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### ► To cite this version:

Eric Dugat-Bony, Pascal Bonnarme, Sébastien Fraud, Jessie Catellote, Anne-Sophie Sarthou, et al.. Effect of sodium chloride reduction or partial substitution with potassium chloride on the microbiological, biochemical and sensory characteristics of semi-hard and soft cheeses. Food Research International, 2019, 125, pp.108643. 10.1016/j.foodres.2019.108643 . hal-02349939

**HAL Id: hal-02349939**

**<https://hal.science/hal-02349939v1>**

Submitted on 20 Jul 2022

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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

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12

13 Declarations of interest: none

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, CaCl<sub>2</sub> (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 10<sup>3</sup>  
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 10<sup>5</sup> 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<sup>-8</sup> 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% K<sub>2</sub>HPO<sub>4</sub>, 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  $-80^{\circ}\text{C}$ , prior to aroma compound  
245 analysis.

246 Before analysis, the cheese samples were thawed at  $4^{\circ}\text{C}$ , 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  $4^{\circ}\text{C}$   
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:  $5^{\circ}\text{C}$  held  
257 for 9 min and then heated to  $230^{\circ}\text{C}$  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

#### 480 **Acknowledgements**

481 This work was supported by FranceAgriMer (RedSel project, grant number 320143436) and  
482 received the financial support of the CNIEL (Centre National Interprofessionnel de l'Economie  
483 Laitière). We thank Armelle Delille (UMR GMPA, INRA) for GC-MS analysis and the INRA  
484 GeT-PlaGE Platform (<http://get.genotoul.fr>) for assistance in 16S rRNA amplicon sequencing.  
485 For RNA sequencing, this work has benefitted from the facilities and expertise of the high-  
486 throughput sequencing platform of I2BC (Centre de Recherche de Gif - [www.i2bc-saclay.fr](http://www.i2bc-saclay.fr)).  
487 We are grateful to the INRA MIGALE bioinformatics platform (<http://migale.jouy.inra.fr>) for  
488 providing computational resources and support, and to our undergraduate students Maxime  
489 Guilleton and Marie Bogusz for bioinformatics analysis.

490

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

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	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	<b>0.001</b>	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	<b>0.005</b>	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	<b>0.039</b>	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	<b>0.025</b>	24.819	<b>0.000</b>	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	<b>0.026</b>	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	<b>0.022</b>	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	<b>0.000</b>	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	<b>0.010</b>	1.656	0.232	10.6 a	9.9 a	9.7 a	9.5 b	10.7 a
Lipolysis index	8.807	<b>0.004</b>	5.944	<b>0.031</b>	5.878	<b>0.017</b>	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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818 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 <sup>a</sup>	4.59 <sup>b</sup>	4.73 <sup>b</sup>
Thickness of rind	3.44 <sup>a</sup>	3.50 <sup>a</sup>	3.36 <sup>a</sup>
Color of cheese core	5.31 <sup>a</sup>	5.31 <sup>a</sup>	5.06 <sup>a</sup>
Flowing aspect	5.10 <sup>a</sup>	6.2 <sup>b</sup>	6.64 <sup>b</sup>
Global intensity of odor	6.25 <sup>a</sup>	7.25 <sup>ab</sup>	6.71 <sup>b</sup>
Mushroom odor	4.59 <sup>a</sup>	3.05 <sup>b</sup>	3.63 <sup>b</sup>
Ammonia odor	2.30 <sup>a</sup>	3.44 <sup>b</sup>	3.21 <sup>b</sup>
Tactile perception of soft texture	4.65 <sup>a</sup>	4.49 <sup>a</sup>	4.63 <sup>a</sup>
Firm texture (in mouth)	2.59 <sup>a</sup>	2.15 <sup>a</sup>	1.18 <sup>b</sup>
Stickiness of core	5.25 <sup>a</sup>	5.51 <sup>a</sup>	5.11 <sup>a</sup>
Granular texture of core	0.66 <sup>a</sup>	0.66 <sup>a</sup>	0.43 <sup>a</sup>
Creaminess of core	6.52 <sup>a</sup>	6.95 <sup>a</sup>	6.94 <sup>a</sup>
Global intensity of taste	6.83 <sup>a</sup>	7.44 <sup>b</sup>	7.36 <sup>b</sup>
Salty taste	5.52 <sup>a</sup>	5.28 <sup>a</sup>	5.57 <sup>a</sup>
Acidic taste	2.49 <sup>a</sup>	2.97 <sup>b</sup>	3.07 <sup>b</sup>
Bitter taste	2.99 <sup>a</sup>	3.57 <sup>ab</sup>	3.84 <sup>b</sup>
Rancid taste	0.71 <sup>a</sup>	0.95 <sup>a</sup>	0.78 <sup>a</sup>
Oxidized/metallic taste	1.27 <sup>a</sup>	1.81 <sup>b</sup>	1.67 <sup>ab</sup>
Aromatic richness	5.33 <sup>a</sup>	5.00 <sup>a</sup>	5.31 <sup>a</sup>
Mushroom aroma of rind	3.52 <sup>a</sup>	2.76 <sup>b</sup>	3.56 <sup>a</sup>

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

820

821

822

823

824

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

Figure 1

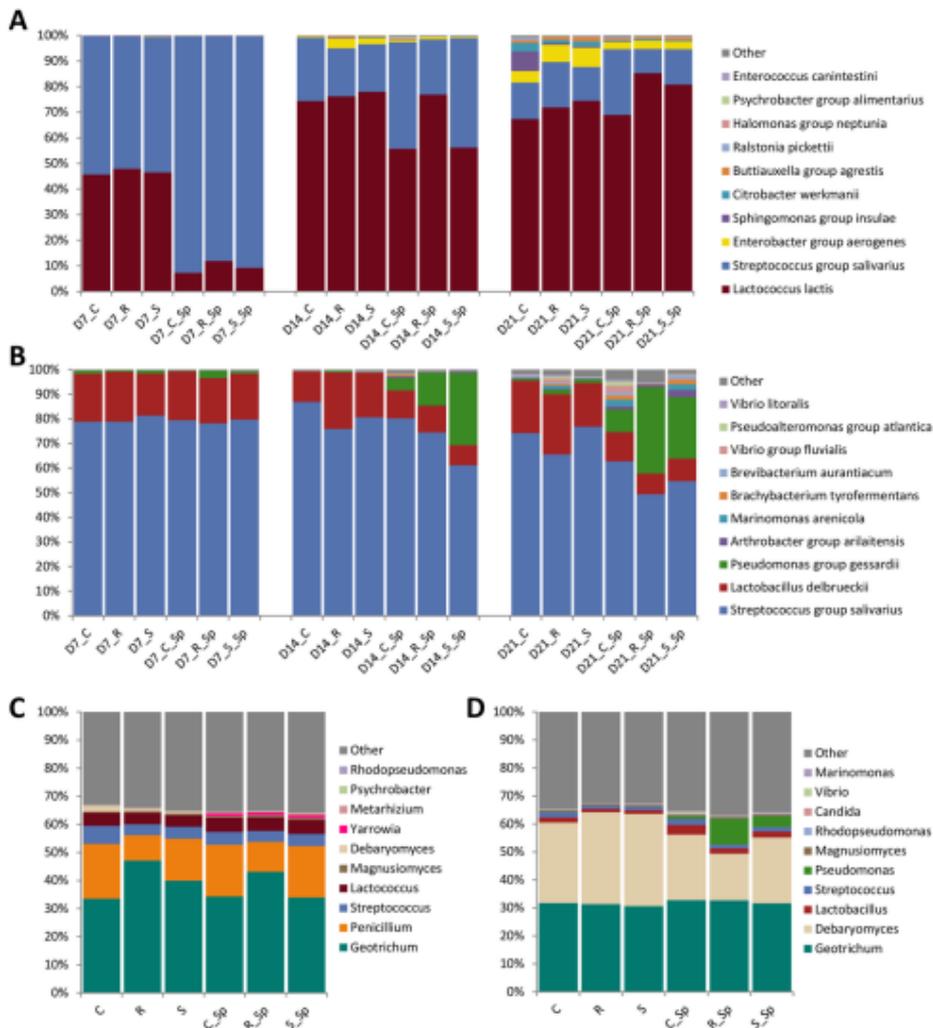


Figure 2

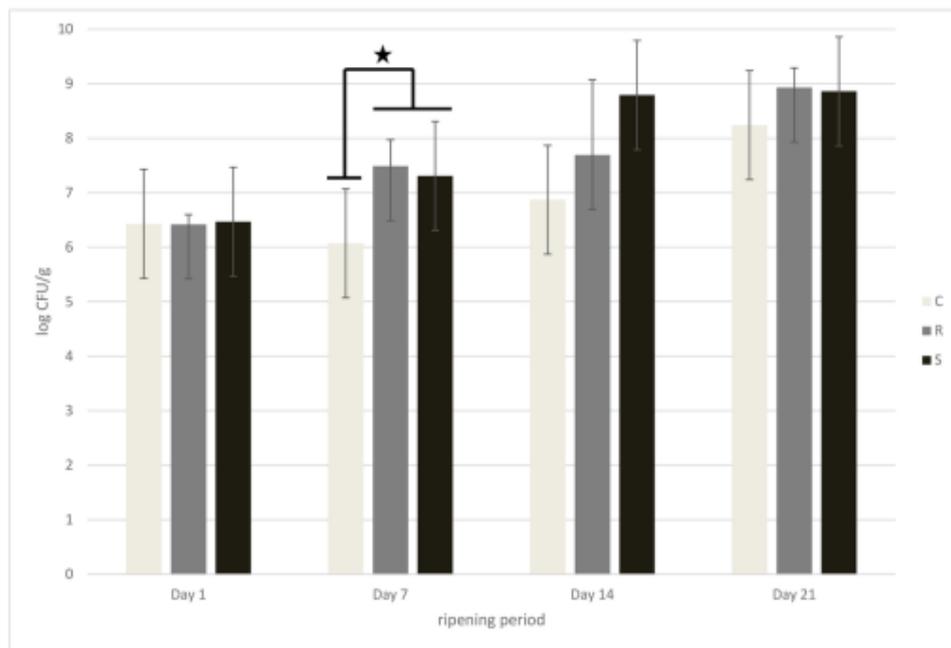


Figure 3

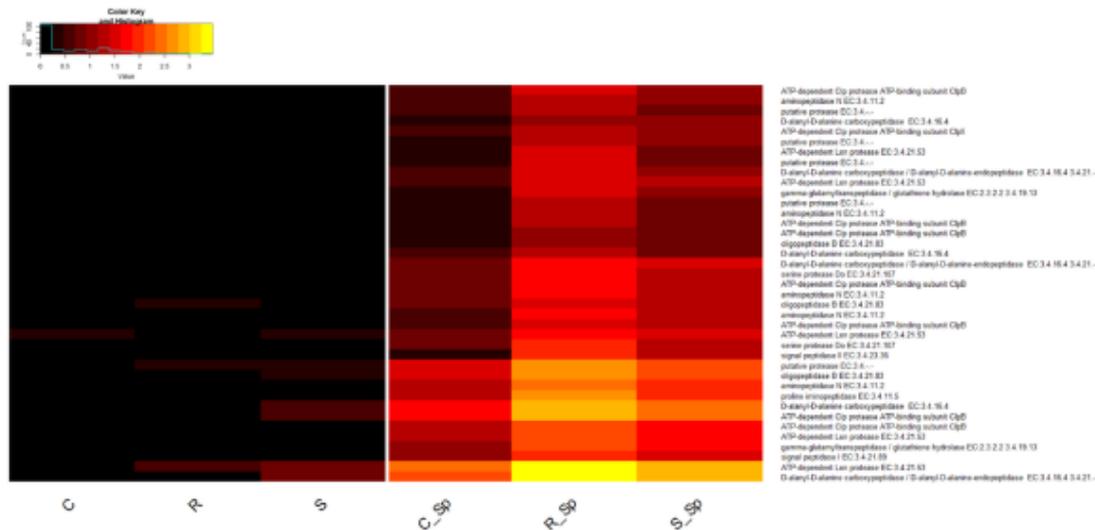
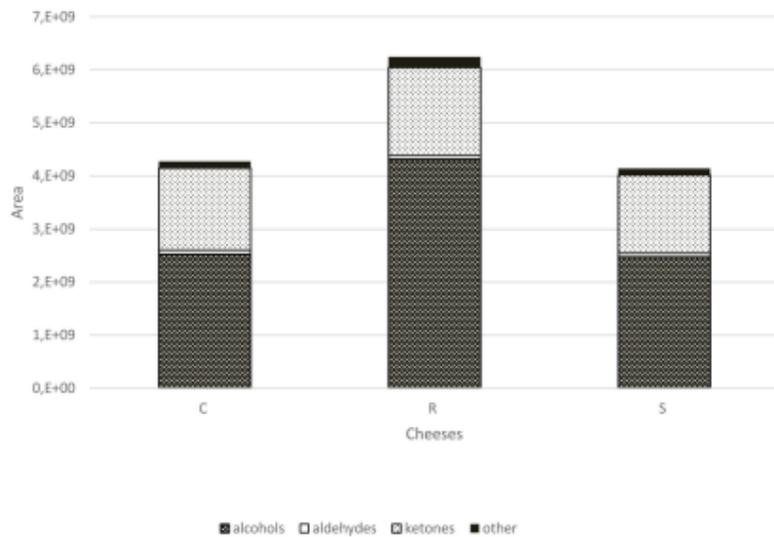


Figure 4



Soft cheese



Semi-hard cheese



Lactic acid bacteria and ripening microflora  
± spoilage microflora

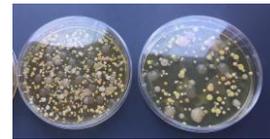
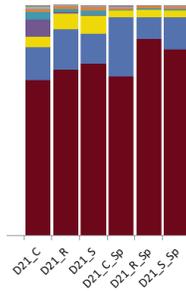
Control cheeses: 15 or 14 g of NaCl/kg of cheese  
Reduced salt cheese: 80% of [NaCl]  
Substituted salt cheeses: 80% NaCl + 20% KCl

21 days of ripening

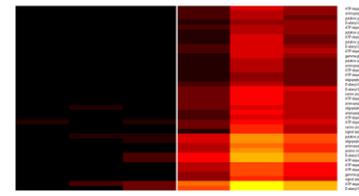
Technological parameters



Composition of ripening microbiota



Metabolism of ripening microbiota



Sensorial quality

