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

3
4 Eric Dugat-Bony¹, Pascal Bonnarme¹, Sébastien Fraud^{2§}, Jessie Castelotte¹, Anne-Sophie
5 Sarthou¹, Valentin Loux³, Olivier Rué³, Nadège Bel², Sarah Chuzeville², Sandra Helinck^{1*}

6 ¹: UMR 787 Génie et Microbiologie des Procédés Alimentaires, AgroParisTech, INRA,
7 Université Paris-Saclay, 78850 Thiverval-Grignon, France

8 ²: Actalia, **Business Unit Dairy Products**, 74801 La Roche-sur-Foron, France

9 ³: MaIAGE, INRA, Université Paris-Saclay, 78350 Jouy-en-Josas, France

10 [§]: present address: General Mills Yoplait, Vienne Technical Center, 38200 Vienne, France

11 * corresponding author: sandra.helinck@agroparistech.fr

12

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14

15 **Abstract**

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

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

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

37

38

39 **Keywords**

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

42 **1. Introduction**

43 Excessive sodium intake is known to cause high blood pressure and to increase the risk of
44 hypertension, cardiovascular disease and gastric cancer (Mohan & Campbell, 2009; Song et al.,
45 2017). The World Health Organization has proposed to reduce sodium intake by 30% in order
46 to obtain the WHO guideline of 2 g/day (i.e., 5 g of salt/day) by 2020 (WHO, 2012). In 2015,
47 75 countries had developed a national sodium reduction strategy (Trieu et al., 2015). This
48 strategy is multifaceted and includes, for example, the establishment of sodium content targets
49 for foods as well as consumer education. Since sodium reduction in the human diet is one of
50 the main objectives for public health agencies, it has become a major challenge for the food
51 industries that have to reformulate products. Indeed, processed foods, including cheese
52 products, are estimated to contribute over 70% to dietary sodium intake (Appel & Anderson,
53 2010; EFSA, 2006). In France, mean daily salt intakes range from approximately 7 to 10 g of
54 salt and are well in excess of dietary needs. Dietary guidelines recommend a 20% salt reduction
55 in foods to obtain a target of 6.5 to 8 g of salt intake per day (ANSES, 2012).

56 Cheese is perceived as being a food source that is highly concentrated in sodium, with amounts
57 that can vary depending on the type of cheese. Soft, semi-hard and hard cheeses contain
58 between 0.5% and 2.5% sodium chloride (NaCl), whereas blue-type cheeses contain between
59 3% and 5% NaCl. Sodium chloride fulfills many important functions in cheese: it modifies the
60 physical properties of the cheese curd and rind (especially maintaining a low **aw**), controls the
61 growth of the cheese-ripening microbiota, limits the development of pathogens or spoilers, and
62 acts as a flavor enhancer (Cruz et al., 2011; Guinee & Fox, 2004; Johnson, Kapoor, McMahon,
63 McCoy, & Narasimmon, 2009). Thus, the sodium reduction strategy must take these parameters
64 into account. This strategy could be achieved either by a reduction in the NaCl level or a partial
65 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

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

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

107 Nevertheless, there is still a lack of information regarding the impact of salt substitution on
108 bacteria, yeast and mold development and metabolic activity during cheese ripening.

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

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

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

123

124 2. MATERIALS & METHODS

125 2.1 Soft and semi-hard cheese production

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

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

141 Coagulation was followed by a 40-min hardening in order to trap intragranular water by
142 achieving a long hardening period. It corresponds to 5 times the rennet clotting time (8 minutes).
143 The cheese curd was then cut (cut size of cheese curd particles: 10 mm x 10 mm) and shaped
144 in polyurethane molds (diameter: 10.5 cm). At the beginning of the molding step, pH was 6.20-
145 6.25 and temperature was 33.5°C. Whey was eliminated and molds were inverted three times.
146 After 20h, cheeses were removed from their mold at pH 4.9-5.10 with a temperature of 16-18°C
147 (day 0). Dry salting then took place (day 1) in order to obtain a final level of 15 g of NaCl/kg
148 of cheese in the control cheese, and 11.5 g of NaCl /kg in the reduced and substituted cheeses.
149 The cheeses (approx. 250 g) were maintained at 13°C and 85% relative humidity (RH) in order
150 to dry their surfaces for 4 hours and were then incubated in a ripening chamber with a relative
151 humidity of 95% for 20 days at 11°C.

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

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

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

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

177

178 **2.2. Microbiological analyses**

179 The cheese samples (10 g) were homogenized in a stomacher for 2 min (AES Chemunex, Easy
180 Mix) and diluted up to 10⁻⁸ by 10-fold serial dilutions. Total microflora were counted according
181 to NF EN ISO 4833, coliforms with NF V 08-050, yeasts and molds with NF V 08-059, and
182 thermophilic lactic acid bacteria with ISO27205. Lactococci were counted on Elliker medium
183 (Difco), *B. linens* on SCB medium (1.5% tryptone, 0.5% soytone, 0.6% yeast extract, 1.2%
184 sodium lactate, 0.25% K₂HPO₄, 4% NaCl and 1.5% agar) and *Pseudomonas spp.* on GSP
185 medium (Glutamate Starch Phenol red agar, VWR).

186

187 **2.3. Bacterial community structure using 16S rRNA gene metabarcoding**

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

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

200

201 ***2.4. Metatranscriptomic analysis***

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

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

225

226 ***2.5 Physicochemical and biochemical analyses***

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

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

240

241 ***2.6 Volatile aroma compound profile***

242 Volatile aroma compounds were quantified at the end of the ripening period (21 days) in three
243 independent cheeses for each condition. The cheese surface was grated and transferred into a
244 10-mL glass bottle that was tightly sealed before storage at -80°C , prior to aroma compound
245 analysis.

246 Before analysis, the cheese samples were thawed at 4°C , vigorously shaken and diluted (1/5 for
247 semi-hard cheese and 1/10 for soft cheese) with a cold solution of 2,5-dimethylpyrazine (546
248 $\mu\text{g/L}$). This solution was used as the internal standard for the experiment. Moreover, NaCl was
249 added to cheese samples with reduced levels of NaCl to have the same NaCl content in order
250 to prevent a salting out effect on the volatile compounds studied. The mixture was stored at 4°C
251 for one night before analysis. Five milliliters of the mixture were then analyzed using a dynamic
252 headspace analyzer (purge-and-trap concentrator, Tekmar-Dohrmann 3100, Tekmar Inc.,
253 Cincinnati, OH, USA) coupled to a gas chromatograph (model 6890, Agilent Technologies,
254 Palo Alto, CA, USA) and a mass spectrometer detector (MSD 5973 inert quadrupole mass
255 spectrometer, Agilent Technologies), and fitted with a $60\text{ m} \times 320\text{ }\mu\text{m}$ i.d. DB-5 MS capillary
256 column (film thickness: $1\text{ }\mu\text{m}$). The oven temperature was programmed as follows: 5°C held
257 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
258 identified by comparison of their mass spectra with those of NIST98 and Wiley libraries. Peak
259 absolute areas (arbitrary units) were calculated from the total ion current (TIC).

260

261 ***2.7 Sensory analysis***

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

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

278

279 ***2.8 Data analysis***

280 All results were reported as mean \pm standard deviation. All data were subjected to one-way or
281 two-way analysis of variance (ANOVA), and differences were considered significant when $p <$
282 0.05. A multiple comparison test (Student-Newman-Keuls test) was then applied. Distinct
283 letters (a, b, c) were assigned to significantly different groups, whereas a combination of the
284 corresponding letters was attributed to those located between several groups. Statistical analysis
285 was carried out with XLStat software (Version 2018.4, Addinsoft, France).

286

287 **3. Results**

288 *3-1 Physicochemical and biochemical characteristics*

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

291 and lactate content at day 21 of ripening (Table 1, two-way ANOVA, $p > 0.05$). Moreover, dry
292 matter and moisture on fat-free dry matter were not affected by the salting conditions (data not
293 shown). However, salting conditions significantly affected both the SN:TN and the NPN:TN
294 ratios (reflecting primary and secondary proteolysis, respectively), and the lipolysis index (two-
295 way ANOVA, $p < 0.05$). For cheeses produced without *Y. lipolytica*, the SN:TN ratio reached
296 43.8% in the control cheese, and 55.4% and 50.5% in R and S cheeses, respectively (Table S3).
297 The lipolysis index was higher in R (10.8) and S cheeses (10.7) than in controls (9.1). This
298 result indicated that reducing NaCl content in soft cheese leads to an increase in both proteolytic
299 and lipolytic activities. The lipolysis index was also significantly modified by the presence of
300 *Y. lipolytica*, independently of the salting conditions (Table 1). For example, it was higher in
301 control cheeses produced without *Y. lipolytica* than in spoiled cheeses (9.1 versus 7.7) (Table
302 S3). Finally, the presence of *Y. lipolytica* also significantly affected the final pH detected in the
303 cheese core (Table 1). For semi-hard cheese salting conditions had no impact on pH, a_w , lactate
304 content and proteolysis at day 21 of ripening (Table 2). Moreover, dry matter and moisture on
305 fat-free dry matter were not affected by the salting conditions (data not shown). The lipolysis
306 index was affected by the interaction of the salting condition and the presence of the spoilage
307 microflora (two-way-ANOVA, $p < 0.05$). It was higher in the R cheeses than in the C and S
308 cheeses and higher in the non-spoiled cheeses than in the spoiled cheeses. The presence of
309 *Pseudomonas* spp., significantly affected the final pH detected in the cheese core, lactate
310 content and proteolysis, considering both SN:TN and NPN:TN ratios. The final pH values
311 obtained in the cheese core and proteolysis were lower in spoiled cheeses than in non-spoiled
312 cheeses, whatever the salting condition (Table S4). On the opposite, the lactate content was
313 higher in spoiled cheeses than in non-spoiled cheeses.

314

315 *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

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

350

351 3-3 Sensory characteristics

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

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

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

369

370 **4. Discussion**

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

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

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

391 in this context. We did not observe higher counts of *G. candidum* in both R and S cheeses than
392 in controls during ripening. A previous study on Torta del Casar, a Spanish soft ewe cheese
393 reported that yeast-molds growth was not affected by NaCl content at levels comprised between
394 0.48 and 2.79% (Tabla, Gómez, Rebollo, & Roa, 2015). However, the relative activity of *G.*
395 *candidum* in our study- as determined by RNA-seq profiles - increased. Reducing salt content
396 did not significantly affect the counts of *P. camemberti* but led to a decrease of its relative
397 activity. In this type of cheese, reducing NaCl content may thus modify the balance between *G.*
398 *candidum* and *P. camemberti*. Although not observed in our study, where the NaCl reduction
399 was only about 20%, it has already been reported that *G. candidum* may outcompete *P.*
400 *camemberti* in cheese produced with low NaCl content and form a surface defect known as
401 'toad skin' (Kindstedt, 2014).

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

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

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

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

441 beginning of ripening, in higher proteolysis, in lower hardness and in higher off-flavor intensity
442 and, more particularly, bitterness and sulfur attributes (Murtaza et al., 2014; Rulikowska et al.,
443 2013). Other authors showed that salt content did not significantly influence the overall
444 bacterial community structure, except that lower salt levels resulted in enhanced starter survival
445 (Porcellato et al., 2014).

446 In this study, we observed a greater development of *Pseudomonas spp.* in both R and S cheeses
447 when the spoiler was deliberately inoculated, which confirms the results obtained in previous
448 studies (Dugat-Bony et al., 2016; Tabla et al., 2015). In the presence of *Pseudomonas spp.*,
449 primary and secondary proteolysis as well as lipolysis were higher than in non-spoiled cheeses.
450 Moreover, the expression of several genes encoding for proteases and peptidases in
451 *Pseudomonas spp.* were overexpressed in low NaCl conditions. It is known that these bacteria
452 are able to produce extracellular, heat-resistant proteolytic and lipolytic enzymes (Baur et al.,
453 2015; Meng et al., 2017) that could generate off-flavors, but genes encoding those enzymes
454 remain to be identified. The expression data presented here could be used in future studies to
455 select a reduced set of candidate genes potentially involved in these functions.

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

463

464 **5. Conclusions**

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

479

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490

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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
aw 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
aw 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).

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

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	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
aw 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
aw 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).

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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 ± 0.74 a

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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

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

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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 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>	8.07 ± 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>	8.09 ± 0.10 <i>a</i>

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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>

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

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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.**

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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

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

Figure 1

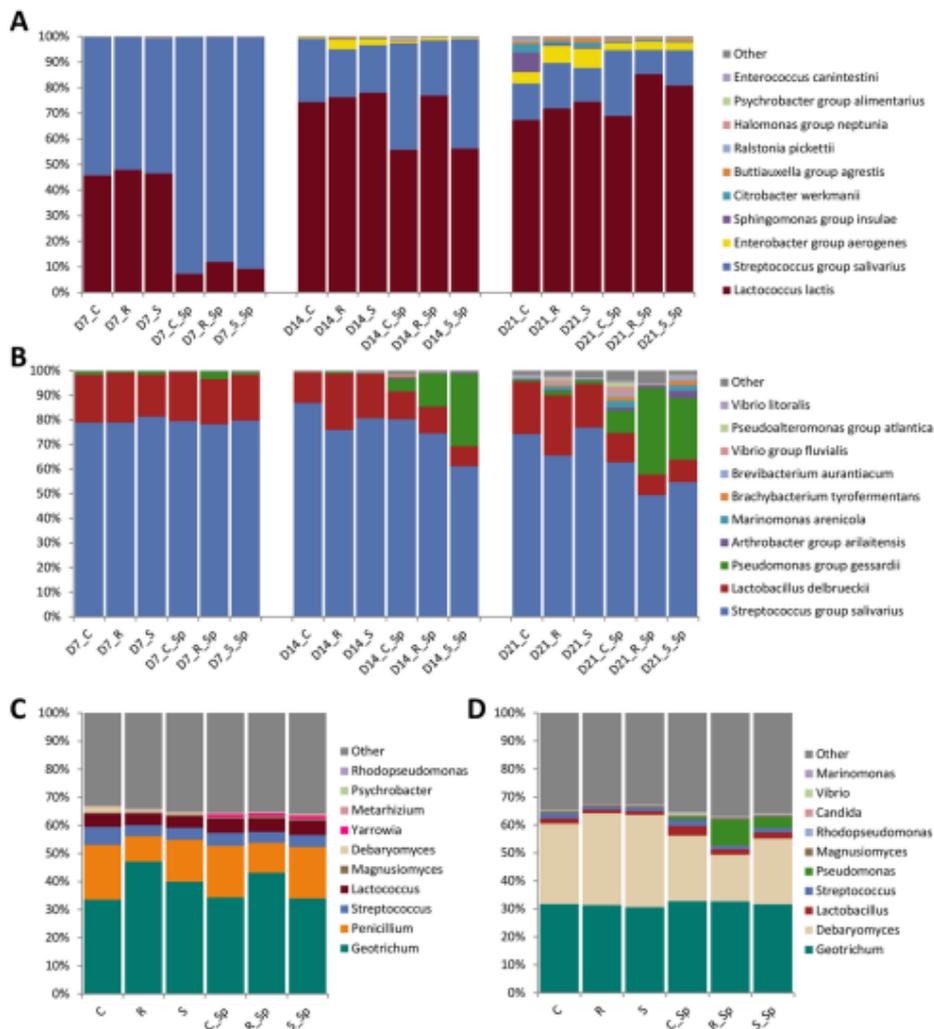


Figure 2

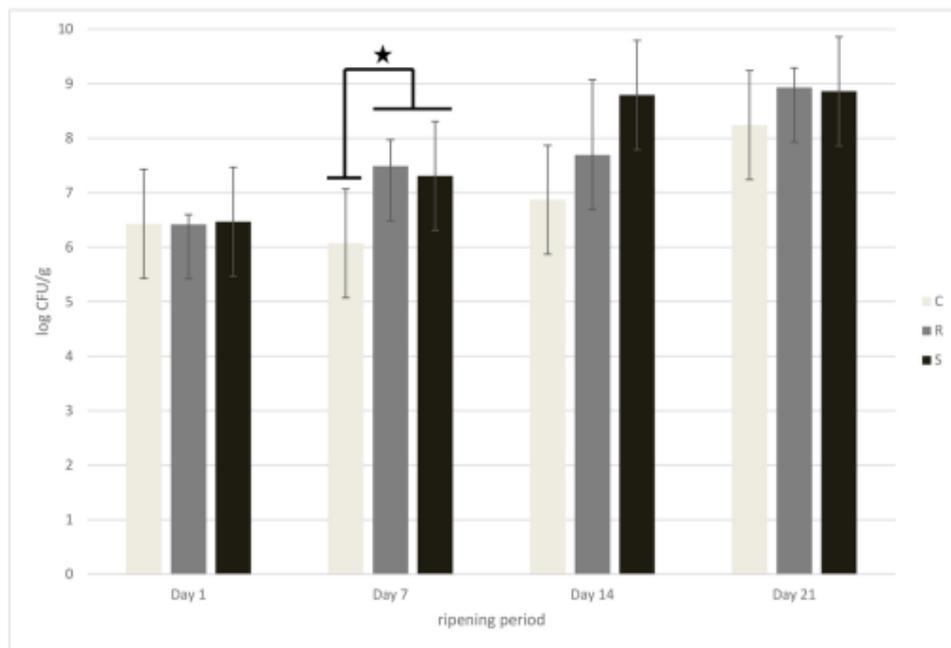


Figure 3

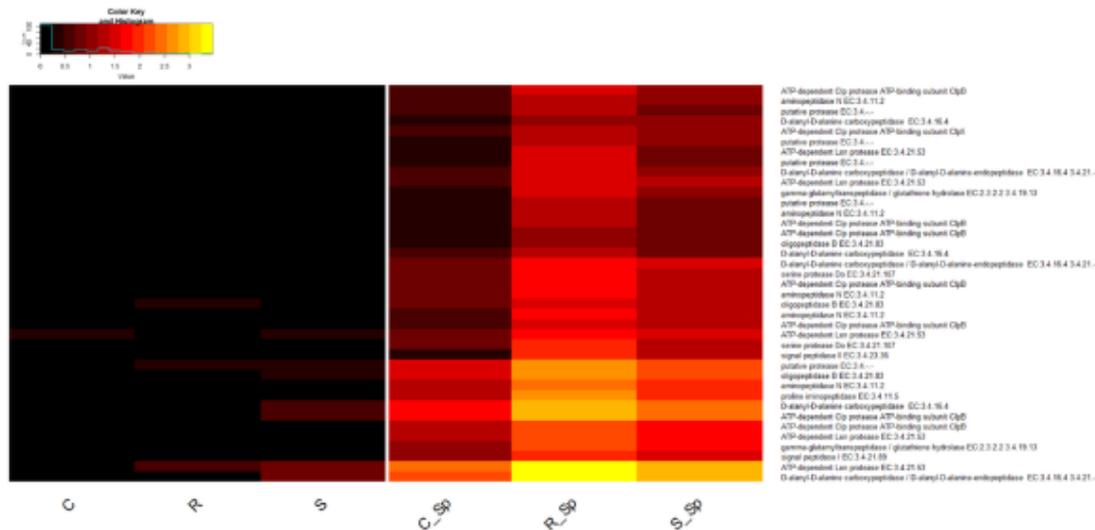
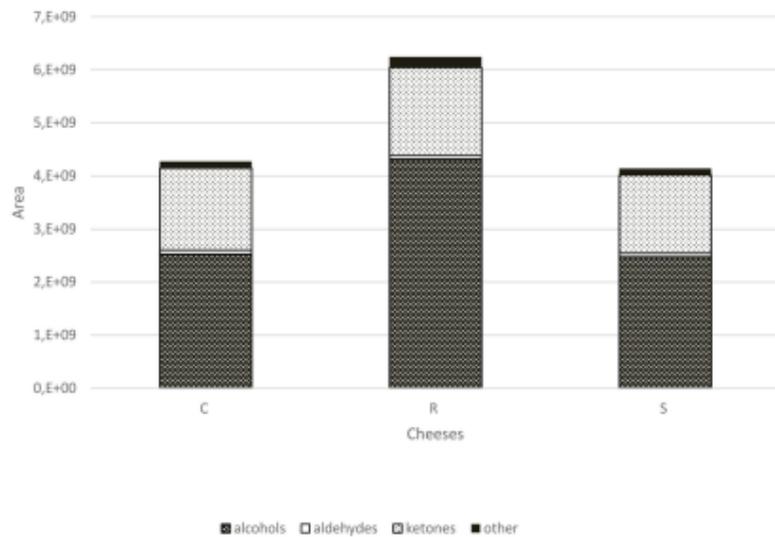


Figure 4



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]

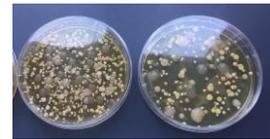
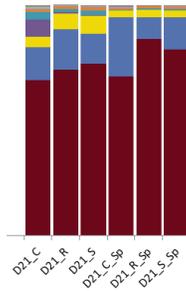
Substituted salt cheeses: 80% NaCl + 20% KCl

21 days of ripening

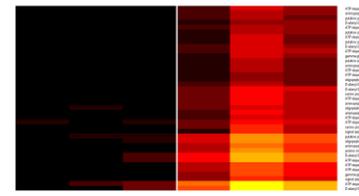
Technological parameters



Composition of ripening microbiota



Metabolism of ripening microbiota



Sensorial quality

