Effect of salt reduction or partial substitution with calcium lactate on the quality of a blue-veined cheese

Imène FERROUKHI [a], Cécile BORD [a], Christophe CHASSARD [a], Julie MARDON [a,*].

[a] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR 545 Fromage, 63370 Lempdes, France.

[*] Corresponding author.

Tel. (+3) 347-398-1339.

E-mail: julie.mardon@vetagro-sup.fr

ABSTRACT

Reducing sodium in food has become a global health concern. In this study we investigated the impact of sprinkling and substitution of salt by calcium lactate during the dry salting of blue-veined cheese. Analyses included physicochemical and biochemical composition, microbial counts, 16rDNA gene metabarcoding analyses and a sensory profile. Salt substitution induced a significant sodium reduction of 40% while sprinkling did not significantly reduce salt (-7%). The mineral migration, B-vitamin evolution and other biochemical parameters were not impacted. No differences in flavour, aroma and odour were detected. However, calcium lactate affected the cheese texture with less hardness and sandy rind. These methods provide new opportunities to reduce salt in dry-salted cheeses, but it is necessary to investigate their effects on other cheese matrixes.

Keywords: Blue-veined cheese, sodium, dry-salt, sensory profile, bacterial community

1 Introduction

Currently, it is obvious that high dietary sodium chloride intake is linked to adverse health effects (Xiao et al., 2022). Sodium intake is associated with high blood pressure which causes 7.5 million deaths annually, the equivalent of about 12.8% of all deaths worldwide. It is a major risk factor for cardiovascular diseases CVD, which in itself is the leading cause of deaths globally (WHO, 2021). The World Health Organization has proposed to reduce sodium intake by 30% in order to reach the guideline of 2g/day (i.e., 5 g of salt/day) by 2025 (WHO, 2021). In France, mean daily salt intakes range from 7 to 10 g of salt and are well in excess of dietary needs (ANSES, 2012). Given that 75% of sodium intake comes from processed foods (Brown et al., 2009), reducing the salt content in these foods is an important lever.

Cheese is well-known sources of sodium, especially blue cheeses, with levels as high as 4% of NaCl (ANSES, 2012). A recent study showed that 40 g of blue-veined cheese could contribute to 23% of the recommended salt intake (Ferroukhi et al., 2022). However, reducing sodium without loss of cheese quality is a challenge due to the specific properties of sodium in flavour, functionality and shelf life extension of cheeses (Guinee, 2004). Moreover, these cheeses can contain an attractive nutritional composition. Ferroukhi et al. (2022) highlighted interesting calcium and vitamin B contents in a blue-veined cheese. It was demonstrated that 40 g of Bleu d'Auvergne PDO cheese contributed to 11% of adequate intake for B12 (Ferroukhi et al., 2022). A link between B vitamins levels and microorganisms has also been established. Except for this last study, no data exists on the evolution of these vitamins in cheese matrix reduced in salt. With increasing demand for more nutritious foods, it is important to improve knowledge of the evolution of the B-vitamin content of dairy products according to different producing factors (LeBlanc et al., 2011).

Numerous attempts have been made by the scientific community and cheese industry to develop an acceptable low-sodium cheese (Bansal & Mishra, 2020). Alternative approaches to achieve sodium-reduced foods include simply reducing NaCl levels or partially replacing NaCl with sodium substitutes, such as KCl, $CaCl_2$, $MgCl_2$ (Chavhan et al., 2015; Dugat-Bony et al., 2019; Grummer et al., 2012; Møller et al., 2012). In the literature, the reduction of added salt is mainly concerned with Cheddar cheese (Møller et al., 2012; Rulikowska et al., 2013). There is a lack of knowledge about salt reduction in blue-veined cheeses which are usually dry salted on the surface. Salt substitution is another interesting practice widely reported contributing to the decrease of salt intake by consumers (Bansal & Mishra, 2020; Grummer et al., 2012). Although KCl has been shown effective in reducing the sodium content of various cheeses (Grummer et al., 2012; Katsiari et al., 1997), adverse effects on the quality of Cheddar have been reported (Gomes et al., 2011; Lindsay et al., 1982). The substitution of NaCl by $MgCl_2$ or $CaCl_2$ has related to off flavours and a decrease in acceptability of Cheddar cheese (Lindsay et al., 1982). Moreover, some of these substitute salts are all acidifying compounds (Remer, 2001). KCl-substituted products should be consumed cautiously by consumers with kidney disease because of the risk of hyperkalaemia (Berthet, 2009). Within this context, organic calcium salts have been used to reduce the acid-forming potential of blue-veined cheeses (Gore et al., 2019). This study found that a partial replacement of NaCl by calcium lactate induced a 19% reduction in Na content in Fourme d'Ambert cheese. However, this research mentioned the use of brining prior to dry salting on the surface. In an exploratory work, different techniques to reduce dry salt on the surface of blue-veined cheese were proposed by Ferroukhi et al. (2023). These authors reported that a substitution of salt by calcium lactate and the sprinkling of a controlled amount of NaCl revealed their technological relevance but deserved to be improved and reproduced in the traditional two-step salting process of blue cheese. Nevertheless, this research mentioned the

use of brining before dry salting on the surface. More recently, Ferroukhi et al (2023) demonstrated that a substitution of salt by 30% calcium lactate was technologically relevant but deserves to be improved in the salting process of blue cheese.

Moreover, during the ripening of cheeses, various biochemical and microbiological mechanisms are involved (McSweeney, 2004), contributing, together with the complexity of the matrix, to the formation of the nutritional and organoleptic properties of blue-veined cheeses. So far, rare works have examined the evolution of these phenomena within a salt reduction context in blue cheeses dry salted on the surface.

Within an important health context, alternatives salting methods are required to reduce sodium in surface dry-salted blue cheeses. Thus, we hypothesise that modifications of the dry surface salting method through a decrease in the quantity of applied salt and a partial replacement of salt by calcium lactate might be relevant alternative techniques to reduce salt content in blue-veined cheeses. The objective of this research was to study the effect of two salt reduction treatments on physicochemical, biochemical, microbiological and sensory quality of this cheese during ripening.

2 MATERIALS AND METHODS

2.1 *Blue cheese production*

Blue cheeses were produced under standard process conditions according to the specifications for Bleu d'Auvergne PDO (INAO, 2021). The raw cow's milk was pasteurised (72 °C, 30s) and standardized for fat content. After calcium chloride addition, the milk then was matured (36 °C, 45 min) and inoculated with a starter cultures (*Lactococcus lactis subsp. Cremoris, Lactococcus lactis subsp. Lactis, Streptococcus thermophiles*) (Danisco, Danemark), *Penicillium roqueforti (SAS LIP, France)* and a rennet extract (Caglificio Clerici,

Italy). The resulting coagulum was cut vertically and horizontally and then stirred to form the 'cap' of the coagulum grains (3 cm^3). The curds were drained in the moulds with several turnings. At day 2 after coagulation, the curds were double dry-salted according to the various salting treatments tested. Six days after the manufacture, the cheeses were pricked. This step aims to create air channels in the core of the cheese to enable the *Penicillium* to develop. Ripening was carried out over 34 days at 8 °C and 95% RH.

2.2 *Salting Treatments and Sampling*

2.2.1 Salting process

As commonly practised in Bleu d'Auvergne cheese, salting process was done by rubbing fine dry salt (Esco, Germany) on the surface of the cheese in two steps. To obtain anhydrous salts, all the fine salt (0.20- 0.23 mm) was previously dried (102 °C, 24h). Surface dry-salting of Bleu d'Auvergne cheese was conducted under three treatments (**Figure S *1***, I). Three cheeses per salting method and per ripening time were collected from the same production batch. The salting treatment (A) represented the control cheese and consisted of a conventional salting method by rubbing the cheese in excess dry salt on the surface. The second salting treatment (B) was a sprinkling of 100 g of fine salt on the cheese surface with a regular rubbing and turning of the cheese. The third salting treatment (C) was a partial substitution of NaCl with 30% calcium lactate (Merck KGaA, Germany). In order to obtain mixtures of salts of homogeneous granulometry with Ca salts, calcium lactate in powder form (0.11-0.15 mm) was also dried and then mixed, ground with NaCl and put into resealable bags. All salting treatments were repeated identically the next day.

2.2.2 Sampling

The sampling plan is presented in **Figure S *1***, (II). A total of 36 Bleu d'Auvergne cheeses were produced. Three blocks of cheeses (2.5 ± 0.1 kg) were sampled per treatment at 6, 13, 21 and 34 days of ripening (corresponding to d6, d13, d21 and d34). The rind and core were separated from each cheese. All samples were ground, homogenised and stored at -20 °C until analysis, except for cheeses collected at 34 days of ripening dedicated to sensory analysis, that were stored at 4 °C.

2.3 *Cheese composition*

2.3.1 Physico-chemical and biochemical analyses

All samples were analysed in triplicate according to the standards of the International Organization for Standardization (AFNOR, 2022). We measured pH, dry matter content, fat content as a proportion of dry matter, total, soluble and non-protein nitrogen contents (SN and NPN), sodium and calcium contents and lactate content at various stages of maturation, according to reference methods (for more information, see the Supplementary Methods). The ratios SN:TN and NPN:TN were used as cheese proteolysis indicators. We measured concentrations of vitamin B2, B6, B9 and B12 in the cheeses throughout ripening as previously described (Ferroukhi et al., 2022).

2.3.2 Microbiological Analyses

The different microorganism flora levels were estimated over all ripening periods. Cheese samples (25 g) were collected aseptically into sterile sample bags (BagMixer CC, France). The initial suspension and further decimal dilutions (10^{-7}) were prepared in 250mL of sterile peptone water. Total aerobic mesophilic bacteria were enumerated as per standard NF EN ISO 4833-2:2013. *Leuconostoc populations* were counted on Mayeux–Sandine–Elliker agar (MSE, Mayeux et al., 1962) *Lactococcus* on M17 agar as described by Terzaghi and Sandine (1975) and *Lactobacillus* on MRS agar according to De MAN et al. (1960).

Yeasts and moulds were counted as per standard NF V 08 – 059. *Enterobacteriaceae* were determined on violet red bile glucose agar (VRBG) according to NF V08-054:2009. All microbiological measurements were done in triplicate (for more information, see the Supplementary Methods).

2.3.3 Bacterial community structure using 16S rRNA gene metabarcoding

The impact of salt reduction on the bacterial community was analysed from samples collected at 6 and 34 days of ripening. As mentioned in a previous study (Ferroukhi et al., 2022), cheeses produced with pasteurised milk did not show large differences in bacterial populations. DNA extraction from the cheese core and PCR amplification of the V3-V4 region of the 16S rRNA gene were performed as previously described (Caron et al., 2021) (for more information, see the Supplementary Methods). Amplicon data from high-throughput sequencing was analysed using the rANOMALY pipeline (Theil & Rifa, 2021). Taxonomic assignment of bacterial sequences was based on two databases, DAIRYdb v2.0 and SILVA 138, keeping the assignment with the highest confidence or the deepest taxonomic rank (Meola et al., 2019). The filtered ASVs count table was used to perform statistical analyses.

2.3.4 Sensory Analysis

Ten subjects were recruited from the trained panel of the sensory analysis laboratory of the VetAgro Sup Institute and trained according to standard ISO 8586-1:1993. They were very familiar with blue cheeses and had good experience in sensory analysis. The sensory evaluation was performed using a sensory profile according to the NF ISO 13299:2016 method. The descriptive analysis evaluates the aspect, aroma, texture and taste properties of cheeses at 34 days of ripening.

Training sessions were conducted before the evaluation sessions wherein the panellists tasted the produced cheeses and other mouldy cheeses. The participants generated a large

number of attributes to describe the blue cheeses tasted. After statistical analysis, a set of 29 attributes was selected and divided into 5 items: appearance, texture, taste, odour and aroma according to Ferroukhi et al. (2022). The performance of the panel was validated during this session in order to control-check for repeatability, discriminative capacity, and consensus according to the guidelines in NF ISO 8586-1:1993. Then, the sensory profile was carried out on cheeses ripened for 34 days and sampled using the method in **Figure S *1***, (III). Slices of cheese (50 g) and removed rind (2 mm) were presented on plates coded with random 3-digit numbers. A vertically-cut slice (rind and core) of each cheese was also presented to the panel to have a representative analysis of the appearance of the cheeses. All cheeses were served at 20 ± 1 °C, presented monadically and distributed according to a Williams Latin Square designed to take into account the first effect of order and carry-over. Scoring was done using a 10-cm linear scale, anchored from 0 (very weak) to 10 (very intense), to record the intensity of the sensory attributes. Between each sample, panellists were asked to rinse their mouth with unsalted crackers then tepid water to remove fatty residuals. There were two evaluation sessions led in a computerized booth according to ISO standard 8589:2010. Data was collected using Tastel software® (version 2019; ABT Informatique, Rouvroy-sur-Marne, France).

2.4 *Data Analysis*

All results were reported as mean ± standard deviation. A Shapiro-Wilk test ($p < 0.05$) was used to check the normality of the data. Two-way mixed-model ANOVA (salting treatment*ripening time) with interaction was carried out on biochemical and microbiological data and a post-hoc comparison (Fisher's test) were applied and differences were considered significant when $p < 0.05$. All statistical analysis of biochemical data was performed using XLSTAT software 2020 (Addinsoft, Paris, France). In addition, a principal component analysis (PCA) was also performed on the mean of all data using a Pearson correlation. For

sensory data, a two-way mixed-model ANOVA (product and assessor) with interaction was carried out on the sensory data, with the assessor as random effect and product as fixed effect, at 95% of confidence levels. Tukey's test was used for multiple comparison tests on the product means, for each attribute, when means were considered different ($P < 0.05$). A hierarchical cluster analysis on the PCA means was performed to estimate the homogeneous groups. The rANOMALY interface was used to investigate the diversity of bacterial communities in the cheeses studied at 6 and 34 days of ripening. Species evenness and richness (α-diversity) of the cheese was evaluated using Shannon, Simpson and InvSimpson indexes to determine evenness and the Observed and Chao1 indexes to determine richness. The compositional dissimilarity between samples (β-diversity) was assessed by the Bray-Curtis method. Differences in the dispersion of bacterial profiles from the centroid between the two ripening times were tested by Tukey's HSD test ($P < 0.05$).

3 Results

3.1 *Physicochemical and Biochemical Characteristics*

The effect of lowering applied salt on the compositional and physicochemical evolution throughout ripening of Bleu d'Auvergne cheese is presented in Table *1*. The dry matter increased during the ripening time in all three cheese models. At d34, the calcium lactate-substituted cheese C had a slightly lower dry matter content (53.79%) than the sprinkled cheese B (54.39%) and the control cheese A (55.05%). An increase in pH in the core and rind of the three cheese models was also observed ($P<0.0001$) (Figure S 2). The pH values reached 6.02, 6.31 and 6.07 at the end of ripening in the core of A, B and C cheeses, respectively. A decreasing pH gradient from the rind to the core of the cheeses was observed during ripening. This gradient was greater at 34 days of ripening, particularly for A and C cheeses with 0.83 and 0.86 points respectively. Concerning nitrogen matter, the salting methods had no impact on crude protein and total nitrogen. In contrast, L-lactate levels

243 decreased during maturation (P<0.0001). The quantity of D-lactate was negligible. However,
244 the crude protein and total nitrogen contents increased during the ripening of the three cheese
245 models, reaching an average crude protein and total nitrogen content of 19.47 and 3.04 g/100g
246 at d 34, respectively.

247 Non-protein nitrogen (NPN) and soluble nitrogen (SN) also increased during ripening
248 in the core of all cheeses. At d 13 and d 21, the NPN and SN values appeared to be higher in
249 the salt-reduced cheeses B and C. At the end of ripening the NPN and SN values were
250 stabilised in the three cheeses with an average value of 0.789 and 2.143 g/100g, respectively.
251 Furthermore, all cheeses were characterised by an increasing level of proteolysis during
252 ripening (Figure S 3). At d 21, the SN:TN and the NPN:TN ratios (reflecting primary and
253 secondary proteolysis, respectively) were higher in the salt-reduced cheeses B and C than in
254 the A cheese. The level of proteolysis plateaued at d34 in all cheeses.

255 Salt content increased in the cheeses core during the ripening period, reaching 2.87%
256 in A cheese, 2.67% in B cheese and 1.73% in C cheese. At d34, the sprinkling technique
257 reduced salt content by 7% compared to the control cheese, which was not significant. In
258 contrast, the substitution of salt with calcium lactate reduced salt content by 40% compared to
259 the control cheese (<0.0001).

260 The evolution of minerals (Figure 1. Evolution of sodium and calcium content of the
261 cheese core and rind during ripening according to three salting methods (mean ± standard
262 deviation of three cheeses). showed an increase of sodium in the cheese core and a decrease in
263 the cheese rind (P<0.001). The sodium content in the rind of cheese A (1443.33 mg /100g)
264 was higher than in cheeses B (1085.00 mg /100g) and C (684.50mg /100g). On the other
265 hand, the calcium content in the rind increased with higher content at d 34 (P<0.0001) in
266 cheese C (1240.00 mg /100g) compared to A (642.50 mg /100g) and B (790 mg/ 100g). In
267 cheeses core, the calcium evolution during ripening was not noticeable. The calcium levels at

d 34 were 613.50, 547.00 and 582.83 mg /100g in the core of A, B and C cheeses respectively. A similar pattern was obtained by estimating the studied parameters on dry matter.

In the present study, the impact of reducing salt on vitamin B2, B6, B9 and B12 changes during ripening was investigated. The trends represented in Figure 2 revealed that the applied salting methods had no effect on B-vitamin composition of the blue cheeses ($P>0.05$). However, the ripening time affected the evolution of these vitamins ($P<0.0001$). Vitamin B2 and B6 contents increased significantly during the ripening period in all cheeses. The average B2 vitamin content of cheeses at d 34 was about 0.38 mg/ 100g. A lower B6 content (0.07 mg/ 100g) was observed in B cheese at d 34. The concentrations of B9 and B12 increased between 6 and 13 days of ripening, afterwards their content decreased to reach an average value of 22.92 µg /100g for B9 and 1.15 µg /100g for B12 in the blue cheeses at the end of ripening.

3.2 *Microbial and 16S rRNA gene Metabarcoding Analyses*

Cheese samples were analysed at each ripening time using classical culture-based counts (Table 2). In parallel, 16S rRNA gene metabarcoding was performed to gain further insight into the bacterial community composition. Finally, 16S rRNA gene Metabarcoding analysis was applied at the beginning was used at the beginning (d 6) and end of ripening (d 34) to assess the impact of salt reductions on the overall microbial community activity. For all bacterial populations studied, the salting method did not induce a major impact on bacterial evolution compared to the ripening time. The total microflora count at the end of the ripening process was higher in B (10.18 log cfu/g) and C (9.42 log cfu/g) cheeses than in A control cheese (8.71 log cfu/g). *Enterobacteriaceae* counts showed no impact of salting treatments. The salt reduction methods did not produce important changes in the count of lactic acid bacteria (LAB) in the cheese core. *Leuconostoc* population tended to increase with the

ripening time. Yeast and moulds increased during ripening in all cheeses, without major differences between the control and the NaCl-reduced cheeses.

Sequencing of the 16S rRNA (V3-V4 regions) of the samples generated a total of 715303 reads that were assigned to 18 ASVs. Taxonomic assignment of each ASV was possible up to the species level (or group of species sharing nearly identical sequences) in most cases. The results of the 16S rRNA gene metabarcoding revealed that the composition of the bacterial communities was similar to the three salting conditions (Table 3). A predominance of *Lactococcus* and *Streptococcus* species in all cheeses were observed with a raw abundance sum of 419585 and 249397 reads, respectively. Sub-dominant species of *Leuconostoc Mesenteroides (*31238 reads), *Brevibacterium (*7568 reads), *Brachybacterium (*2410 reads) and *Lactobacillus* (3852 reads) were also present in all cheeses. Community richness and diversity were assessed for each salting method at 6 and 34 days of ripening using four alpha diversity parameters (Observed, Chao1, Shannon and Simpson). The results in *Table S 1* showed that the salting techniques had no impact on the richness and diversity of the bacterial community at 6 and 34 days of ripening ($p>0.05$). The mean richness indices (Observed and Chao1) of blue cheeses were 19.333 for both ripening times. The mean Shannon and Simpson indices of the cheeses were 1.337 and 0.637 at 6 days and 1.273 and 0.583 at 34 days, respectively.

Species dissimilarity between the salting methods at d 6 and d 34 was assessed based on beta diversity using the Bray-Curtis method. The results revealed that the same species were present in all cheeses at both 6 days ($p=0.886$) and 34 days ($p=0.444$) of ripening (data not shown).

3.3 *Sensory Characteristics*

Significant differences in texture and marbling were highlighted by the panel between the A, B and C cheeses. The sensory profile results showed a significant ($P <0.05$) product effect

for 8 sensory attributes over 28 total attributes (Figure 3). The reliability of the panel was also demonstrated by a significant product × assessor interaction ($P < 0.05$) only for two sensory attributes (lactic aroma and cavities-quantity_C). Surprisingly, no significant salty taste and bitterness difference was observed between cheeses. The observation plot (Figure 3, A and B) and the dendrogram from the agglomerative hierarchical clustering (Figure 3, C) revealed that the calcium lactate substituted cheese constituted a distinctive cluster from the sprinkled and control cheeses. The A and B cheeses were perceived as being significantly firmer than the C cheese, which was characterised by a creamier texture in the core and sandy in the rind. On the other hand, marbling appeared to be more pronounced in C cheese than in A and B cheeses.

4 Discussion

Reducing salt in the diet is becoming a global concern (WHO, 2021). Given the major role of salt in cheese production, it is necessary to examine the impact of salt reduction in the changes in physico-chemical, biochemical and microbiological parameters and the consequences that may have on sensory characteristics. To date, the studies conducted in this way have been mainly interested in hard and semi-hard cheeses (Gomes et al., 2011; Grummer et al., 2012; Lindsay et al., 1982). We decided to provide comparable data on blue-veined cheeses, which are very little studied but rather popular with consumers. The present research investigated the impact of salt reduction by sprinkling method and partial replacement of NaCl by 30% calcium lactate on all physico-chemical, biochemical, microbiological, and sensory aspects of blue-veined cheeses during ripening.

Biochemical and physicochemical changes in blue cheese matrix under salt reduction conditions

The mineral exchange observed in the current work between the rind and the core (Na-ion inflow and Ca-ion outflow) has been demonstrated in previous studies (Ferroukhi et al.,

2022; Guinee, 2004). This reaction is induced by the application of dry salt on the surface, which causes an exudation of the mineral-laden water, while salt (sodium) moves into the cheese matrix (Guinee, 2004; Le Graet & Brulé, 1988). The increasing pH gradient found was negatively correlated ($r = -0.73$, $p<0.05$) to a decreasing lactate content. According to McSweeney (2004), the bacteria on the surface metabolise lactate into CO_2 and O_2, thereby deacidifying the cheese surface and creating a pH gradient developed from the centre to the rind. As the surface pH increases, Ca and phosphates move irreversibly from the core to the cheese surface. The same pattern has been found in other studies (Ferroukhi et al., 2022; Gore et al., 2019; Le Graet & Brulé, 1988). The pH gradient is thus responsible for an accumulation of these minerals on the cheese surface which is even more important with the application of calcium lactate, impacting the texture of the cheese rind. Indeed, in the present work, the calcium lactate-substituted cheeses had the highest calcium content in the rind. However, there was no calcium contribution to the cheese core as found by Ferroukhi et al. (2022) and Gore et al. (2019) in blue-veined-cheeses, suggesting that the calcium brought by the salt mixture remained on the cheese surface.

In this work, a technological relevance of the sprinkling and calcium lactate substitution methods was demonstrated during the dry salting of blue-veined cheese in two steps. This is consistent with the previous findings of Ferroukhi et al. (2023). However, these authors reported a 45% decrease in sodium in cheese with the sprinkling technique, contrary to what was obtained in the present paper (-7%). This can be attributed to the double salting applied here and conventionally in the salting of blue cheeses, unlike the exploratory study of Ferroukhi et al. (2023). In addition, we can hypothesise that the quantity of salt sprinkled in two steps was not sufficiently decreased to allow a marked reduction of sodium in the cheese matrix, and the wet surface of the blue cheeses retains a large proportion of salt. Calcium lactate was an effective salt substitute as already reported in another blue-veined cheese (Gore

et al., 2019). The significant decrease of sodium caused by the double salting the cheese with the mixture of salt and 30% calcium lactate has supported the assumptions made by Ferroukhi et al. (2023) . We can suppose that the application of calcium lactate on the surface would prevent some of the salt from penetrating, thus leading to a reduction in sodium content.

Blue-veined cheeses are known to have a high proteolysis rate (McSweeney, 2004). In our experience, the increase in proteolysis, positively correlated with the increase in the mould count and pH ($r=0.881$ for moulds, $r=0.909$ for pH, $p<0.05$), corroborated the literature data (Cantor et al., 2017; Dugat-Bony et al., 2016; Ferroukhi et al., 2022). As described in Bleu d'Auvergne cheese (Ferroukhi et al. 2022) and other blue-veined cheeses (Fernandez-Salguero et al., 1989; Ferroukhi et al., 2022; Prieto et al., 2000), a high and increasing rate of proteolysis marked the three cheeses produced (A, B and C). The rapid proteolysis of salt-reduced cheeses suggested that moisture conditions in salt-reduced cheeses accelerated the proteolytic activity of moulds, particularly at 21 days of ripening. A similar effect was reported in Minas cheese (Gomes et al., 2011) and soft cheeses substituted with potassium chloride (Dugat-Bony et al., 2019). At 34 days, there was no difference between the control cheese and the reduced salt cheeses.

In addition, the impact of reducing salt on the water-soluble vitamin composition of blue-veined cheeses during ripening was investigated. We have highlighted in a previous study that Bleu d'Auvergne cheese may contain interesting quantities of B vitamins (Ferroukhi et al. 2022). Nevertheless, before this study, no data were available on the evolution of water-soluble vitamins during the ripening of blue-veined cheeses. The salt reduction applied here did not affect the levels of vitamins B2, B6, B9 and B12 and this is relevant for the nutritional value of these cheeses. Indeed, B vitamins have a number of major roles in metabolic processes such as energy production and red blood cell formation (LeBlanc

et al., 2011). B vitamins, normally present in many foods, are easily removed or destroyed during food processing, so inadequate intakes are common in many populations.

The average results obtained here for vitamin B2 and B6 content were comparable to those found in other blue-veined cheeses (Altangerel et al., 2011; Ferroukhi et al., 2022; O'Brien & O'Connor, 2017). The pyridoxine and riboflavin contents depend largely on the milk's initial composition and the cheese-making technology. Moreover, it has been reported that blue cheeses, with a high proteolysis rate, contain a higher content of B vitamins (Gregory, 1967). Regarding folates, the level of this vitamin in the cheeses seems lower than those found in blue-veined cheeses such as Roquefort or Stilton (O'Brien & O'Connor, 2017). The increase in folate content and subsequent decrease after 13 days of ripening may result from synthesis followed by consumption of folate by cheese microbiota. For instance, most authors claim that *Streptococcus thermophilus* normally produce folates whereas *Lactobacillus delbrueckii subsp*. *bulgaricus* would be a folate consumer (LeBlanc et al., 2011), so the selection of adequate combination of strains is essential to develop fermented foods with increased vitamin concentrations. Attractive amounts of vitamin B12 were also found in the three cheeses (control and salt-reduced cheeses) at the end of the ripening process. A previous investigation demonstrated that a 40 g portion of Bleu d'Auvergne could significantly contribute to the nutritional requirements of vitamin B12 (Ferroukhi et al. 2022).

Impacts of Reducing Salt on Sensory Properties

Salt has a key role in the development of sensory characteristics during the ripening of cheese and its decrease can affect cheese organoleptic and functional quality (Bansal & Mishra, 2020; Guinee, 2004). The sensory analysis of this work revealed no difference in flavour, odour and aroma of the salt-reduced cheeses and the control cheese. Similar observations were reported during a flash profile in a previous investigation (Ferroukhi et al., 2023). Reducing the added salt content in cheeses is often related to the appearance of

417 bitterness probably caused by a high proteolysis indexes (Møller et al., 2012). In spite of the
418 increasing proteolysis that was observed in experimental cheeses, there was no difference in
419 bitterness between the control and salt-reduced cheeses, presumably owing to the stabilisation
420 of this phenomenon at the end of the ripening process.

421 The significant salt reduction in the cheese substituted with calcium lactate (-40%) did
422 not change the flavour. This result is consistent with observations made on Fourme d'Ambert
423 cheese (Gore et al., 2019). These authors concluded that calcium lactate could substitute NaCl
424 up to 75% without affecting the bitterness and salinity of the cheeses. On the other hand, in
425 this same study, Fourme d'Ambert cheeses had previously received salt by brining.

426 Here, the reduction of salt in the dry salting of blue cheeses showed that using calcium
427 lactate could impact the texture of the cheeses. In general, it has been reported that low
428 sodium cheeses tend to produce softer and creamier cheeses (Guinee & Fox, 2004). Here, the
429 effect of salt reduction methods on the sensory quality of blue cheeses was less important as
430 compared to the salt reduction of Cheddar cheese (Lindsay et al. 1982; Rulikowska et al.
431 2013). The nature of the calcium lactate (white powder) remaining on the surface may explain
432 the sandy texture of the rind of B cheese. The same observation was reported for Fourme
433 d'Ambert (Gore et al., 2019). Furthermore, calcium lactate applied to the cheese surface may
434 block the moisture release. Hence, the softer texture of the calcium lactate substituted cheese
435 is probably due to a more significant presence of moisture (reflected by the slight lower dry
436 matter for calcium lactate substituted cheese) and casein hydration. As reported by Guinee.
437 (2004) and Johnson (2000), salt increases the water-holding capacity of cheese matrix by
438 increasing casein hydration, matrix volume and casein solubilisation. Consequently, the
439 reduction in casein hydration decreased the ability of the cheese matrix to resist deformation,
440 resulting in a decrease in hardness.

On the other hand, the sensory analysis also indicated that the Ca-lactate substituted cheese contained more marbling than the other cheeses. This doesn't necessarily mean a higher growth of *Penicillium roqueforti*, but could be related to the heterogeneity of the matrix of a blue-veined cheese, which leads to heterogeneous cheese slices served during the testing (Ferroukhi et al., 2023).

Impacts of Reducing Salt on Microbial Growth

A significant salt lowering in cheeses is often associated with an alteration of their microbiological quality (Guinee et Fox, 2004). The microbial data highlighted a total absence of *Enterobacteriacae*. Given that the presence of these bacteria is recognised as an indicator of contamination and hygiene in cheeses (Mladenović et al., 2021), this may suggest that the salting treatments applied did not alter the sanitary quality of the cheeses. These confirmed the results obtained by Ferroukhi et al. (2022). Contrary to the reported increase of *Pseudomonas* in semi-hard and soft-surface ripened cheeses reduced in salt (Dugat-Bony et al., 2016, 2019), in this study applied reduction methods did not induce an increase of spoilage microbiota.

The cheeses reduced in salt had more total microflora at the end of the ripening process, probably owing to more favourable biochemical conditions (moisture, pH, salt.. etc.). In essence, the applied sodium lowering treatments did not have a major impact on bacterial evolution in the produced blue cheeses. The rapid growth of yeasts and moulds observed was a typical feature of blue-veined cheeses, previously reported in Bleu d'Auvergne (Ferroukhi et al., 2022), Gorgonzola (Gobbetti et al., 1997) and Roquefort cheese (El Dairouty et al., 1990) for example. The activity and growth of this fungus is related to some environmental conditions inside the cheese such as prickling (oxygen in cavities) and salt level ($r=0.622$, $p<0.05$) (Caron et al., 2021). Our data revealed that reducing salt by 7% and 40% in this research did not interfere with the growth and activity of this species.

Penicillium roqueforti is used in blue cheeses as a secondary starter for flavour production, mostly through proteolysis and lipolysis during ripening (Cantor et al., 2017; Caron et al., 2021). The correlation found between increased proteolysis and mould growth confirmed the high proteolytic activity of *Penicillium roqueforti.* Through a combination of intra- and extracellular enzymes, this fungus is able to degrade most proteins. The resulting peptides contribute to cheese flavour, and their breakdown into amino acids further influences cheese aromas and the proliferation of other microorganisms (McSweeney, 2004).

Among the bacterial populations studied, lactic acid bacteria (LAB) did not show a large variation with salt reduction. Comparable findings have been made in Halloumi cheese (Kamleh et al., 2012), and other semi-hard and soft cheeses substituted with potassium chloride (Dugat-Bony et al., 2019). In contrast, LAB counts were consistently higher in the salt reduced Cheddar cheese (Rulikowska et al., 2013; Schroeder et al., 1988). This group of microorganisms can influence the ripening process and the sensory characteristics of cheeses due to the production of different organic acids and flavour compounds (McSweeney, 2004).

As expected, starter bacteria (*Lactococcus* and *Streptococcus*) inoculated into the milk during manufacture were highly dominant over the other species. As reported in a previous study (Ferroukhi et al., 2022), *Lactococcus species* were positively correlated with B2 and B6 contents ($r>0.960$, $p<0.05$) in contrast to a negative correlation with *Streptococcus species* ($r<-0.950$, $p<0.05$) in all produced cheeses. It is of great interest to highlight that the salt reductions tested in this work did not seem to interfere in the activity of certain micro-organisms involved in the metabolism of these nutrients. However, depending on the bacterial strain, the metabolism of vitamins can be different and remains to be investigated.

CONCLUSION

In conclusion, in this paper we proposed new methods to reduce Na content in a blue-veined cheese. In practice, sprinkling and partial substitution of salt by calcium lactate appear to be suitable alternatives to conventional dry-salting methods. The cheeses obtained using a 30% substitution of salt with calcium lactate contained 40% less sodium than the control cheese. The minor effect of salt sprinkling on sodium content suggests that the salting process should be improved by decreasing the amount of salt applied. Regarding the sensory aspect, the main changes concerned textural modifications of the cheeses brought by the substitution of calcium lactate. All other organoleptic qualities remained unchanged with the proposed salt reduction techniques. The impacts of the salt reduction procedures proposed in this research on the physicochemical, biochemical and microbiological criteria of the cheeses were insignificant, suggesting a preservation of intrinsic properties of the cheeses. This paper highlights that salt reduction did not affect the nutritional quality of blue-veined cheeses in terms of B vitamins. The B-vitamin results also provide interesting data that were lacking in the literature and that can assist in the nutritional optimization of cheese. In prospect, it would be interesting to investigate these salting methods on another cheese dry salted on the surface to estimate their impact on the quality of the ripened cheese.

Declarations of competing interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper**.**

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Figures Captions

Figure 1. Evolution of sodium and calcium content of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses). A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate. The figure shows an increase in the amount of sodium in the cheese core and a decrease in the rind with the three salting modalities. Simultaneously the calcium content decreases in the core and increases in the rind of the cheeses with a high level in the cheese substituted with calcium lactate. [a-g] Values with the same letter are statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

Figure 2. Evolution of B-vitamins content of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses). A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate. The figure demonstrates a similar trend in the evolution of B vitamins in three salting treatments. Vitamins B2 and B6 increased during ripening while vitamin B9 and B12 tended to decrease. [a-g] Values with the same letter are statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

Figure 3. Principal component analysis performed on sensory profile: plot of principal axes F1 – F2. A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate. (A) Correlation circle, (B) Representation of cheeses, (C) Dendrogram derived from hierarchical classification ascendant performed on the first three components of principal component analysis, obtained from sensory profile. The dashed line shows the level of truncation. In the correlation circle (A), only 8 descriptors out of 28 studied had a significant 'product' effect. The representation (B) shows an opposition of cheese substituted with calcium lactate compared to control and sprinkled cheese. This is confirmed by the homogeneous groups created in the dendrogram (C), where A and B cheeses from the same group differentiated on C cheese.

Figure S 1. Sampling plan. Sampling of cheeses during the ripening periods (I). Sampling method for the rind and core of Bleu d'Auvergne cheese (II). Sampling method for the sensory analysis (III). A: Standard cheese salted in excess of salt by a traditional method, B: Cheeses salted by sprinkling 100 g of fine salt, C: Cheeses salted with a mix of NaCl and 30% of calcium lactate, W: Cheeses unmoulded before salting. Sampling of the rind and core was performed to follow the biochemical and physico-chemical parameters during the ripening of the cheeses. For each ripening time, triplicates of cheeses were produced by salting method.

Figure S 2. Evolution of pH of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses). The figures show an increasing pH in the rind and core of the manufactured cheeses. The pH of the rind was higher than the pH of the core creating an increasing pH gradient from core to rind cheese. $^{a-h}$ Values with the same letter are statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

Figure S 3. Evolution of proteolysis of the cheese core during ripening according to three salting methods (mean ± standard deviation of three cheeses). Primary and secondary proteolysis increased throughout the ripening of all cheeses. The level of proteolysis was higher in the salt-reduced cheeses at 21 days of ripening and then stabilised at the end of ripening. $^{a-e}$ Values with the same letter are statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

TABLES

Table 1. Evolution of the composition of the cheese core during ripening according to three salting methods (mean ± standard deviation of three cheeses).

Ripening	Methods	DM (%)	Fat [1]	FDM (%)	Lactate[2]	Salt (%)	Crude protein [1]	TN [1]	NPN [1]	SN [1]
Day 6	A	51,81±0.96 ^{ab}	29,15±0.43 ^{c}	0,61±0.61 ^{fgh}	151,10±29.64 ^{de}	0,80±0.20 ^{a}	18,62±0.37 ^{a}	2,92±0.06 ^{a}	0,17±0.01 ^{a}	0,59±0.03 ^{a}
	B	52,04±0.38 ^{b}	28,83±0.23 ^{abc}	0,65±0.65 ^{cdef}	158,39±12.48 ^{e}	0,71±0.04 ^{a}	18,80±0.04 ^{ab}	2,95±0.01 ^{ab}	0,18±0.01 ^{c}	0,63±0.03 ^{a}
	C	51,21±0.22 ^{a}	28,92±0.23 ^{bc}	0,71±0.71 ^{gh}	162,30±6.19 ^{e}	0,88±0.50 ^{a}	18,99±0.04 ^{bc}	2,98±0.01 ^{bc}	0,17±0.01 ^{a}	0,62±0.05 ^{a}
Day 13	A	53,64±0.35 ^{cd}	28,53±0.06 ^{a}	0,45±0.45 ^{a}	171,46±8.66 ^{e}	3,01±0.52 ^{f}	18,72±0.14 ^{a}	2,93±0.02 ^{ab}	0,18±0.01 ^{bc}	0,65±0.04 ^{a}
	B	52,250.47 ^{b}	28,73±0.35 ^{ab}	1,16±1.16 ^{cd}	211,19±24.93 ^{f}	2,34±0.60 ^{de}	18,84±0.10 ^{ab}	2,99±0.07 ^{bcd}	0,17±0.01 ^{ab}	0,63±0.03 ^{a}
	C	52,04±0.44 ^{b}	29,70±0.10 ^{de}	0,62±0.62 ^{h}	169,34±9.50 ^{e}	1,35±0.03 ^{b}	19,65±0.33 ^{f}	3,08±0.05 ^{f}	0,21±0.03 ^{d}	0,72±0.08 ^{a}
Day 21	A	54,74±0.24 ^{e}	29,12±0.14 ^{c}	0,48±0.48 ^{a}	117,68±8.14 ^{cd}	2,57±0.11 ^{e}	19,18±0.07 ^{cd}	3,01±0.01 ^{cde}	0,41±0.06 ^{e}	1,16±0.13 ^{b}
	B	53,35±0.65 ^{c}	29,17±0.06 ^{c}	0,73±0.73 ^{bc}	65,39±26.70 ^{ab}	2,21±0.08 ^{d}	19,29±0.25 ^{de}	3,01±0.04 ^{cde}	0,61±0.07 ^{f}	1,62±0.16 ^{c}
	C	53,19±0.42 ^{c}	29,867±0.15 ^{de}	0,62±0.62 ^{efgh}	87,62±28.25 ^{bc}	1,61±0.32 ^{c}	19,51±0.46 ^{ef}	3,05±0.08 ^{ef}	0,68±0.15 ^{fg}	1,70±0.59 ^{c}
Day 34	A	55,05±0.20 ^{e}	29,63±0.45 ^{d}	0,71±0.71 ^{ab}	62,18±11.85 ^{ab}	2,87±0.30 ^{f}	19,54±0.27 ^{ef}	3,04±0.08 ^{def}	0,80±0.13 ^{g}	2,11±0.21 ^{d}
	B	54,39±0.19 ^{de}	30,00±0.27 ^{e}	0,59±0.59 ^{cde}	61,19±7.13 ^{ab}	2,67±0.14 ^{ef}	19,52±0.13 ^{ef}	3,06±0.02 ^{ef}	0,79±0.11 ^{g}	2,24±0.12 ^{d}
	C	53,79±0.36 ^{cd}	30,03±0.32 ^{e}	0,43±0.43 ^{defg}	36,09±15.16 ^{a}	1,73±0.14 ^{c}	19,37±0.15 ^{def}	3,04±0.02 ^{cdef}	0,78±0.16 ^{g}	2,08±0.29 ^{d}
P-Value Ripening*Methods		0.129	<0.0001	0.000		0.000	<0.0001	0.034	<0.0001	0.088

A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate.

$^{a-h}$ Means in a column sharing common superscripts are similar as tested by Fisher test (Two-way ANOVA, P <0.05).

[1] Expressed as g/100 g.

[2] Expressed as mg/100 g.

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745 Table 2. Microbiological counts (log cfu/g) of cheese core samples during ripening (mean ± standard deviation of three cheeses).

Ripening	Salting methods	*Lactobacillus*	*Lactococcus*	Total microbiota	*Leuconostoc*	Yeasts	Moulds	*Enterobacteriaceae*
Day 6	A	6,64± 0.10^{cd}	7,18±0.08^{f}	9,06±0.13^{abc}	4,76±0.16^{a}	1,46±0.02^{a}	1,58±0.10^{a}	ND
	B	6,91±0.17^{ef}	6,96±0.29^{ef}	8,99±0.08^{ab}	4,67±0.03^{a}	1,41±0.05^{a}	1,59±0.04^{a}	ND
	C	6,80±0.08^{de}	6,94±0.20^{def}	9,10±0.11^{abc}	5,75±0.16^{b}	1,53±0.03^{b}	1,57±0.03^{a}	ND
Day 13	A	5,78±0.17^{a}	7,22±0.29^{f}	8,73±0.08^{a}	6,14±0.03^{cd}	1,80±0.05^{d}	2,23±0.04^{b}	ND
	B	5,99± 0.25^{b}	7,26±0.19^{f}	8,84±0.40^{a}	6,04±0.26^{c}	1,76±0.02^{cd}	2,18±0.12^{b}	ND
	C	6,07±0.12^{b}	6,61±0.41^{bcd}	9,10±0.04^{abc}	6,26±0.14^{d}	1,72±0.07^{c}	3,16±0.14^{c}	ND
Day 21	A	6,21±0.17^{b}	6,66±0.55^{bc}	9,46±0.75^{c}	6,20±0.05^{d}	5,10±0.02^{ef}	5,38±0.00^{d}	ND
	B	6,14± 0.11^{b}	6,36±0.75^{b}	9,34±0.79^{bc}	6,15±0.05^{cd}	5,08±0.04^{ef}	5,37±0.09^{d}	ND
	C	6,46±0.57^{c}	5,97±0.24^{a}	8,92±0.35^{a}	6,24±0.06^{d}	5,22±0.04^{g}	5,87±0.46^{e}	ND
Day 34	A	6,20±0.06^{b}	6,73±0.08^{cde}	8,71±0.15^{a}	6,14±0.12^{d}	5,12±0.02^{f}	6,15±0.02^{f}	ND
	B	7,07±0.21^{fg}	7,84±0.23^{g}	10,18±0.02^{d}	6,02±0.13^{c}	4,94±0.23^{e}	6,18±0.04^{f}	ND
	C	7,15± 0.10^{g}	7,14±0.10^{f}	9,42±0.48^{c}	5,73±0.10^{b}	5,31±0.04^{h}	6,04±0.02^{f}	ND
P-Value Ripening*Methods		<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	

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747 A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular
748 rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate.
749 $^{a-h}$ Means in a column sharing common superscripts are similar as tested by Fisher test (Two-way ANOVA, P <0.05).
750 ND : No detected
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755 Table 3. Sum of relative abundance of each species produced blue-cheese cores (mean ± standard deviation of three cheeses).

Methods	Ripening time					
	6 Days			34 Days		
	A	B	C	A	B	C
Lactococcus_species	46,96±0.02^{a}	51,11±0.10^{a}	45,99±0.03^{a}	68,78±0.10^{b}	64,91±0.08^{b}	74,44±0.09^{b}
Streptococcus_species	45,34±0.01^{b}	42,84±0.10^{b}	47,99±0.03^{b}	25,39±0.09^{a}	29,31±0.08^{a}	18,36±0.09^{a}
Leuconostoc_mesenteroides	4,31±0.01^{ab}	4,06±0.00^{a}	4,46±0.00 ^{ab}	3,65±0.01^{a}	4,57±0.01^{ab}	5,08±0.01^{a}
Brachybacterium_species	1,02±0.00^{b}	0,58±0.00^{b}	0,41±0.00^{a}	0,50±0.01^{ab}	0,35±0.00^{a}	0,31±0.00^{a}
Brevibacterium_species	2,00±0.01^{b}	1,06±0.00^{ab}	0,82±0.00^{a}	1,08±0.01^{ab}	0,65±0.00^{a}	0,64±0.00^{a}
Lactobacillus_species	0,11±0.00^{ab}	0,19±0.00^{bc}	0,18±0.00^{bc}	0,39±0.00^{c}	0,07±0.00^{a}	1,03±0.01^{d}
Enterobacteriaceae_species	0,11±0.00^{a}	0,08±0.00^{a}	0,09±0.00^{a}	0,14±0.00^{a}	0,06±0.00^{a}	0,07±0.00^{a}
Lentilactobacillus_species	0,13±0.00^{b}	0,06±0.00^{a}	0,06±0.00^{a}	0,05±0.00^{a}	0,05±0.00^{a}	0,04±0.00^{a}
Romboutsia_species	0,01±0.00^{b}	0,01±0.00^{ab}	0,01±0.00^{ab}	0,00±0.00^{a}	0,01±0.00^{ab}	0,01±0.00^{ab}
Acinetobacter_johnsonii	0,02±0.00^{a}	0,00±0.00^{a}	0,00±0.00^{a}	0,01±0.00^{a}	0,01±0.00^{a}	0,00±0.00^{a}

756 A: control cheese salted by rubbing the cheese in excess dry salt on the surface.; B: Reduction of salt by sprinkling of 100 g of fine salt on the cheese surface with a regular
757 rubbing and turning of the cheese.; C: Reduction of salt by a partial substitution of NaCl with 30% calcium lactate.
758 $^{a-d}$ Means in a line sharing common superscripts are similar as tested by Fisher test (Two-way ANOVA, P <0.05)

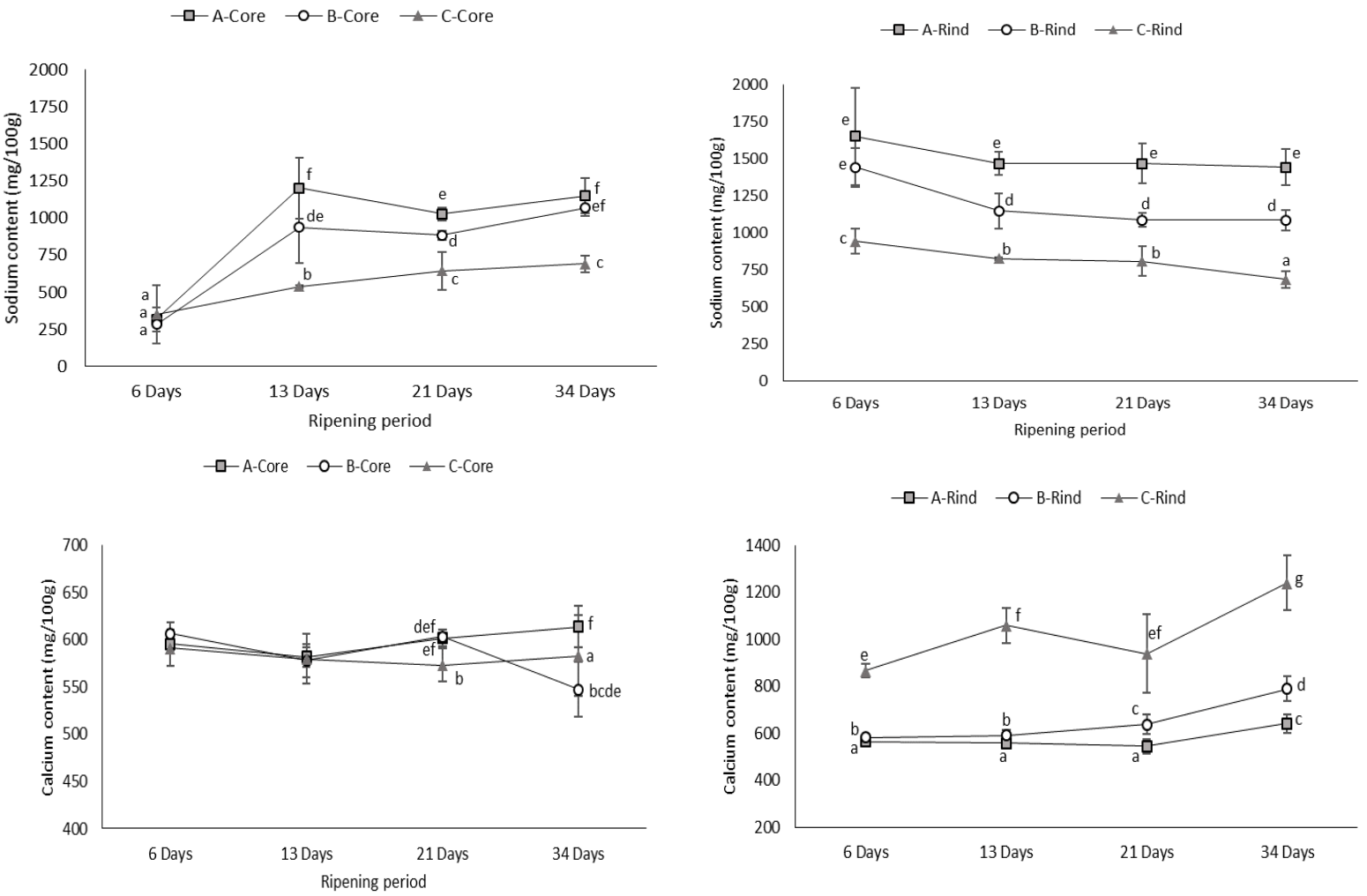

Figure 4. Evolution of sodium and calcium content of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses).
[a-g] Values with the same letter are in statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

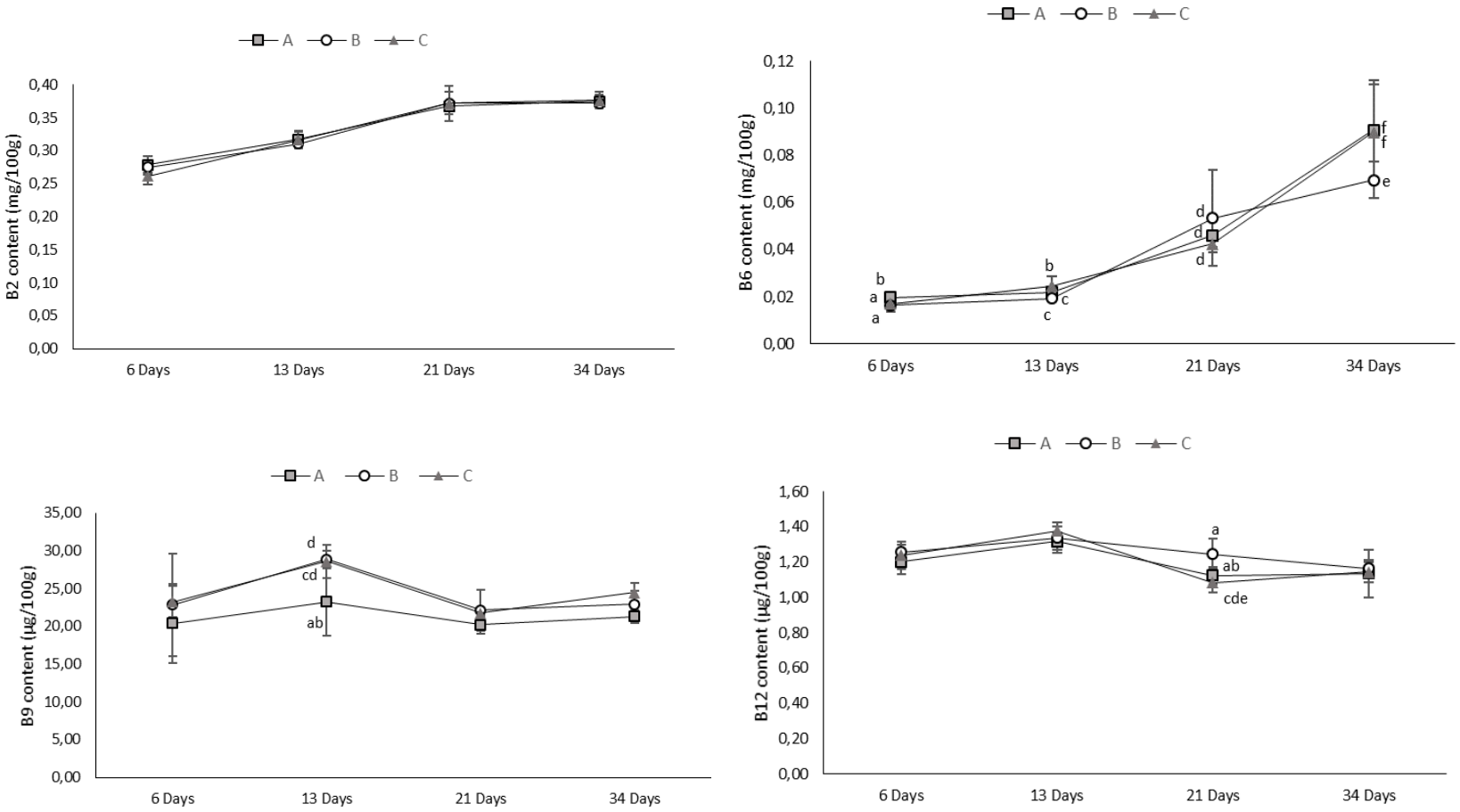

Figure 5. Evolution of B-vitamins content of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses). [a-f] Values with the same letter are in statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

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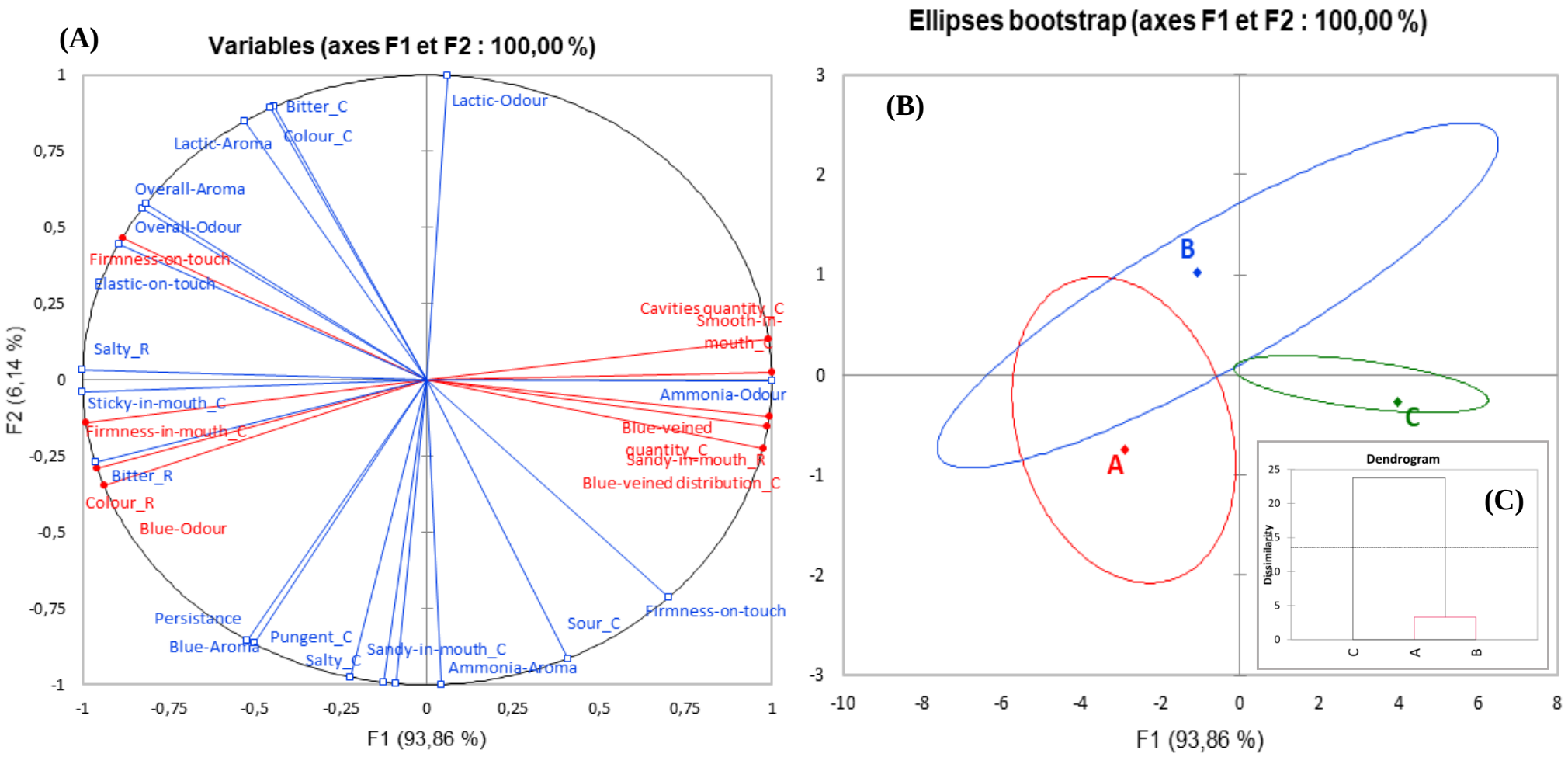

Figure 6. Principal component analysis performed on sensory profile: plot of principal axes F1 – F2. (A) Correlation circle, (B) Representation of cheeses, (C) Dendrogram derived from hierarchical classification ascendant performed on the first three components of principal component analysis, obtained from sensory profile. The dashed line shows the level of truncation.

Supplementary Data

Table S 2. Alpha diversity indices obtained from blue-cheese cores at 6 and 34 days of ripening

	ripening time.							
	6 days				34 Days			
Methods	Observed	Chao1	Shannon	Simpson	Observed	Chao1	Shannon	Simpson
A	19,000	19,000	1,380	0,650	18,670	18,670	1,250	0,570
B	19,330	19,330	1,310	0,630	20,000	20,000	1,310	0,580
C	19,670	19,670	1,320	0,630	19,330	19,330	1,260	0,600
P-Value	0,868				0,402			

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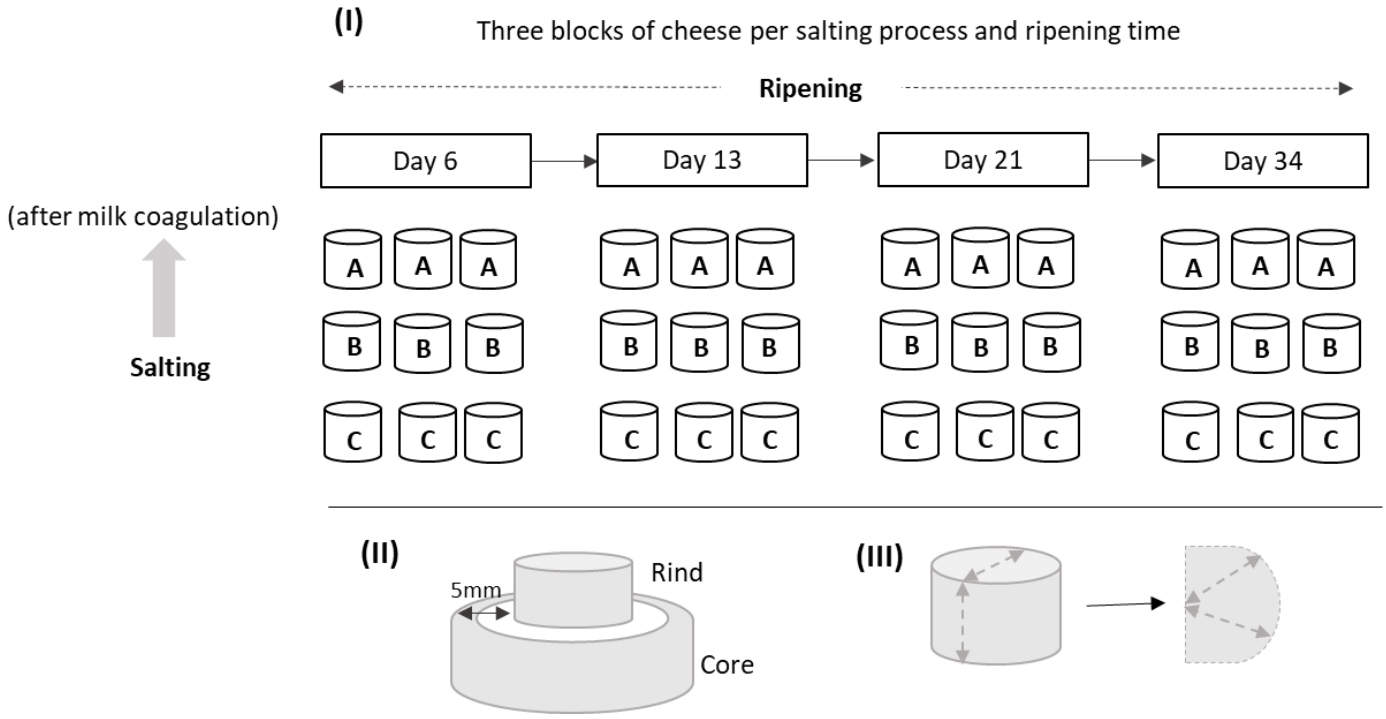

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Figure S 1. Sampling plan. Sampling of cheeses during the ripening periods (I). Sampling method for the rind and core of Bleu d'Auvergne cheese (II). Sampling method for the sensory analysis (III). A: Standard cheese salted in excess of salt by a traditional method, B: Cheeses salted by sprinkling 100 g of fine salt, C: Cheeses salted with a mix of NaCl and 30% of calcium lactate, W: Cheeses unmoulded before salting. Sampling of the rind and core was performed to follow the biochemical and physico-chemical parameters during the ripening of the cheeses. For each ripening time, triplicates of cheeses were produced by salting method.

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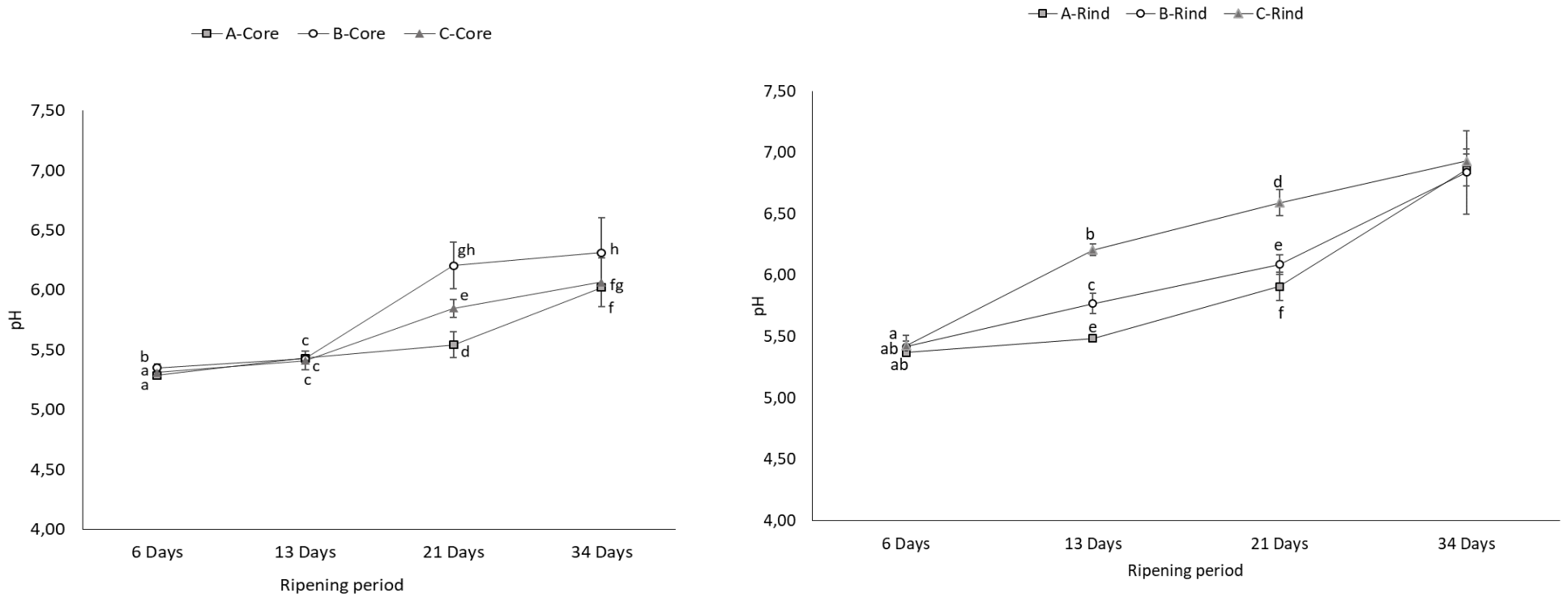

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Figure S 2. Evolution of pH of the cheese core and rind during ripening according to three salting methods (mean ± standard deviation of three cheeses). [a-h] Values with the same letter are in statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

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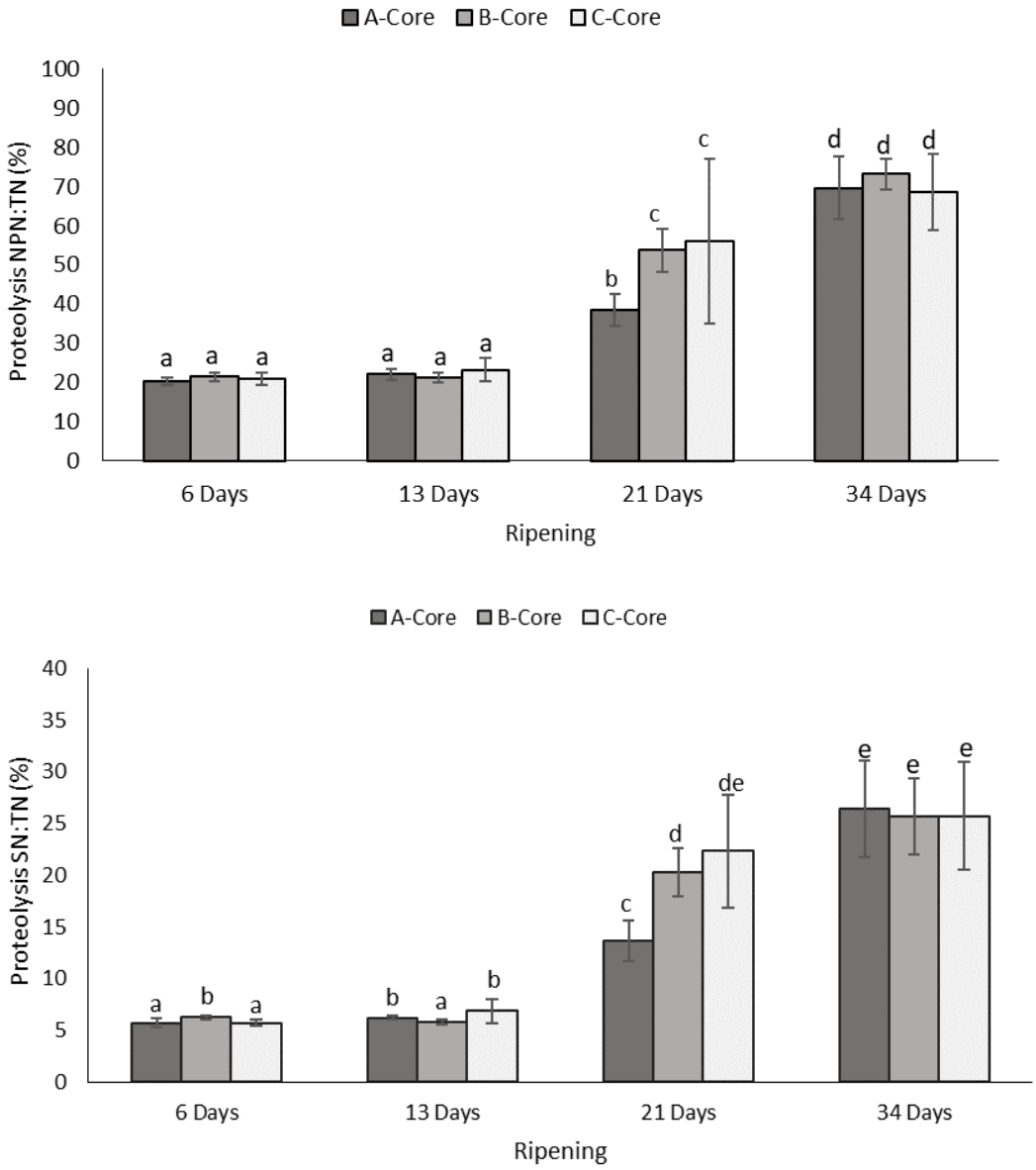

Figure S 3. Evolution of proteolysis of the cheese core during ripening according to three salting methods (mean ± standard deviation of three cheeses). [a-e] Values with the same letter are in statistically homogeneous groups on the basis of a two-way ANOVA ('ripening time*salting method' interaction).

Supplementary Materials

1. Physico-chemical and biochemical analysis

Standard physicochemical measurements of the cheeses were performed: All samples for analysis had been previously ground and stored at -20 °C. Cheese pH was measured using a penetration pH electrode CG 840 (Schott, Mainz, Germany). The dry matter (DM) was analysed by desiccation. In compliance with the standard NF ISO 5534:2004, a weighed test portion mixed with sand was dried by heating it for 24 h at 102 °C. The dried test portion was then weighed to determine the mass loss. Fat content was determined using the HEISS method according to the procedure described in standard NF V 04-287:2019. In a cheese butyrometer the proteins were first dissolved with a combination of acetic acid and perchloric acid and then separated from the fat by centrifugation. The fat content was read directly on the butyrometric scale with correction equation. Total nitrogen matter (TNM) was determined by the Kjeldahl method in compliance with the standards NF ISO 8968-1:2014. About 1g of the sample was mineralised with a mix of concentrated sulfuric acid and potassium sulfate. Copper sulfate was used as a catalyst to convert the organic nitrogen present to ammonium sulfate. Then excess sodium hydroxide was added to the cooled mineralization to release ammonia which was steam distilled into excess boric acid solution. Hydrochloric acid titration was performed to calculate the nitrogen content from the amount of ammonia produced. Total Nitrogen content was converted in crude protein by a factor of 6.38.

Calcium (Ca) and sodium (Na) contents were measured by inductively coupled plasma optical emission spectroscopy according to NF EN 16943:2017. The samples were first digested with nitric acid (60%) and hydrochloric acid (30%). After nebulisation, the aerosol was directed to a high frequency induced argon plasma, in which the elements were atomised and excited for irradiation. Sodium content was converted into salt percent. All measurements were performed in triplicate. Lactate levels were measured by enzymes assay kit (Megazyme, Ireland). As described in

834 the instructions, cheese samples (1 g) were homogenised in a 100 mL volumetric flask with distilled
835 water heated to 60°C, with stirring for 20 min or until complete dispersion.

836 **2. Microbiological Analyses**

837 We estimated the different microorganism flora levels over all ripening periods in order to
838 assess the effect of salt reduction on the evolution of the microorganisms. Cheese samples (25 g)
839 were collected aseptically into sterile sample bags and homogenised with 250 mL of peptone water
840 for 2 min in a stomacher (BagMixer CC, France). Total aerobic mesophilic bacteria number was
841 counted after three days at 30 °C on plate count agar (PCA) as per standard NF EN ISO 4833-
842 2:2013. *Leuconostocs* were counted on Mayeux–Sandine–Elliker agar (MSE, Mayeux et al., 1962)
843 after 2 days at 30 °C. *Lactococcus* were counted on M17 agar as described by Terzaghi and Sandine
844 (1975) after 2 days at 30 °C. *Lactobacillus* were enumerated on Man Rogosa Sharpe agar (MRS)
845 incubated at 30 °C for 3 days according to De MAN et al. (1960).

846 Yeasts and moulds were counted on Oxytetracycline-Glucose-Yeast Extract Agar (OGA) as per
847 standard NF V 08 – 059. The medium was supplemented with oxytetracycline before plating and
848 incubated at 25 °C for 5 days. Enterobacteria were enumerated on violet red bile glucose (VRBG)
849 incubated in anaerobic conditions during 24h according to NF V08-054:2009. All media were
850 purchased from Biokar Diagnostics (Biokar, Beauvais, France). All microbiological measurements
851 were done in triplicate.

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