



## The effect of reduced sodium chloride content on the microbiological and biochemical properties of a soft surface-ripened cheese

E. Dugat-Bony,\* A.-S. Sarthou,\* M.-C. Perello,†‡ G. de Revel,†‡ P. Bonnarne,\* and S. Helinck\*<sup>1</sup>

\*Unité Mixte de Recherche, Génie et Microbiologie des Procédés Alimentaires, AgroParisTech, INRA, Université Paris-Saclay, 78850, Thiverval-Grignon, France

†Institut des Sciences de la Vigne et du Vin, Unité de recherche Œnologie, University of Bordeaux, F-33882 Villenave d'Ornon, France

‡INRA, Institut des Sciences de la Vigne et du Vin, USC 1366 Œnologie, University of Bordeaux, F-33882 Villenave d'Ornon, France

### ABSTRACT

Many health authorities have targeted salt reduction in food products as a means to reduce dietary sodium intake due to the harmful effects associated with its excessive consumption. In the present work, we evaluated the effect of reducing sodium chloride (NaCl) content on the microbiological and biochemical characteristics of an experimental surface-ripened cheese. A control cheese (1.8% NaCl) and a cheese with a reduced NaCl content (1.3% NaCl) were sampled weekly over a period of 27 d. Reducing NaCl content induced microbial perturbations such as the lesser development of the yeast *Debaryomyces hansenii* and the greater development of the gram-negative bacterium *Hafnia alvei*. This was accompanied by changes in proteolytic kinetics and in profiles of volatile aroma compounds and biogenic amine production. Finally, the development of the spoilage microorganism *Pseudomonas fragi* was significantly higher in the cheese with a reduced salt content.

**Key words:** salt reduction, cheese microflora, aroma compound, biogenic amine

### INTRODUCTION

Excessive sodium intake is known to cause high blood pressure and its associated heart diseases (Mohan and Campbell, 2009). In Europe, mean daily sodium intakes range from approximately 3 to 5 g (approximately 8 to 11 g of salt) and are well in excess of dietary needs (approximately 1.5 g of sodium/day for adults; EFSA, 2006). The World Health Organization has proposed a limit of dietary sodium intake by restricting daily salt intake to less than 5 g/d (WHO, 2003). In European and North American countries, processed foods, including cheese products, are estimated to contribute over

70% to dietary sodium intake. Thus, sodium reduction in the human diet is currently one of the main objectives for public health agencies (Elliott and Brown, 2007) and, consequently has become a major challenge facing the food industries. In France, dietary guidelines recommend a 20% reduction of salt in foods to obtain a target of 6.5 to 8 g of salt intake per day (ANSES, 2012). Cheese is perceived as being a food source that is high in sodium, with amounts that vary depending on the type of cheese. In fact, cheeses such as soft, semi-hard, and hard cheeses contain between 0.5 and 2.5% sodium chloride (NaCl), and blue-type cheeses contain between 3 and 5% NaCl.

Sodium chloride fulfills many important functions in cheese: it modifies the physical properties of the cheese curd and rind, controls the growth of the cheese-ripening microflora, limits the development of undesirable species (comprising both pathogens and spoilage microbes), and contributes to taste and overall consumer satisfaction (Guinee and Fox, 2004; Cruz et al., 2011). Several studies have been conducted to characterize the effect of reducing NaCl content or partially substituting NaCl with KCl on various quality aspects of hard cheeses (Shrestha et al., 2011a,b; Grummer et al., 2012; Hystead et al., 2013; Rulikowska et al., 2013; Porcellato et al., 2014), white brined cheeses (Melilli et al., 2004; Ayyash et al., 2011; Ayyash and Shah, 2011a,b,c; Kamleh et al., 2012; Osaili et al., 2014; Thibaudeau et al., 2015), and cream cheeses (Møller et al., 2012). However, there is still a lack of information regarding soft cheeses and, specifically, surface-ripened cheeses.

Soft cheeses represent 40% of the total ripened cheese produced in France. Such products generally contain NaCl at levels that vary from 1.5 to 2.3%. The quality of surface-ripened soft cheeses essentially relies on good development of a rind microbial community, originating from several reservoirs (Irlinger et al., 2015). In soft smear-ripened cheeses, the surface microflora is composed of various species of yeasts, as well as gram-positive and gram-negative bacteria. Salt level has an effect on the growth of bacteria isolated

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<sup>1</sup>Corresponding author: [sandra.helinck@agroparistech.fr](mailto:sandra.helinck@agroparistech.fr)

from surface-ripened cheeses, including *Brevibacterium*, *Corynebacterium*, *Microbacterium*, and *Staphylococcus* (Mounier et al., 2007). Thus, NaCl content could be a major technological factor controlling underlying ecosystem assembly and behavior. Modifying NaCl content in soft smear-ripened cheeses may affect all biochemical processes sustained by surface microorganism activity, including proteolysis and the production of various secondary metabolites (e.g., aroma compounds and biogenic amines). Furthermore, by changing the indigenous microflora, the natural barrier effect against undesirable species may be altered as well.

The objective of this study was to investigate the effect of reduced NaCl content on microbiological balance with respect to both the cheese-ripening microflora and a spoilage microorganism (*Pseudomonas fragi*), proteolysis, aromatic qualities, and the biogenic amine production profile of an experimental surface-ripened cheese similar to Munster-Livarot-type cheese.

## MATERIALS AND METHODS

### Strains and Growth Conditions

The 9 microorganisms that composed the model microbial community were *Corynebacterium casei* UCMA 3821 (obtained from the Laboratoire des Microorganismes d'Intérêt Laitier et Alimentaire, Caen, France), *Brevibacterium aurantiacum* ATCC 9174, *Geotrichum candidum* ATCC 204307 (both from the American Type Culture Collection, Rockville, MD), *Arthrobacter arilaitensis* CIP 108037 (from the collection of the Institut Pasteur, Paris, France), and *Staphylococcus equorum* Mu2, *Hafnia alvei* GB001, *Lactococcus lactis* ssp. *lactis* S3+ and its protease-negative variant S3-, *Kluyveromyces lactis* 3550, and *Debaryomyces hansenii* 304 (from the culture collection of the Génie et Microbiologie des Procédés Alimentaires, INRA, Thiverval-Grignon, France). The spoilage microorganism, *Pseudomonas fragi* 1E26, was kindly provided by C. Delbes-Paus (from the culture collection of the Unité de Recherches Fromagères, INRA, Aurillac, France). All strains were originally isolated from cheese. The lactic acid bacteria *Lactococcus lactis* ssp. *lactis* S3+ and S3- were grown for 24 h at 30°C under static conditions in M17 lactose (0.5%) broth (Biokar Diagnostics, Beauvais, France), inoculated at 3% in reconstituted skim milk (100 g/L, Difco Laboratories, Detroit, MI), and then incubated for 16 h at 30°C. All other bacteria were grown under aerobic conditions (using a rotary shaker at 150 rpm) at 25°C for 48 h in 50-mL conical flasks containing 10 mL of brain heart infusion broth (Biokar Diagnostics). The yeasts were grown under the same conditions, ex-

cept that potato dextrose broth (Biokar Diagnostics) was used as the growth medium.

### Cheese Production

Sterile model surface-ripened cheeses were produced in a manner similar to that described in previous studies (Mounier et al., 2008; Dugat-Bony et al., 2015). Such a model cheese has been shown to be representative of Munster-Livarot-type cheese in terms of its sensory properties (Bonaïti et al., 2005). Briefly, pilot-scale cheese production (coagulation, cutting, draining, and molding of the curd) was carried out under aseptic conditions in a sterilized 3-m<sup>3</sup> chamber, as previously described (Mounier et al., 2008). Only *Lactococcus lactis* ssp. *lactis* S3+/S3- were inoculated at this stage. One kilogram of the resulting cheese curd was crumbled under sterile conditions and mixed 3 times with 234 mL of 90 or 64 g/L NaCl for 10 s at maximum speed using a Waring blender to obtain a homogeneous cheese paste with a salt content of approximately 18 (control level) and 13 (reduced level) g/kg, respectively. Freshly prepared microbial solutions ( $2 \times 10^9$  cfu/mL in physiological saline solution for bacteria, and  $2 \times 10^7$  cfu/mL for *D. hansenii* and *K. lactis*, and  $2 \times 10^4$  cfu/mL for *G. candidum*) were then added to the cheese. The 5 ripening bacteria were inoculated at a concentration of  $10^6$  cfu/g of cheese; *D. hansenii* and *K. lactis* were inoculated at  $10^4$  cfu/g of cheese; and *G. candidum* was inoculated at  $10^2$  cfu/g of cheese. In some experiments, *P. fragi* was inoculated at  $10^4$  cfu/g of cheese. Twenty-five grams of the inoculated cheese paste was then transferred to sterile crystallizing basins with a diameter of 5.6 cm. The model cheeses were ripened for 1 d at 17°C and 93% relative humidity and then for 26 d at 12°C and 97% relative humidity. At least 3 cheeses for each salting condition were taken at regular time intervals during the ripening process (0, 6, 13, 20, and 27 d). The entire cheese was homogenized at 4°C under sterile conditions, and 1 g was sampled and transferred to a sterile container for microbiological analysis. Cheeses were then stored at -80°C until quantitative PCR assay, biogenic amine, and aroma compound analysis.

### Physicochemical Analysis

Samples were analyzed for pH, water activity ( $a_w$ ), and NaCl content. Cheese pH was measured after homogenization of the entire cheese (both surface and core) with a BlueLine 27 surface electrode (Schott, VWR, Fontenay-sous-Bois, France). Water activity was measured on 5 g of cheese using an electronic water activity meter (Novasina, Labmaster, Lachen, Switzer-

land). For NaCl content, the sodium ion concentration was determined on a cheese suspension prepared from 1 g of cheese and 9 mL of distilled water using an Orion Dual Star pH/ISE meter (Thermo Scientific, Villebon-sur-Yvette, France). The NaCl content was measured at d 6 when diffusion of salt was stable within the cheese.

### Microbiological Analysis

One gram of the cheese was diluted to 1:10 in sterile saline solution (9 g/L NaCl) and homogenized with an Ultra Turrax Homogenizer (Labortechnik, Staufen, Germany) at 8,000 rpm for 1 min. Microorganisms were enumerated by surface plating in duplicate on specific agar base medium. Total ripening bacteria were enumerated on brain heart infusion agar supplemented with 22.5 mg/L amphotericin B after 3 to 5 d of incubation at 25°C under aerobic conditions, and then transferred for exposure to natural light for colony pigmentation. All bacterial strains were differentiated by their different morphotypes. The yeast population was determined on yeast-glucose-chloramphenicol agar supplemented with 0.01 g/L tetrazolium chloride after 2 to 3 d of incubation at 25°C under aerobic conditions. All yeast strains were differentiated by their different morphotypes. A population of *P. fragi* 1E26 was selectively determined on CFC medium (Biokar Diagnostics) supplemented with 5 mg/L vancomycin.

### Quantitative PCR Assay

**DNA Extraction from Cheese.** A cheese sample (0.5 g) was homogenized with 500  $\mu$ L of 1 $\times$  Tris-EDTA buffer (Tris 10 mM, EDTA 1 mM) for 20 s at 6,500 rpm using a Precellys homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) without beads. Five hundred microliters of the cheese suspension was transferred to a 2-mL tube containing 0.2 g of zirconium beads (diameter: 0.1 and 0.5 mm, 50/50; BioSpec Products, Bartlesville, OK) and 75  $\mu$ L of an enzymatic solution containing lysozyme (54.5 mg/mL, Sigma, St. Louis, MO) and lyticase (1,300 U/mL, Sigma). After incubation at 37°C for 30 min, 40  $\mu$ L of proteinase K (14 mg/mL, Sigma) and 100  $\mu$ L of SDS (200 g/L, Sigma) were added. The tube was incubated at 55°C for 30 min. The following steps of the DNA extraction procedure, including phenol/chloroform extraction, RNaseA treatment, and ethanol precipitation, were performed as previously described (Monnet et al., 2006). The DNA pellet was resuspended in 100  $\mu$ L of 1 $\times$  Tris-EDTA buffer and quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

**Quantitative PCR.** Two primer pairs targeting the 16S rRNA gene, and already described (Bergmark et al., 2012), were used in this study: Pse435F (ACTT-TAAGTTGGGAGGAAGGG) and Pse686R (ACA-CAGGAAATTCACCACCC), which is specific to the *Pseudomonas* genus, and the universal bacterial primer pair Eub 338F (ACTCCTACGGGAGGCAGCAG) and Eub 518 R (ATTACCGCGGCTGCTGG). The PCR amplifications and analyses were done using a Bio-Rad CFX Connect real-time PCR detection system (Bio-Rad, Marnes-la-Coquette, France) and a SYBR Green real-time PCR kit (SsoAdvanced Universal SYBR Green Supermix, Bio-Rad, Hercules, CA). The thermocycling program consisted of initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation (95°C/5 s) and annealing/extension (60°C/20 s for the universal primer pair and 65°C/20 s for the *Pseudomonas*-specific primer pair). Fluorescence acquisition was done at the end of each extension. After real-time PCR, a melting curve analysis was performed by measuring fluorescence during heating from 65 to 95°C with 0.2°C increments. Quantification cycle (C<sub>q</sub>) values were determined with CFX manager software (version 3.1; Bio-Rad), using the regression determination mode. For each sample, the C<sub>q</sub> value obtained with the universal primer pair was used to normalize the value obtained with that specific to *Pseudomonas* using the log<sub>2</sub>( $\Delta\Delta$ C<sub>q</sub>) method. The control sample used for the comparison corresponded to a cheese without *P. fragi*.

### Biochemical Analysis

**Proteolysis Index.** A cheese suspension was prepared from 2 g of cheese and 18 mL of distilled water. It was homogenized for 2 min at 25,000 rpm using a mechanical blender (Ultra-Turrax model T25, Labortechnik). This suspension was used for total nitrogen (TN), noncasein nitrogen (referred to as acid-soluble nitrogen, ASN), and NPN content measures, as previously described (Leclercq-Perlat et al., 2000b). The TN content was directly measured on 2 mL of the cheese suspension using the Kjeldahl method according to standard NF EN ISO 8968-1 (ISO, 2014). The ASN content was measured according to the procedure described in standard NF ISO 27871 (ISO, 2011). Five milliliters of the cheese suspension was adjusted to pH 4.6 using 5 N HCl and centrifuged for 30 min at 2,600  $\times$  g. The supernatant was filtered through a nylon syringe filter with a porosity of 0.22  $\mu$ m (AIT, Houilles, France). The nitrogen content of this soluble fraction was measured using the Kjeldahl method according to standard NF EN ISO 8968-1 (ISO, 2014). For NPN, 7 mL of the cheese suspension was mixed with 10 mL

of distilled water and 7 mL of trichloroacetic acid (240 g/L) and then homogenized using a mechanical blender (2 min, 25,000 rpm). The mixture was incubated for 20 min at room temperature and filtered through a nylon syringe filter with a porosity of 0.22  $\mu\text{m}$  (AIT). The ratios ASN:TN and NPN:TN were used as cheese-ripening indicators.

**Volatile Aroma Compound Profile.** Volatile aroma compounds were quantified at the end of ripening (d 27) in 5 independent cheeses for each condition. Before analysis, the cheese samples were thawed at 4°C, vigorously shaken, and diluted (1/6) with a cold solution of 2,5-dimethylpyrazine (324  $\mu\text{g/L}$ ). This solution was used as the internal standard for the experiment. Five milliliters of the mixture was analyzed using a dynamic headspace analyzer (purge-and-trap concentrator, Tekmar-Dohrmann 3100, Tekmar Inc., Cincinnati, OH) coupled to a gas chromatograph (model 6890, Agilent Technologies, Palo Alto, CA) and a mass spectrometer detector (MSD 5973 inert quadrupole mass spectrometer, Agilent Technologies), as previously described (Irlinger et al., 2012). The concentrations of the volatile compounds produced were quantified from a standard curve made with 5 pure compounds purchased from Sigma-Aldrich (St. Louis, MO), incorporated into curd. Before the addition of compounds, the curd was diluted (1/6) with a solution of 2,5-dimethylpyrazine (324  $\mu\text{g/L}$ ). The concentrations of volatile sulfur compounds, aldehydes, alcohols, esters, and ketones were quantified from a standard curve made with dimethyl disulfide (DMDS), 3-methyl butanal, 3-methyl butanol, acetic acid 3-methylbutyl acetate, and 2-heptanone, respectively. The results were expressed in micrograms of compound per kilogram of cheese.

**Biogenic Amine Concentration.** Biogenic amines (BA) were quantified in 4 different culture conditions at the end of ripening (d 27): in model cheeses with 1.8 and 1.3% of salt content, and in the presence and absence of *P. fragi*. The BA were quantified in 4 independent cheeses for each condition. Cheese samples (5 g) were homogenized in 10 mL of 0.1 N HCl and centrifuged at 12,000  $\times g$  (20 min, 4°C). The supernatant was recovered and extraction was performed again with 10 mL of 0.1 N HCl. The 2 acid extracts were then mixed and made up to 20 mL with 0.1 N HCl. This extract was filtered (0.45  $\mu\text{m}$ ) and stored at -20°C until analysis of BA. A standard solution containing a mixture of histamine, tyramine, tryptamine, phenylethylamine, putrescine, isoamylamine, and cadaverine (500 mg/L each) in a hydroalcoholic solution (50% vol/vol) was used, in different dilutions, to quantify the BA present in the samples. Separation, identification, and quantification of these compounds were performed with

HPLC following the procedure previously described by Helinck et al. (2013).

### Statistical Analysis

Differences in the results obtained between the control cheeses and those with reduced NaCl content were assessed using a dependent Student test for paired-samples. Means with a *P*-value < 0.05 were considered significantly different between the 2 groups.

## RESULTS

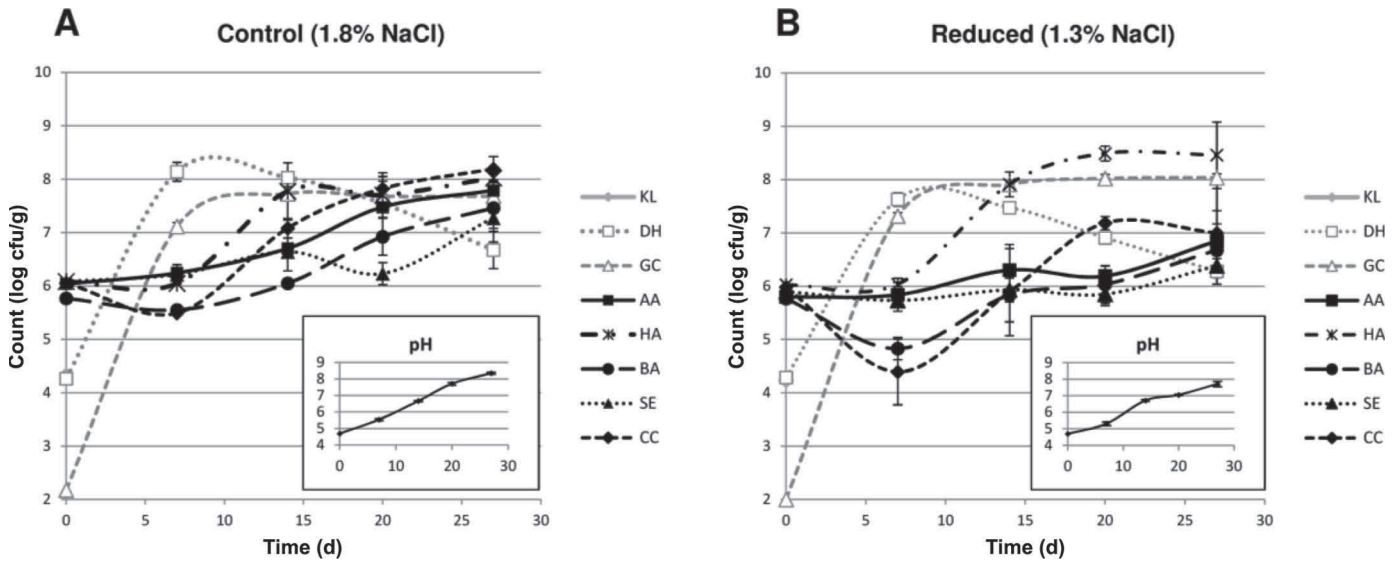
### Physicochemical Parameters of Cheeses

The NaCl content was 17.9  $\pm$  0.1 g/kg in the control cheese and 12.9  $\pm$  0.2 g/kg in the reduced-salt cheese. The pH values ranged from 4.69  $\pm$  0.01 at d 0 to 8.35  $\pm$  0.06 in the control cheese and to 7.71  $\pm$  0.16 in the cheese with a reduced NaCl content at d 27 (Figure 1). No major difference was observed between  $a_w$  in the control cheese (1.8% NaCl) and in the reduced-salt cheese (1.3% NaCl) during ripening. At d 27, the  $a_w$  value was 0.962  $\pm$  0.001 for the control cheese and 0.967  $\pm$  0.001 for the cheese with reduced NaCl content.

### Effect of Reducing Salt Content on the Technological Microflora

The evolution of yeast and bacterial counts over time was similar during the entire ripening period in the control and reduced-NaCl cheeses (Figure 1). In both cheeses, growth measurements revealed the early development of yeasts during the first 2 wk of ripening, especially *D. hansenii* and *G. candidum*, followed by the development of acid-sensitive bacteria (*A. arilaitensis*, *H. alvei*, *B. aurantiacum*, *S. equorum*, and *C. casei*) between d 6 and 27.

However, reducing the NaCl content had a strong effect on the development of each individual cheese-ripening microorganism (Figure 1). Reducing the NaCl content resulted in lesser development of *D. hansenii* (7.63  $\pm$  0.12 vs. 8.14  $\pm$  0.18 log cfu/g at d 6, Student's test, *P* < 0.05; 7.47  $\pm$  0.09 vs. 8.03  $\pm$  0.11 log cfu/g at d 13, Student's test, *P* < 0.05) and slightly greater development of *G. candidum* (7.31  $\pm$  0.01 vs. 7.12  $\pm$  0.08 log cfu/g at d 6, not statistically significant; 7.91  $\pm$  0.08 vs. 7.68  $\pm$  0.03 log cfu/g at d 13, not statistically significant). This was accompanied by a slower increase in the pH values at d 20 (7.05  $\pm$  0.04 vs. 7.71  $\pm$  0.08, Student's test, *P* < 0.05). Furthermore, the lesser development of most ripening bacteria except *H. alvei* was reported in the reduced-NaCl cheese. The



**Figure 1.** Changes in the microbial community structure during surface-ripened cheese maturation. Microbiological counts and pH values were measured for (A) control cheeses (1.8% NaCl) and (B) cheeses with a reduced NaCl content (1.3% NaCl). KL = *Kluyveromyces lactis*, DH = *Debaryomyces hansenii*, GC = *Geotrichum candidum*, AA = *Arthrobacter arilaitensis*, HA = *Hafnia alvei*, BA = *Brevibacterium aurantiacum*, SE = *Staphylococcus equorum*, CC = *Corynebacterium casei*. Error bars represent SD ( $n = 3$ ).

population of *H. alvei* was, in fact, significantly higher in the reduced-NaCl cheese from d 20 to the end of ripening, with counts of  $8.49 \pm 0.14$  and  $8.46 \pm 0.62$  log cfu/g at d 20 and 27, respectively, compared with  $7.70 \pm 0.42$  and  $8.02 \pm 0.09$  log cfu/g in the control cheese (Student's test,  $P < 0.05$ ).

#### Effect of Reducing Salt Content on the Growth of *P. fragi*

In this experiment, *P. fragi* was added to model cheeses (both control and reduced-salt cheeses) containing the cheese-ripening microflora. The development of cheese-ripening yeasts and bacteria was similar in the presence and absence of *P. fragi* in cheeses (data not shown). Microbial counts indicated greater development of *P. fragi* in cheeses with a reduced NaCl content (Table 1). This species reached  $6.19 \pm 0.47$  log cfu/g at the end of ripening in the cheese with 1.3% NaCl, which was significantly higher than  $4.94 \pm 0.41$  log cfu/g in the cheese with 1.8% NaCl (Student's test,  $P < 0.05$ ). This result was confirmed by quantitative PCR (Student's test,  $P < 0.05$ ).

#### Effect of Reducing Salt Content on Proteolysis

The concentrations of different nitrogen fractions were determined to evaluate the influence of reducing NaCl content on proteolysis activity. Changes in the ASN:TN and NPN:TN ratios during the ripening

period of both controls and reduced-salt cheeses are shown in Figure 2.

The ASN:TN ratio is used as an index of the degree of primary proteolysis in cheese. This index increased from d 1 to 27 in our experimental cheeses. No significant difference was observed until d 27, when it reached 71% in the control cheese and 76% in the reduced-NaCl cheese (Student's test,  $P < 0.05$ ). The NPN:TN ratio, reflecting secondary proteolysis, increased more rapidly in cheese with a reduced NaCl content than in the control cheese. Significant differences were observed for this ratio from d 6 to 27, except at d 13 (Student's test,  $P < 0.05$ ). At d 27, the NPN:TN ratio reached 57% in the control cheese and 61% in the reduced-NaCl cheese. Overall, these results indicate that reducing NaCl con-

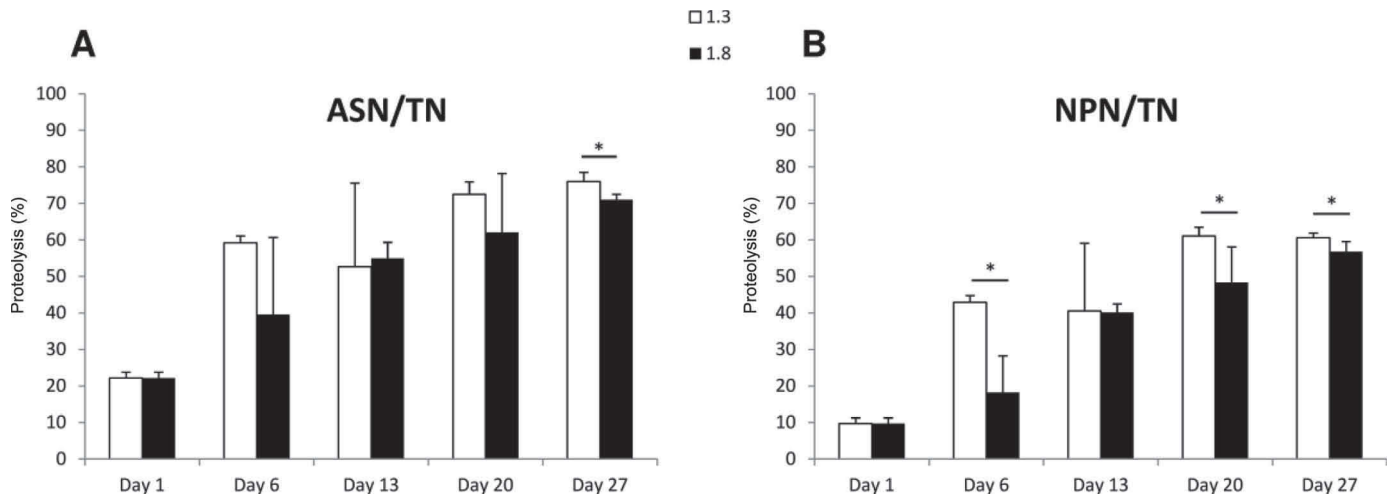
**Table 1.** Development of *Pseudomonas fragi* in control cheeses (1.8% NaCl) and reduced-NaCl cheese (1.3% NaCl)

Ripening time (d)	Average microbial count (log)		qPCR [ $\log_2(\Delta\Delta Cq)$ ] <sup>1</sup>	
	1.8% NaCl	1.3% NaCl	1.8% NaCl	1.3% NaCl
6	4.41*	4.53*	NA <sup>2</sup>	NA
13	4.87	5.08	NA	NA
20	4.57*	5.56*	1.12	1.95
27	4.94*	6.19*	0.86*	4.97*

<sup>1</sup>Quantitative PCR as measured by quantification cycle (Cq) method.

<sup>2</sup>NA = data not available.

\*Statistically significant difference between control and reduced-NaCl cheeses at  $P < 0.05$  (by Student's *t*-test).



**Figure 2.** Comparison of the evolution of the proteolysis index during the maturation of control cheeses (1.8% NaCl) and cheeses with a reduced salt content (1.3% NaCl). (A) Primary proteolysis index, expressed as a percentage, was calculated by dividing the concentration measured in the acid-soluble nitrogen fraction (ASN) by the concentration measured in the total nitrogen fraction (TN) and multiplied by 100; (B) secondary proteolysis index, expressed as a percentage, was calculated by dividing the concentration measured in the NPN fraction by the concentration measured in the TN fraction. Statistically significant differences between the 2 conditions (Student's test,  $P < 0.05$ ) are highlighted with asterisks. Error bars represent SD ( $n = 3$ ).

tent in our model cheese would accelerate and increase proteolytic activities, especially secondary proteolysis.

#### Effect of Reducing Salt Content on Volatile Aroma Compound Profiles

The total volatile compounds produced by the microflora after 27 d of ripening in the control cheese and in the cheese with reduced salt content were significantly different (Student's test,  $P < 0.05$ ). As shown in Table 2, the highest levels of aroma compounds were detected in cheese with reduced salt content. High levels of sulfur compounds (e.g., DMDS, methanethiol, and methylthioacetate) and alcohols (e.g., ethanol, 2-propanol, 3-methyl-1-butanol, and 2-methyl-1-butanol) were measured in the cheese with reduced salt content. Moreover, we detected variations with respect to a variety of volatile compounds produced, depending on the salt level. For example, 2 esters, hexanoic acid ethyl ester and acetic acid ethyl ester, were only found in the cheese with reduced salt content.

#### Effect of Reducing Salt Content on Production of Biogenic Amines

Biogenic amines were quantified at the end of ripening in model cheeses with 1.8% and 1.3% salt contents, in the presence and absence of *P. fragi* (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2015-10502>). At d 0, BA were detected at trace levels with a total level of 1.7 mg/kg of cheese. At d 27, among the 7 BA

analyzed, histamine and isoamylamine were detected at low levels ( $<3.8$  and  $0.1$  mg/kg, respectively), whereas tyramine, tryptamine, and phenylethylamine were not detected. Only putrescine and cadaverine were detected at high levels, and differences were observed between control cheeses and reduced-NaCl cheeses, regardless of the presence or absence of *P. fragi*. Putrescine was detected at significantly higher levels in reduced-NaCl cheese than in control cheese (in the absence of *P. fragi*):  $54.1 \pm 40.1$  versus  $11.4 \pm 5.7$  mg/kg of cheese (Student's test,  $P < 0.05$ ). Similarly, cadaverine was detected at significantly higher levels in reduced-NaCl cheese than in control cheeses:  $460.3 \pm 153.8$  versus  $335.9 \pm 164.9$  mg/kg of cheese (Student's test,  $P < 0.05$ ).

## DISCUSSION

Reduction of salt levels in food products such as cheese has been undertaken in various countries. Doyle and Glass (2010) described the significant positive economic benefit that widespread reduction in dietary sodium would achieve. Physicochemical, microbiological, and sensory studies are therefore needed to evaluate the potential effect of reducing sodium content in cheese. To date, such studies have been carried out essentially on hard and semi-hard cheeses (Rulikowska et al., 2013; Porcellato et al., 2014; Soares et al., 2015) and Mozzarella cheese (Arboatti et al., 2014). We decided to provide similar data on soft surface-ripened cheese, which represents an important part of cheese

**Table 2.** Volatile compound levels ( $\mu\text{g}/\text{kg}$ ) detected in cheeses (means and SD of 5 independent cheeses) after 27 d of ripening<sup>1</sup>

Compound	Control cheese		Cheese with 1.3% NaCl	
	Mean	SD	Mean	SD
Methanethiol	62*	18	159*	63
Dimethyl disulfide	1,262*	406	3,235*	1,269
Dimethyl trisulfide	39	21	140	97
S-Methyl thioacetate	63*	16	351*	180
2,4-Dithiapentane	27*	12	66*	25
Total	1,453		3,950	
2-Methyl propanal	4*	1	22*	8
3-Methyl butanal	43*	27	95*	30
2-Methyl butanal	3*	2	25*	6
Total	50		142	
Ethanol	7*	7	316*	75
2-Propanol	291*	164	616*	331
2-Butanol	4*	3	0*	0
2-Methyl-1-propanol	10*	6	160*	31
3-Methyl-1-butanol	109*	61	515*	99
2-Methyl-1-butanol	14*	6	179*	26
Total	435		1,785	
Thiobis-methane	43	20	120	114
3-Methyl pentane	2	3	18	18
2-Propanone	971	518	586	436
2-Pentanone	12*	4	4*	5
2-Heptanone	32*	7	49*	12
Octane	14*	3	54*	13
Total	1,073		831	
Hexanoic acid ethyl ester	0	0	244	311
Acetic acid ethyl ester	0*	0	39*	19
Total	0		282	

\*Statistically significant between control and reduced-NaCl cheeses at  $P < 0.05$  (by Student's  $t$ -test).

production in France. Compared with other types of cheeses, soft cheeses harbor a rich and diverse nonlactic microflora that successively establishes itself at the surface of the cheese (Irlinger and Mounier, 2009; Irlinger et al., 2015). Thus, modifying environmental conditions by reducing NaCl content might induce significant changes in the development of such microflora and, consequently, in cheese quality.

Water activity, which depends on the level of moisture and the concentration of low-molecular-weight solutes including NaCl, is a key physical parameter controlling the development of microbial species in cheese (Guinee and Fox, 2004). It is commonly accepted that reduction in NaCl content results in modification of  $a_w$ . Rulikowska et al. (2013) reported a strong influence of NaCl concentration (0.5 or 3%) on  $a_w$  in Cheddar cheese. However,  $a_w$  values were very similar for salt contents ranging from 1.2 to 1.8% in Cheddar cheese (Rulikowska et al., 2013) and Mozzarella (Arboatti et al., 2014). In our experiments, no major difference in  $a_w$  was observed between control cheese and cheese with a reduced NaCl content during the entire ripening period.

We nevertheless observed differences in the growth kinetics of some of the cheese-ripening microorganisms; for example, *D. hansenii* was found to develop less in

cheese with a reduced NaCl content (Figure 1). Because this species actively consumes lactate in cheese products (Leclercq-Perlat et al., 2000a), this could explain the slower increase in the pH of the cheese with reduced NaCl content ( $\text{pH } 7.71 \pm 0.16$  at d 27) compared with the control cheese ( $\text{pH } 8.35 \pm 0.06$  at d 27). *Debaryomyces hansenii* is recognized as a halotolerant yeast with a halotolerance ranging from 0 to 3 M, and an optimal salinity of 0.5 to 1 M, corresponding to 2.9 and 5.9% NaCl in cheese (Gunde-Cimerman et al., 2009). Concomitantly, we observed greater development of *H. alvei* in cheese with a reduced NaCl content. This gram-negative bacterium, which is frequently detected in smear-ripened cheese (Larpin-Laborde et al., 2011) and hard cheese (Maifreni et al., 2013), is also used as a commercial adjunct to improve cheese flavor (Skovmose, 2003). In fact, *H. alvei* can produce high levels of aroma compounds including volatile sulfur compounds (e.g., methanethiol, DMDS, dimethyl trisulfide) and branched-chain alcohols (Morales et al., 2004; Irlinger et al., 2012; Pogačić et al., 2015). Accordingly, the levels of some of these compounds at the end of the ripening period was significantly higher in the reduced-NaCl cheese containing higher levels of *H. alvei*. Some of these compounds, such as sulfur compounds, have

very low perception thresholds and give their uniqueness to a variety of ripened cheeses. However, it is possible that these compounds have a strong effect on the global aroma perception of cheese. Thus, sensory analysis is still needed to evaluate the effect of reducing salt content on such type of cheese.

It is important to point out that the microbial consortia could produce beneficial compounds from amino acids such as aroma compounds, together with undesirable compounds such as biogenic amines. Putrescine and cadaverine were detected at higher levels in cheeses with a reduced NaCl content than in control cheeses. No appreciable amount of histamine or tyramine was produced in the cheese models, these being considered the most toxic BA (Shalaby, 1996). Putrescine and cadaverine have toxicological effects that are generally considered lower than those of histamine and tyramine, but they may potentiate histamine and tyramine toxicities (Shalaby, 1996). Little is known about the ability of cheese-ripening microorganisms to produce BA. The ability to produce putrescine and cadaverine is present in various *H. alvei* strains isolated from cheeses (Marino et al., 2000; Coton et al., 2012; Maifreni et al., 2013). Moreover, gene sequences for lysine and ornithine decarboxylase, responsible for the production of these 2 BA, were identified in the genome of *H. alvei* strain FB1 (Tan et al., 2014). Some strains of *D. hansenii* isolated from cheese are also able to decarboxylate ornithine and histidine (Gardini et al., 2006). We suspect that the production of putrescine and cadaverine in our study could be due to the greater development of *H. alvei*. The biosynthesis of BA in foods requires the availability of free AA. The NPN:TN index was higher in cheeses with a reduced NaCl content than in the control cheese from d 6 to 27. Thus, the greater development of *H. alvei* combined with higher free AA availability could result in higher BA production in our model cheeses with reduced NaCl content.

Finally, salt is considered a critical hurdle to control the growth of pathogenic and spoilage microorganisms. *Pseudomonas* species are considered major cheese spoilage microorganisms that might negatively affect the sensory characteristics of cheese. For example, some strains belonging to this genus can produce pigments (Martin et al., 2011), BA (Martuscelli et al., 2005; Coton et al., 2012), and a wide variety of volatile compounds (Morales et al., 2005b) during ripening, leading to undesirable changes in appearance, texture, flavor, and odor that reduce cheese quality and make products unsaleable. Moreover, this may lead to considerable waste and losses for the food industry (Martin et al., 2011). We detected greater development of *P. fragi* in cheeses with a reduced NaCl content than in control cheeses. This is in agreement with results obtained

by Tabla et al. (2015), who demonstrated that NaCl concentration significantly influenced growth of *Pseudomonas* in a soft ewe cheese.

The presence of *P. fragi* did not affect the overall production of BA and volatile aroma compounds in our cheeses (Supplemental Tables S1 and S2; <http://dx.doi.org/10.3168/jds.2015-10502>). This is likely because of the low development of this species ( $6.19 \pm 0.47$  log cfu/g at d 27) compared with other microbial species such as *H. alvei*. Moreover, the production of BA (tyramine, putrescine, and cadaverine) by some strains of *Pseudomonas* isolated from Pecorino Abruzzese was shown to be strain-dependent (Martuscelli et al., 2005). The production of volatile compounds such as alcohols, esters, and sulfur compounds by *P. fragi* during ripening, with counts close to 10 log cfu/g in the outer part of semi-hard cheeses, is also strain-dependent (Morales et al., 2005a).

## CONCLUSIONS

Reducing NaCl content, as recommended by many health organizations worldwide, by 30% in an experimental surface-ripened cheese resulted in significant modifications of its microbiological and biochemical properties. Because these properties contribute to cheese quality and consumer acceptability, we recommend further investigations to evaluate the sensory and textural characteristics of representative soft cheeses produced in real conditions with a reduced NaCl content. Partial substitution of sodium chloride by other chloride salts such as potassium chloride represents an alternative option to limit the negative effects of reducing NaCl content in soft cheese and should thus be further investigated.

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