substitution of salt should be the subject of specific studies for each type of product while remaining vigilant about the quality of raw material, the microbiological stability of the products and their organoleptic characteristics.

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References

- ANSES. (2012). Avis de l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif au suivi des teneurs en sel des principaux vecteurs entre 2003 et 2011 et simulation des impacts sur les apports en sel de la population française. (No. saisine n°2012-SA-0052).
<https://www.anses.fr/fr/system/files/NUT2012sa0052.pdf>. Accessed 7 March 2019
- Appel, L. J., & Anderson, C. A. M. (2010). Compelling Evidence for Public Health Action to Reduce Salt Intake. *New England Journal of Medicine*, 362(7), 650–652.
<https://doi.org/10.1056/NEJMe0910352>
- Arboatti, A. S., Olivares, M. L., Sabbag, N. G., Costa, S. C., Zorrilla, S. E., & Sihufe, G. A. (2014). The influence of sodium chloride reduction on physicochemical, biochemical, rheological and sensory characteristics of Mozzarella cheese. *Dairy Science & Technology*, 94(4), 373–386. <https://doi.org/10.1007/s13594-014-0169-2>
- Ayyash, M. M., Sherkat, F., Francis, P., Williams, R. P. W., & Shah, N. P. (2011). The effect of sodium chloride substitution with potassium chloride on texture profile and microstructure of Halloumi cheese. *Journal of Dairy Science*, 94(1), 37–42.
<https://doi.org/10.3168/jds.2010-3407>
- Baptista, D. P., Araújo, F. D. da S., Eberlin, M. N., & Gigante, M. L. (2017). Reduction of 25% salt in Prato cheese does not affect proteolysis and sensory acceptance. *International Dairy Journal*, 75, 101–110.
<https://doi.org/10.1016/j.idairyj.2017.08.001>
- Baur, C., Krewinkel, M., Kranz, B., von Neubeck, M., Wenning, M., Scherer, S., ... Fischer, L. (2015). Quantification of the proteolytic and lipolytic activity of microorganisms

514 isolated from raw milk. *International Dairy Journal*, 49, 23–29.

515 <https://doi.org/10.1016/j.idairyj.2015.04.005>

516 Berger, C., Khan, J. A., Molimard, P., Martin, N., & Spinnler, H. E. (1999). Production of

517 Sulfur Flavors by Ten Strains of *Geotrichum candidum*. *Applied and Environmental*

518 *Microbiology*, 65(12), 5510–5514.

519 Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., ...

520 Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from

521 Illumina amplicon sequencing. *Nature Methods*, 10(1), 57–59.

522 <https://doi.org/10.1038/nmeth.2276>

523 Boutrou, R., & Guéguen, M. (2005). Interests in *Geotrichum candidum* for cheese

524 technology. *International Journal of Food Microbiology*, 102(1), 1–20.

525 <https://doi.org/10.1016/j.ijfoodmicro.2004.12.028>

526 Boutrou, R., Kerriou, L., & Gassi, J.-Y. (2006). Contribution of *Geotrichum candidum* to the

527 proteolysis of soft cheese. *International Dairy Journal*, 16(7), 775–783.

528 <https://doi.org/10.1016/j.idairyj.2005.07.007>

529 Cogan, T. M., Chamba, J.-F., Hohenegger, M., Guéguen, M., Ward, A. C., Gelsomino, R., ...

530 Sebastiani, H. (2014). Biodiversity of the Surface Microbial Consortia from

531 Limburger, Reblochon, Livarot, Tilsit, and Gubbeen Cheeses. In C. W. Donnelly

532 (Ed.), *Cheese and Microbes* (pp. 219–250). Retrieved from

533 <http://www.asmscience.org/content/book/10.1128/9781555818593.chap10>

534 Costa, R. G. B., Alves, R. C., Cruz, A. G. da, Sobral, D., Teodoro, V. A. M., Costa Junior, L.

535 C. G., ... Miguel, E. M. (2018). Manufacture of reduced-sodium Coalho cheese by

536 partial replacement of NaCl with KCl. *International Dairy Journal*, 87, 37–43.

537 <https://doi.org/10.1016/j.idairyj.2018.07.012>

538 Costa, R. G. B., Caiafa Junior, A., Gomes da Cruz, A., Sobral, D., Gonçalves Costa Júnior, L.
539 C., Jacinto de Paula, J. C., ... Martins Teodoro, V. A. (2019). Effect of partial
540 replacement of sodium chloride with potassium chloride on the characteristics of
541 Minas Padrão cheese. *International Dairy Journal*, 91, 48–54.
542 <https://doi.org/10.1016/j.idairyj.2018.12.002>

543 Coton, M., Delbés-Paus, C., Irlinger, F., Desmasures, N., Le Fleche, A., Stahl, V., ... Coton,
544 E. (2012). Diversity and assessment of potential risk factors of Gram-negative isolates
545 associated with French cheeses. *Food Microbiology*, 29(1), 88–98.
546 <https://doi.org/10.1016/j.fm.2011.08.020>

547 Cruz, A. G., Faria, J. A. F., Pollonio, M. A. R., Bolini, H. M. A., Celeghini, R. M. S.,
548 Granato, D., & Shah, N. P. (2011). Cheeses with reduced sodium content: Effects on
549 functionality, public health benefits and sensory properties. *Trends in Food Science &*
550 *Technology*, 22(6), 276–291. <https://doi.org/10.1016/j.tifs.2011.02.003>

551 Czarnacka-Szymani, J., & Jezewska-Zychowicz, M. (2015). Impact of nutritional information
552 on consumers' acceptance of cheese with reduced sodium chloride content.
553 *International Dairy Journal*, 40, 47–53. <https://doi.org/10.1016/j.idairyj.2014.08.017>

554 De Filippis, F., Genovese, A., Ferranti, P., Gilbert, J. A., & Ercolini, D. (2016).
555 Metatranscriptomics reveals temperature-driven functional changes in microbiome
556 impacting cheese maturation rate. *Scientific Reports*, 6.
557 <https://doi.org/10.1038/srep21871>

558 De Filippis, F., Parente, E., & Ercolini, D. (2018). Recent Past, Present, and Future of the
559 Food Microbiome. *Annual Review of Food Science and Technology*, 9(1), 589–608.
560 <https://doi.org/10.1146/annurev-food-030117-012312>

561 Dugat-Bony, E., Sarthou, A.-S., Perello, M.-C., de Revel, G., Bonnarme, P., & Helinck, S.
562 (2016). The effect of reduced sodium chloride content on the microbiological and

563 biochemical properties of a soft surface-ripened cheese. *Journal of Dairy Science*,
 564 99(4), 2502–2511. <https://doi.org/10.3168/jds.2015-10502>
 565 Dugat-Bony, Eric, Garnier, L., Denonfoux, J., Ferreira, S., Sarthou, A.-S., Bonnarme, P., &
 566 Irlinger, F. (2016). Highlighting the microbial diversity of 12 French cheese varieties.
 567 *International Journal of Food Microbiology*, 238, 265–273.
 568 <https://doi.org/10.1016/j.ijfoodmicro.2016.09.026>
 569 Dugat-Bony, Eric, Straub, C., Teissandier, A., Onésime, D., Loux, V., Monnet, C., ...
 570 Bonnarme, P. (2015). Overview of a Surface-Ripened Cheese Community
 571 Functioning by Meta-Omics Analyses. *PLOS ONE*, 10(4), e0124360.
 572 <https://doi.org/10.1371/journal.pone.0124360>
 573 EFSA. (2006). Tolerable upper intake levels for vitamins and minerals.
 574 http://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableui
 575 l.pdf. Accessed 7 March 2019
 576 Engel, E., Septier, C., Leconte, N., Salles, C., & Quere, J.-L. L. (2001). Determination of
 577 taste-active compounds of a bitter Camembert cheese by omission tests. *Journal of*
 578 *Dairy Research*, 68(4), 675–688. <https://doi.org/10.1017/S0022029901005209>
 579 Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., ... Pascal, G.
 580 (2018). FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics*, 34(8),
 581 1287–1294. <https://doi.org/10.1093/bioinformatics/btx791>
 582 Faccia, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2012). Influence of the
 583 different sodium chloride concentrations on microbiological and physico-chemical
 584 characteristics of mozzarella cheese. *Journal of Dairy Research*, 79(04), 390–396.
 585 <https://doi.org/10.1017/S0022029912000209>
 586 Felicio, T. L., Esmerino, E. A., Vidal, V. A. S., Cappato, L. P., Garcia, R. K. A., Cavalcanti,
 587 R. N., ... Cruz, A. G. (2016). Physico-chemical changes during storage and sensory

588 acceptance of low sodium probiotic Minas cheese added with arginine. *Food*
589 *Chemistry*, 196, 628–637. <https://doi.org/10.1016/j.foodchem.2015.09.102>

590 Ferrocino, I., & Cocolin, L. (2017). Current perspectives in food-based studies exploiting
591 multi-omics approaches. *Current Opinion in Food Science*, 13, 10–15.
592 <https://doi.org/10.1016/j.cofs.2017.01.002>

593 Galli, B. D., Martin, J. G. P., da Silva, P. P. M., Porto, E., & Spoto, M. H. F. (2016). Sensory
594 quality of Camembert-type cheese: Relationship between starter cultures and ripening
595 molds. *International Journal of Food Microbiology*, 234, 71–75.
596 <https://doi.org/10.1016/j.ijfoodmicro.2016.06.025>

597 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., ... Regev,
598 A. (2011). Full-length transcriptome assembly from RNA-Seq data without a
599 reference genome. *Nature Biotechnology*, 29(7), 644–652.
600 <https://doi.org/10.1038/nbt.1883>

601 Grummer, J., Bobowski, N., Karalus, M., Vickers, Z., & Schoenfuss, T. (2013). Use of
602 potassium chloride and flavor enhancers in low sodium Cheddar cheese. *Journal of*
603 *Dairy Science*, 96(3), 1401–1418. <https://doi.org/10.3168/jds.2012-6057>

604 Grummer, J., Karalus, M., Zhang, K., Vickers, Z., & Schoenfuss, T. C. (2012). Manufacture
605 of reduced-sodium Cheddar-style cheese with mineral salt replacers. *Journal of Dairy*
606 *Science*, 95(6), 2830–2839. <https://doi.org/10.3168/jds.2011-4851>

607 Grummer, J., Bobowski, N., Karalus, M., Vickers, Z., & Schoenfuss, T. C. (2013). Use of
608 potassium chloride and flavor enhancers in low sodium Cheddar cheese. *Journal of*
609 *Dairy Science*, 96(3), 1401–1418. <https://doi.org/10.3168/jds.2012-6057>

610 Guinee, T. P., & Fox, P. F. (2004). Salt in Cheese: Physical, Chemical and Biological
611 Aspects. In P. L. H. M. Patrick F. Fox Timothy M. Cogan and Timothy P. Guinee

612 (Ed.), *Cheese: Chemistry, Physics and Microbiology* (pp. 207–259). Retrieved from
 613 <http://www.sciencedirect.com/science/article/pii/S1874558X04800691>

614 He, F. J., & MacGregor, G. A. (2001). Beneficial effects of potassium. *BMJ : British Medical*
 615 *Journal*, 323(7311), 497–501.

616 Hystead, E., Diez-Gonzalez, F., & Schoenfuss, T. C. (2013). The effect of sodium reduction
 617 with and without potassium chloride on the survival of *Listeria monocytogenes* in
 618 Cheddar cheese. *Journal of Dairy Science*, 96(10), 6172–6185.
 619 <https://doi.org/10.3168/jds.2013-6675>

620 Jaenke, R., Barzi, F., McMahon, E., Webster, J., & Brimblecombe, J. (2017). Consumer
 621 acceptance of reformulated food products: A systematic review and meta-analysis of
 622 salt-reduced foods. *Critical Reviews in Food Science and Nutrition*, 57(16), 3357–
 623 3372. <https://doi.org/10.1080/10408398.2015.1118009>

624 Johnson, M. E., Kapoor, R., McMahon, D. J., McCoy, D. R., & Narasimmon, R. G. (2009).
 625 Reduction of Sodium and Fat Levels in Natural and Processed Cheeses: Scientific and
 626 Technological Aspects. *Comprehensive Reviews in Food Science and Food Safety*,
 627 8(3), 252–268. <https://doi.org/10.1111/j.1541-4337.2009.00080.x>

628 Jonnala, Y., R. B., McSweeney, P. L. H., Sheehan, J. J., & Cotter, P. D. (2018). Sequencing
 629 of the Cheese Microbiome and Its Relevance to Industry. *Frontiers in Microbiology*,
 630 9. <https://doi.org/10.3389/fmicb.2018.01020>

631 Kamleh, R., Olabi, A., Toufeili, I., Najm, N. E. O., Younis, T., & Ajib, R. (2012). The effect
 632 of substitution of sodium chloride with potassium chloride on the physicochemical,
 633 microbiological, and sensory properties of Halloumi cheese. *Journal of Dairy Science*,
 634 95(3), 1140–1151. <https://doi.org/10.3168/jds.2011-4878>

635 Kanehisa, M., Sato, Y., & Morishima, K. (2016). BlastKOALA and GhostKOALA: KEGG
 636 Tools for Functional Characterization of Genome and Metagenome Sequences.

637 *Journal of Molecular Biology*, 428(4), 726–731.

638 <https://doi.org/10.1016/j.jmb.2015.11.006>

639 Khetra, Y., Kanawjia, S. K., & Puri, R. (2016). Selection and optimization of salt replacer,
 640 flavour enhancer and bitter blocker for manufacturing low sodium Cheddar cheese
 641 using response surface methodology. *LWT - Food Science and Technology*, 72, 99–
 642 106. <https://doi.org/10.1016/j.lwt.2016.04.035>

643 Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013).
 644 TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions
 645 and gene fusions. *Genome Biology*, 14(4), R36. [https://doi.org/10.1186/gb-2013-14-4-](https://doi.org/10.1186/gb-2013-14-4-r36)
 646 [r36](https://doi.org/10.1186/gb-2013-14-4-r36)

647 Kindstedt, P. S. (2014). The Basics of Cheesemaking. In C.W.Donnely (Ed), *Cheese and*
 648 *Microbes* (pp 17–38). ASM Press. <https://doi.org/10.1128/microbiolspec.CM-0002-12>

649 Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: fast and accurate filtering of
 650 ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, 28(24), 3211–3217.
 651 <https://doi.org/10.1093/bioinformatics/bts611>

652 Lessard, M.-H., Bélanger, G., St-Gelais, D., & Labrie, S. (2012). The Composition of
 653 Camembert Cheese-Ripening Cultures Modulates both Mycelial Growth and
 654 Appearance. *Applied and Environmental Microbiology*, 78(6), 1813–1819.
 655 <https://doi.org/10.1128/AEM.06645-11>

656 Lessard, M.-H., Viel, C., Boyle, B., St-Gelais, D., & Labrie, S. (2014). Metatranscriptome
 657 analysis of fungal strains *Penicillium camemberti* and *Geotrichum candidum* reveal
 658 cheese matrix breakdown and potential development of sensory properties of ripened
 659 Camembert-type cheese. *BMC Genomics*, 15(1), 235.

660 Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and
661 dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 550.
662 <https://doi.org/10.1186/s13059-014-0550-8>

663 Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast Length Adjustment of Short Reads to
664 Improve Genome Assemblies. *Bioinformatics*, btr507.
665 <https://doi.org/10.1093/bioinformatics/btr507>

666 Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2014). Swarm: robust and
667 fast clustering method for amplicon-based studies. *PeerJ*, 2, e593.
668 <https://doi.org/10.7717/peerj.593>

669 Marcellino, N., Beuvier, E., Grappin, R., Guéguen, M., & Benson, D. R. (2001). Diversity of
670 *Geotrichum candidum* Strains Isolated from Traditional Cheesemaking Fabrications in
671 France. *Applied and Environmental Microbiology*, 67(10), 4752–4759.
672 <https://doi.org/10.1128/AEM.67.10.4752-4759.2001>

673 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing
674 reads. *EMBnet.Journal*, 17(1), 10–12. <https://doi.org/10.14806/ej.17.1.200>

675 McCarthy, C. M., Kelly, P. M., Wilkinson, M. G., & Guinee, T. P. (2017). Effect of fat and
676 salt reduction on the changes in the concentrations of free amino acids and free fatty
677 acids in Cheddar-style cheeses during maturation. *Journal of Food Composition and*
678 *Analysis*, 59, 132–140. <https://doi.org/10.1016/j.jfca.2017.01.007>

679 McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., & Guinee, T. P. (2015). Effect of salt and
680 fat reduction on the composition, lactose metabolism, water activity and microbiology
681 of Cheddar cheese. *Dairy Science & Technology*, 95(5), 587–611.
682 <https://doi.org/10.1007/s13594-015-0245-2>

683 McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., & Guinee, T. P. (2016). Effect of salt and
684 fat reduction on proteolysis, rheology and cooking properties of Cheddar cheese.
685 *International Dairy Journal*, 56, 74–86. <https://doi.org/10.1016/j.idairyj.2016.01.001>

686 Mdaini, N., Gargouri, M., Hammami, M., Monser, L., & Hamdi, M. (2006). Production of
687 natural fruity aroma by *Geotrichum candidum*. *Applied Biochemistry and*
688 *Biotechnology*, 128(3), 227–235. <https://doi.org/10.1385/ABAB:128:3:227>

689 Meng, L., Zhang, Y., Liu, H., Zhao, S., Wang, J., & Zheng, N. (2017). Characterization of
690 *Pseudomonas* spp. and Associated Proteolytic Properties in Raw Milk Stored at Low
691 Temperatures. *Frontiers in Microbiology*, 8, 2158.
692 <https://doi.org/10.3389/fmicb.2017.02158>

693 Menzel, P., Ng, K. L., & Krogh, A. (2016). Fast and sensitive taxonomic classification for
694 metagenomics with Kaiju. *Nature Communications*, 7, 11257.
695 <https://doi.org/10.1038/ncomms11257>

696 Mohan, S., & Campbell, N. R. C. (2009). Salt and high blood pressure. *Clinical Science*,
697 117(1), 1–11. <https://doi.org/10.1042/CS20080207>

698 Monnet, C., Dugat-Bony, E., Swennen, D., Beckerich, J.-M., Irlinger, F., Fraud, S., &
699 Bonnarne, P. (2016). Investigation of the Activity of the Microorganisms in a
700 Reblochon-Style Cheese by Metatranscriptomic Analysis. *Frontiers in Microbiology*,
701 7, 536. <https://doi.org/10.3389/fmicb.2016.00536>

702 Morales, P., Fernandez-Garcia, E., & Nunez, M. (2005). Production of volatile compounds in
703 cheese by *Pseudomonas fragi* strains of dairy origin. *Journal of Food Protection*,
704 68(7), 1399–1407.

705 Mounier, J., Rea, M. C., O'Connor, P. M., Fitzgerald, G. F., & Cogan, T. M. (2007). Growth
706 Characteristics of *Brevibacterium*, *Corynebacterium*, *Microbacterium*, and
707 *Staphylococcus* spp. Isolated from Surface-Ripened Cheese. *Applied and*

Environmental Microbiology, 73(23), 7732–7739.

<https://doi.org/10.1128/AEM.01260-07>

Murtaza, M. A., Huma, N., Sameen, A., Murtaza, M. S., Mahmood, S., Mueen-ud-Din, G., & 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(11), 6700–6707. <https://doi.org/10.3168/jds.2014-8046>

Porcellato, D., Brighton, C., McMahon, D. J., Oberg, C. J., Lefevre, M., Broadbent, J. R., & Steele, J. L. (2014). Application of ARISA to assess the influence of salt content and cation type on microbiological diversity of Cheddar cheese. *Letters in Applied Microbiology*, 59(2), 207–216. <https://doi.org/10.1111/lam.12263>

Rho, M., Tang, H., & Ye, Y. (2010). FragGeneScan: predicting genes in short and error-prone reads. *Nucleic Acids Research*, 38(20), e191–e191. <https://doi.org/10.1093/nar/gkq747>

Rulikowska, A., Kilcawley, K. N., Doolan, I. A., Alonso-Gomez, M., Nongonierma, A. B., Hannon, J. A., & Wilkinson, M. G. (2013). The impact of reduced sodium chloride content on Cheddar cheese quality. *International Dairy Journal*, 28(2), 45–55. <https://doi.org/10.1016/j.idairyj.2012.08.007>

Sihufe, G. A., De Piante Vicín, D. A., Marino, F., Ramos, E. L., Nieto, I. G., Karlen, J. G., & Zorrilla, 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. <https://doi.org/10.1016/j.idairyj.2018.02.006>

Soares, C., Fernando, A. L., Alvarenga, N., & Martins, A. P. L. (2016). Substitution of sodium chloride by potassium chloride in São João cheese of Pico Island. *Dairy Science & Technology*, 96(5), 637–655. <https://doi.org/10.1007/s13594-016-0293-2>

Soares, C., Fernando, A. L., Mendes, B., & Martins, A. P. L. (2015). The effect of lowering salt on the physicochemical, microbiological and sensory properties of São João

733 cheese of Pico Island. *International Journal of Dairy Technology*, 86(3), 409–419.
734 <https://doi.org/10.1111/1471-0307.12198>

735 Søndergaard, L., Ryssel, M., Svendsen, C., Høier, E., Andersen, U., Hammershøj, M., ...
736 Jespersen, L. (2015). Impact of NaCl reduction in Danish semi-hard Samsøe cheeses
737 on proliferation and autolysis of DL-starter cultures. *International Journal of Food*
738 *Microbiology*, 213, 59–70. <https://doi.org/10.1016/j.ijfoodmicro.2015.06.031>

739 Song, J. H., Kim, Y. S., Heo, N. J., Lim, J. H., Yang, S. Y., Chung, G. E., & Kim, J. S.
740 (2017). High Salt Intake Is Associated with Atrophic Gastritis with Intestinal
741 Metaplasia. *Cancer Epidemiology and Prevention Biomarkers*, 26(7), 1133–1138.
742 <https://doi.org/10.1158/1055-9965.EPI-16-1024>

743 Tabla, R., Gómez, A., Rebollo, J. E., & Roa, I. (2015). Salt influence on surface
744 microorganisms and ripening of soft ewe cheese. *Journal of Dairy Research*, 82(02),
745 215–221. <https://doi.org/10.1017/S0022029915000023>

746 Tabla, R., Gómez, A., Simancas, A., Rebollo, J. E., Molina, F., & Roa, I. (2016).
747 Enterobacteriaceae species during manufacturing and ripening of semi-hard and soft
748 raw ewe's milk cheese: Gas production capacity. *Small Ruminant Research*, 145, 123–
749 129. <https://doi.org/10.1016/j.smallrumres.2016.11.008>

750 Taormina, P. J. (2010). Implications of Salt and Sodium Reduction on Microbial Food Safety.
751 *Critical Reviews in Food Science and Nutrition*, 50(3), 209–227.
752 <https://doi.org/10.1080/10408391003626207>

753 Thibaudeau, E., Roy, D., & St-Gelais, D. (2015). Production of brine-salted Mozzarella
754 cheese with different ratios of NaCl/KCl. *International Dairy Journal*, 40, 54–61.
755 <https://doi.org/10.1016/j.idairyj.2014.07.013>

756 Trieu, K., Neal, B., Hawkes, C., Dunford, E., Campbell, N., Rodriguez-Fernandez, R., ...
757 Webster, J. (2015). Salt Reduction Initiatives around the World – A Systematic

758 Review of Progress towards the Global Target. *PLOS ONE*, 10(7), e0130247.
759 <https://doi.org/10.1371/journal.pone.0130247>
760 WHO. (2012). Guideline: sodium intake for adults and children. Retrieved from
761 <https://apps.who.int/iris/handle/10665/77985>
762 Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing
763 EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and
764 whole-genome assemblies. *International Journal of Systematic and Evolutionary*
765 *Microbiology*, 67(5), 1613–1617. <https://doi.org/10.1099/ijsem.0.001755>

766 Table 1 : Biochemical composition of soft cheeses at day 21 of ripening.

767

	cheese		spoilage <i>(Yarrowia lipolytica)</i>		cheese*spoilage		cheese			spoilage <i>(Yarrowia lipolytica)</i>	
	F	p-value	F	p-value	F	p-value	R cheese	S cheese	C cheese	not spoiled	spoiled
pH core	1.968	0.182	20.776	0.001	2.258	0.147	5.32 a	5.38 a	5.30 a	5.421 a	5.259 b
pH rind	0.970	0.407	0.282	0.605	1.279	0.314	6.05 a	6.18 a	6.10 a	6.133 a	6.092 a
a w core	3.793	0.053	1.656	0.222	0.003	0.997	0.977 a	0.971 a	0.973 a	0.975 a	0.973 a
a w rind	0.809	0.468	7.048	0.021	0.085	0.919	0.969 a	0.965 a	0.966 a	0.970 a	0.964 a
Lactate (mg/100g)	1.046	0.381	0.049	0.829	0.355	0.708	576 a	672 a	650 a	639 b	627 a
Proteolysis (mg/100g)											
SN:TN ratio	8.616	0.005	0.006	0.937	1.221	0.329	54.1 a	49.7 ab	45.5 b	49.8 a	49.7 b
Proteolysis (mg/100g)											
NPN:TN ratio	4.283	0.039	0.554	0.471	1.212	0.332	26.9 a	23.3 ab	20.9 b	23.1 a	24.3 b
Lipolysis index	5.119	0.025	24.819	0.000	1.630	0.236	10.0 a	9.2 ab	8.4 b	10.2 a	8.2 b

768 C: control cheese; R: reduced salt cheese; S: substituted salt cheese.

769 All data were subjected to two-way ANOVA (p-value threshold = 0.05).

770

771 Table 2 : Biochemical composition of semi-hard cheeses at day 21 of ripening.

772

	cheese		spoilage <i>(Pseudomonas fluorescens)</i>		cheese*spoilage		cheese			spoilage <i>(Pseudomonas fluorescens)</i>	
	F	p-value	F	p-value	F	p-value	R cheese	S cheese	C cheese	not spoiled	spoiled
pH core	0.466	0.638	6.424	0.026	0.122	0.887	5.50 a	5.55 a	5.49 a	5.45 b	5.58 a
pH rind	1.265	0.317	0.874	0.368	0.330	0.725	5.83 a	5.86 a	5.67 a	5.74 a	5.84 a
a w core	0.642	0.544	0.853	0.374	0.818	0.465	0.969 a	0.977 a	0.97 a	0.978 a	0.972 a
a w rind	1.387	0.287	0.010	0.921	0.235	0.794	0.981 a	0.978 a	0.979 a	0.979 a	0.979 a
Lactate (mg/100g)	0.482	0.629	6.919	0.022	0.084	0.920	285 a	267 a	270 a	296 a	252 b
Proteolysis (mg/100g) SN:TN ratio	2.342	0.138	24.843	0.000	3.555	0.061	23.1 a	21.4 a	21.0 a	19.7 b	24.0 a
Proteolysis (mg/100g) NPN:TN ratio	1.902	0.192	9.401	0.010	1.656	0.232	10.6 a	9.9 a	9.7 a	9.5 b	10.7 a
Lipolysis index	8.807	0.004	5.944	0.031	5.878	0.017	5.9 a	4.2 b	4.5 b	4.4 b	5.3 a

773 C: control cheese; R: reduced salt cheese; S: substituted salt cheese.

774 All data were subjected to two-way ANOVA (p-value threshold = 0.05).

775

Table 3A : Microbiological concentrations (log CFU/g) of soft cheese during ripening.

Cheese samples	Day 1					Day 7				
	Lactococci	Streptococci	Coliforms	PC	GC	Lactococci	Streptococci	Coliforms	PC	GC
Soft C	7.91 ± 0.53 a	7.79 ± 0.91 a	3.01 ± 1.06 a	3.64 ± 0.31 a	3.93 ± 0.29 a	7.82 ± 0.20 a	8.70 ± 0.07 a	3.36 ± 0.79 a	5.38 ± 0.35 a	6.08 ± 0.78 a
Soft R	7.91 ± 0.53 a	7.79 ± 0.91 a	3.01 ± 1.06 a	3.64 ± 0.31 a	3.93 ± 0.29 a	7.72 ± 0.77 a	8.76 ± 0.04 a	3.02 ± 0.44 a	5.67 ± 0.16 a	6.62 ± 0.23 a
Soft S	7.91 ± 0.53 a	7.79 ± 0.91 a	3.01 ± 1.06 a	3.64 ± 0.31 a	3.93 ± 0.29 a	7.87 ± 0.62 a	8.77 ± 0.18 a	3.05 ± 0.42 a	5.41 ± 0.47 a	5.80 ± 0.54 a

Cheese samples	Day 14					Day 21				
	Lactococci	Streptococci	Coliforms	PC	GC	Lactococci	Streptococci	Coliforms	PC	GC
Soft C	7.83 ± 0.35 a	8.62 ± 0.50 a	3.36 ± 1.30 a	6.57 ± 0.26 a	6.24 ± 0.21 a	7.87 ± 0.32 a	8.84 ± 0.25 a	4.16 ± 1.18 a	7.08 ± 0.40 a	7.00 ± 0.22 a
Soft R	7.89 ± 0.45 a	8.86 ± 0.10 a	4.66 ± 0.91 a	7.06 ± 0.37 a	7.17 ± 0.63 a	8.05 ± 0.68 a	8.69 ± 0.23 a	4.69 ± 0.90 a	7.55 ± 0.12 a	7.06 ± 0.37 a
Soft S	7.75 ± 0.68 a	8.78 ± 0.09 a	3.47 ± 1.69 a	6.72 ± 0.11 a	7.02 ± 0.37 a	7.57 ± 0.70 a	8.93 ± 0.09 a	4.36 ± 0.38 a	6.92 ± 0.21 a	7.36 ± 0.08 a

C: control cheese; R: reduced salt cheese; S: substituted salt cheese; PC: *Penicillium camemberti*; GC: *Geotrichum candidum*.

Values are the means of triplicate trials ± standard deviation. Means with columns without a common superscript letter differ significantly (ANOVA, $p < 0.05$).

Table 3B : Microbiological concentrations (log CFU/g) of semi-hard cheeses during ripening.

Cheese samples	Day 1						Day 7					
	Lactococci	Streptococci	Coliforms	Brevi	DH	Molds	Lactococci	Streptococci	Coliforms	Brevi	DH	Molds
Semi-hard C	7.44 ± 0.06 <i>a</i>	8.87 ± 0.16 <i>a</i>	2.63 ± 0.39 <i>a</i>	3.56 ± 0.07 <i>a</i>	4.78 ± 0.18 <i>a</i>	2.49 ± 0.50 <i>a</i>	7.88 ± 0.14 <i>a</i>	9.29 ± 0.33 <i>a</i>	2.35 ± 0.58 <i>a</i>	3.97 ± 0.10 <i>a</i>	7.92 ± 0.66 <i>a</i>	8.04 ± 0.05 <i>a</i>
Semi-hard R	7.63 ± 0.06 <i>a</i>	8.87 ± 0.12 <i>a</i>	2.49 ± 0.89 <i>a</i>	3.60 ± 0.25 <i>a</i>	4.93 ± 0.19 <i>a</i>	2.49 ± 0.20 <i>a</i>	7.92 ± 0.21 <i>a</i>	9.17 ± 0.13 <i>a</i>	2.45 ± 1.31 <i>a</i>	4.04 ± 0.26 <i>a</i>	8.47 ± 0.1 <i>a</i>	8.75 ± 0.07 <i>a</i>
Semi-hard S	7.57 ± 0.18 <i>a</i>	8.84 ± 0.12 <i>a</i>	2.44 ± 0.85 <i>a</i>	3.63 ± 0.14 <i>a</i>	5.34 ± 0.45 <i>a</i>	2.46 ± 0.15 <i>a</i>	7.76 ± 0.08 <i>a</i>	9.33 ± 0.22 <i>a</i>	2.24 ± 0.65 <i>a</i>	3.99 ± 0.15 <i>a</i>	8.55 ± 0.04 <i>a</i>	8.88 ± 0.09 <i>a</i>

Cheese samples	Day 14						Day 21					
	Lactococci	Streptococci	Coliforms	Brevi	DH	Molds	Lactococci	Streptococci	Coliforms	Brevi	DH	Molds
Semi-hard C	7.61 ± 0.22 <i>a</i>	9.19 ± 0.63 <i>a</i>	2.79 ± 0.84 <i>a</i>	4.84 ± 0.46 <i>a</i>	8.65 ± 0.04 <i>a</i>	8.01 ± 0.15 <i>a</i>	6.16 ± 0.89 <i>a</i>	9.19 ± 0.33 <i>a</i>	2.80 ± 0.70 <i>a</i>	5.65 ± 1.29 <i>a</i>	8.67 ± 0.08 <i>a</i>	8.23 ± 0.18 <i>a</i>
Semi-hard R	7.09 ± 0.13 <i>a</i>	9.04 ± 0.10 <i>a</i>	2.20 ± 0.17 <i>a</i>	4.76 ± 0.85 <i>a</i>	8.62 ± 0.10 <i>a</i>	8.09 ± 0.18 <i>a</i>	6.85 ± 0.53 <i>a</i>	9.48 ± 0.04 <i>a</i>	2.04 ± 0.90 <i>a</i>	6.05 ± 1.05 <i>a</i>	8.83 ± 0.04 <i>a</i>	8.40 ± 0.08 <i>a</i>
Semi-hard S	6.70 ± 0.57 <i>a</i>	8.97 ± 0.06 <i>a</i>	3.11 ± 0.84 <i>a</i>	4.92 ± 0.67 <i>a</i>	8.75 ± 0.09 <i>a</i>	7.99 ± 0.15 <i>a</i>	6.53 ± 0.73 <i>a</i>	9.49 ± 0.04 <i>a</i>	3.13 ± 0.88 <i>a</i>	5.59 ± 1.24 <i>a</i>	8.74 ± 0.08 <i>a</i>	8.15 ± 0.27 <i>a</i>

C: control cheese; R: reduced salt cheese; S: substituted salt cheese; Brevi: *Brevibacterium sp.*; DH: *Debaryomyces hansenii*.

Values are the means of triplicate trials ± standard deviation. Means with columns without a common superscript letter differ significantly (ANOVA, $p < 0.05$).

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Table 4 : Average **intensity** of sensory attributes determined for soft cheese at day 21 of ripening **using a scale ranging from 0 to 10.**

Attributes	Control cheese (C)	Reduced salt cheese (R)	Substituted salt cheese (S)
Color of cheese rind	3.72 ^a	4.59 ^b	4.73 ^b
Thickness of rind	3.44 ^a	3.50 ^a	3.36 ^a
Color of cheese core	5.31 ^a	5.31 ^a	5.06 ^a
Flowing aspect	5.10 ^a	6.2 ^b	6.64 ^b
Global intensity of odor	6.25 ^a	7.25 ^{ab}	6.71 ^b
Mushroom odor	4.59 ^a	3.05 ^b	3.63 ^b
Ammonia odor	2.30 ^a	3.44 ^b	3.21 ^b
Tactile perception of soft texture	4.65 ^a	4.49 ^a	4.63 ^a
Firm texture (in mouth)	2.59 ^a	2.15 ^a	1.18 ^b
Stickiness of core	5.25 ^a	5.51 ^a	5.11 ^a
Granular texture of core	0.66 ^a	0.66 ^a	0.43 ^a
Creaminess of core	6.52 ^a	6.95 ^a	6.94 ^a
Global intensity of taste	6.83 ^a	7.44 ^b	7.36 ^b
Salty taste	5.52 ^a	5.28 ^a	5.57 ^a
Acidic taste	2.49 ^a	2.97 ^b	3.07 ^b
Bitter taste	2.99 ^a	3.57 ^{ab}	3.84 ^b
Rancid taste	0.71 ^a	0.95 ^a	0.78 ^a
Oxidized/metallic taste	1.27 ^a	1.81 ^b	1.67 ^{ab}
Aromatic richness	5.33 ^a	5.00 ^a	5.31 ^a
Mushroom aroma of rind	3.52 ^a	2.76 ^b	3.56 ^a

Milky aroma	3.75 ^a	3.89 ^a	4.04 ^a
Animal/cowshed aroma	2.35 ^a	3.14 ^b	3.12 ^b
Soap aroma	0.91 ^a	1.33 ^a	1.23 ^a
Ammonia aroma	1.65 ^a	2.47 ^b	2.41 ^b

Means in the same line without a common superscript letter are significantly different (ANOVA, $p<0.05$).

Figure 1

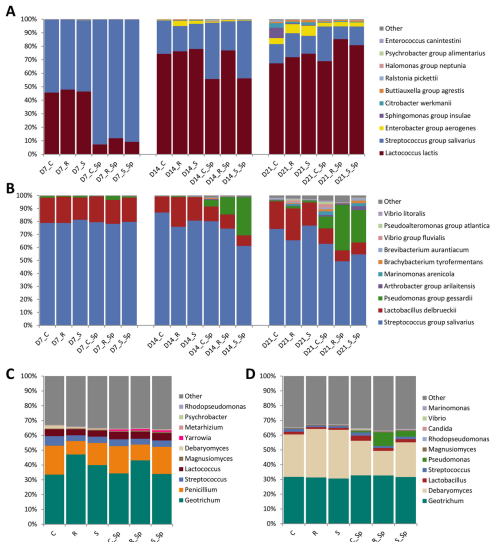


Figure 2

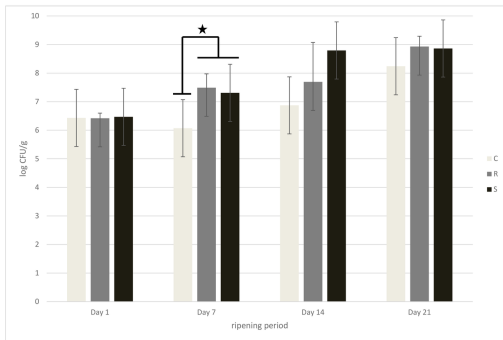


Figure 3

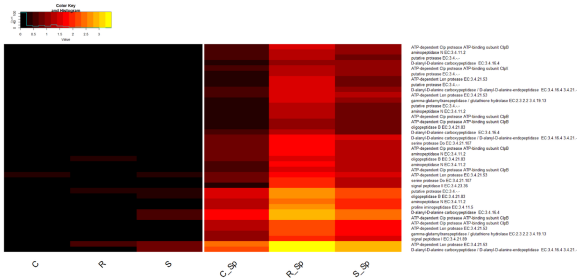
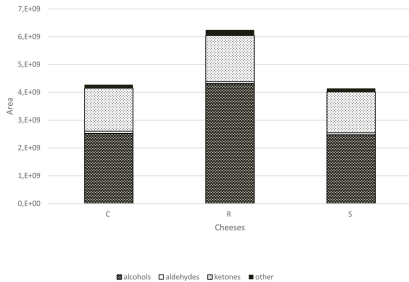


Figure 4



Soft cheese



Semi-hard cheese



Lactic acid bacteria and ripening microflora
± spoilage microflora

Control cheeses: 15 or 14 g of NaCl/kg of cheese

Reduced salt cheese: 80% of [NaCl]

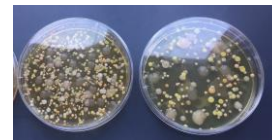
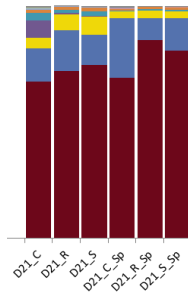
Substituted salt cheeses: 80% NaCl + 20% KCl

21 days of ripening

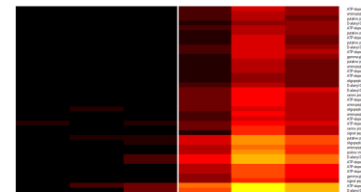
Technological parameters



Composition of ripening microbiota



Metabolism of ripening microbiota



Sensorial quality

