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ABSTRACT

Reducing sodium content in food is a major public health challenge, especially for cheeses. This study investigates different salt reduction methods in surface dry-salted blue cheese and their effect on biochemical, physicochemical, microbiological, rheological and sensory characteristics of cheese. The produced cheeses were salted with two granulometries and various methods. All methods decreased sodium content in cheese core (-11% to -67%) and induced higher water activity. Sprinkling (-45% NaCl content) and calcium lactate substitution (-10% NaCl content) were technically feasible. The sanitary quality and cheeses' microbiota were not impacted except for a reduction of yeasts and moulds. Higher indexes of richness and diversity was found on salt-reduced cheeses with a dominance of *Streptococcus*, *Lactococcus* and *Leuconostoc mesenteroides*. Texture and appearance of the cheeses were also affected. Finally, this research identified the limits of salt reduction in blue-veined cheeses. Sprinkling and partial substitution with calcium lactate should be further studied.

Key words: salt reduction, blue cheese, food microbiota, calcium lactate, alternative methods.

1 Introduction

The sodium content of processed foods is considered a public health concern. There is strong evidence for a causal relationship between salt intake and blood pressure, leading to cardiovascular disease (Doyle & Glass, 2010). The World Health Organisation has suggested reducing sodium intake by 30% in order to obtain the WHO guideline of 2 g/day (i.e., 5 g of salt/day) by 2025 (WHO, 2021). It was estimated that 75% of sodium intake comes from processed foods (Brown et al., 2009). In France, bread, meat products, soups and cheese are the main contributors to sodium chloride (NaCl) intake in the population (ANSES, 2012). Blue-veined cheeses appear as the saltiest variety of cheeses with salt content up to 4% (ANSES, 2002). Despite its nutritional value (Ferroukhi et al., 2022), one serving of blue cheese may contribute up to 40% of the recommended dietary Na intake (Guinee & O'Kennedy, 2007).

Thus, reducing NaCl content of blue cheese is necessary to help improve health outcomes. However, the reduction of salt is difficult to achieve because of its major functions in cheese: it modulates the physico-chemical and biochemical properties of the cheese during ripening, maintains a low a_w which controls the development of micro-organisms, and is involved in the sensory characteristics of finished products (Fox et al., 2004; Guinee, 2004).

Many varieties of blue cheese are salted with an excess of salt by repeated surface application of dry NaC1 while others are brine-salted (Guinee, 2004). Surface dry-salting process is complex and poorly studied. Dry salt is dissolved on the surface and diffuses slowly into the matrix, creating a counterflow of moisture from the centre to the outside (Guinee, 2004). Salt diffusion depends on the intrinsic properties of the cheese, the quantity of salt added, the degree of salt rehydration on the surface and the salt granulometry (Guinee &

Fox, 2017). Consequently, studying salt reduction strategies in such salted products implies to investigate these parameters.

One of the salt reduction strategies is based on the simple reduction of the level of added NaCl (or direct decrease of NaCl) as already performed in soft and semi-hard cheeses, Feta and Cheddar cheese (Aly, 1995; Dugat-Bony et al., 2019; Møller et al., 2012). (Dugat-Bony et al., 2019) reported that salt reduction in soft cheese induced more sensory changes than in semi-hard cheese. In contrast, salt reduction increased the growth of spoilage agent in semi-hard cheese. Nevertheless, there is still a lack of information on reducing the salt content of other cheeses such as dry-salted cheeses. The direct reduction of salt quantities has so far been applied in cheese technologies where salting is done in coagulum and the common quantity of salt used is known. This is different from dry surface salting of blue-veined cheeses where an uncontrolled quantity of dry salt is rubbed and retained on the cheese surface.

The second strategy focuses on the total or partial replacement of NaCl by other salts. In Cheddar, Mozzarella and Kefalograviera cheeses it has been shown that potassium chloride (KCl) can be used successfully to achieve a large reduction in sodium without adverse effect on the quality of cheeses (Chavhan et al., 2015; Grummer et al., 2012, 2013; Katsiari et al., 1998). In contrast, other authors have reported that substitution of salt by KCl increases the bitterness of Cheddar cheese and disrupt the biochemical reactions of the cheese such as lipolysis and proteolysis (Fitzgerald & Buckley, 1985; Lindsay et al., 1982; Rulikowska et al., 2013). However, because of hyperkalemia risks, KCl-substituted products should be consumed with caution by consumers with renal disease (Berthet, 2009). The substitution of NaCl by $MgCl_2$ or $CaCl_2$ has been reported imparting off flavours (sour, bitter, metallic, and soapy) and decreases Cheddar cheese acceptability (Fitzgerald & Buckley, 1985; Grummer et al., 2012). In addition, from a nutritional standpoint $MgCl_2$ and $CaCl_2$ are acid-forming compounds (Remer, 2001). Little research has been done on the salt substitution in blue-veined cheeses. One recent study used organic calcium salts to reduce the acid-forming potential of blue-veined cheeses (Gore et al., 2019). This research revealed that a partial NaCl replacement by calcium lactate induced a reduction of Na content by 19% in Fourme d'Ambert. Except for Gore et al. study (2019), calcium lactate has been used so far in calcium fortification of milk (39%), cottage cheese and yogurt (36%) (Reykdal & Lee, 1993; Singh & Muthukumarappan, 2008; Yonis et al., 2013). Therefore, this substitute could be of nutritional interest.

In this context, it's mandatory to further explore salt reduction strategies in surface dry-salted products to provide relevant alternative process and allow blue cheeses to better fulfil nutritional guidelines. Thus, the objective of this study was to investigate the relevance of alternative salting methods to reduce the salt level in a surface dry salted blue-veined cheese. Various salting processes and salt granulometries were tested through two kinds of reduction strategies: simple reduction of NaCl quantities and partial substitution of NaCl by calcium lactate. The different effects of these salt reductions on physicochemical, biochemical, microbiological, rheological and sensory parameters of finished products were monitored.

2 Materials and methods

2.1 *Cheese Making*

Cheesemaking of a blue-veined cheese 'Bleu d'Auvergne' was carried out according to the PDO specifications and under industrial conditions (Figure S *1*) (INAO, 2021). Raw cow milk (about 1000 L) was pasteurised (72 °C, 30 s) and standardised for fat content and calcium chloride. Milk then was matured (36 °C, 45 min) and added with a classical starter culture (*Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Streptococcus thermophilus)* (Danisco, Danemark), hétérofermentative bacteria (*Leuconostoc mesenteroides* *subsp. Mesenteroides)* (Lallemand, France) and *Penicillium roqueforti* (SAS LIP, France). A quantity (30mL/100L) of liquid rennet extract (Caglificio Clerici, Italy) was also introduced shortly thereafter at 34,6°C. After that, the coagulum was cut vertically and horizontally and then stirred to form 'the cap' of the coagulum grains (3 cm^3). The syneresis was carried out in moulds to obtain drained curds. After the moulding, the cheese curds were 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 roqueforti* to grow and colonize it. The ripening was carried out at 8 °C and 95% RH over a period of 28 days.

2.2 *Salting Treatments and Sampling*

Surface dry-salting of Bleu d'Auvergne are described in Table 1. To obtain anhydrous salts, all the salts were previously dried (102 °C, 24h). Two control treatments (Cc and Cf) varying in the salt granulometry (coarse salt (0.9–3.15mm) or fine salt (0.22 mm)) were applied according to the traditional method by rubbing the cheese surface with excess of salt. Then, five methods of net reduction of NaCl quantities were tested by changing salt granulometry and application procedure (STc, SRc, STf, SRf and SP). STc and STf consisted of rolling cheese on a layer of coarse or fine salt spread on a tray. SRc and SRf were to remove the excess of salt from the surface by rubbing. The SP method was to sprinkle 100g of fine salt over the whole surface of the cheese. One method of partial substitution of NaCl with calcium lactate (S30) was carried out. The substitution (S30) was carried out using fine salt replaced with 30% of calcium lactate (0.162 mm) (Merck KGaA, Germany), in order to have a homogeneous grain size mixture. The mixture of NaCl-calcium lactate was homogenised in a mixer and placed in sealed containers until applications.

Eight blocks of cheese (2 ±0.5kg) were sampled at 28 days of ripening. The rind was cut to 5mm using a cutting wire and the core was cut into pieces and ground in a blender (Moulinex, France). In order to limit the variability due to the heterogeneity of blue cheese matrix, the core samples were homogenised by mechanical agitation and frozen at -20 °C in sealed bags until analysed. For each salting modality, one ripened cheese was held in cold storage at 1°C prior to sensory analysis. A commercial cheese (Std) was included to compare the produced cheeses to a reference, particularly for sensory evaluation. This cheese was only studied for physicochemical and sensory analysis.

2.3 *Physicochemical and Biochemical Analyses*

The physicochemical composition of the cheeses was analysed in compliance with ISO standards. Cheese pH was measured using a penetration pH electrode CG 840 (Schott, Mainz, Germany). 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 hours 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. Water activity (a_w) was measured in ≈ 5 g grated cheese samples at 20 °C, as per the manufacturer's instructions (HYGROLAB C1, Rotronic, Bassersdorf, Schweiz). Ratio of fat in dry matter (FDM) was calculated. Total nitrogen matter (TNM) was determined by the Kjeldahl method in compliance with the standard NF ISO 8968-1:2014. Total Nitrogen content was converted to 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 and hydrochloric acid. 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.

2.4 *Microbiological analyses*

Cheese samples (25 g) were homogenised in a stomacher for 2 min with 250mL of peptone water (BagMixer CC, France). Serial dilutions (10^{-7}) and the sowing were made with spiral method (EasySpiral Dilute, interscience, France). Total microflora was counted according to NF EN ISO 4833-2:2013. *Leuconostocs* were enumerated on Mayeux, Sandine and Elliker agar (MSE), *Lactococcus* on M17 agar and *Lactobacillus* on MRS agar according to NF EN 15787.Yeasts and moulds were counted with NF V 08‑059. *Enterobacteriaceae* were also screened on VRBG agar. Cell counts on media were performed in triplicate.

In order to investigate microbial communities in blue cheese, a metagenomic analysis was performed on cheeses after 28 days of ripening. DNA was extracted from the cheeses (*Penicillium*-free zone) using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Illkirch, France). DNA was quantified using Qubit Fluorometric Quantitation (Invitrogen, Thermo Fisher Scientific, Waltham, MA) method. Amplification and sequencing were carried out in the V3-V4 region of the 16S rDNA gene. Amplicons were sequenced with Illumina technology in 2x250bp. Amplicon data from high-throughput sequencing was analysed using the rANOMALY pipeline (Theil & Rifa, 2021), which relies on the dada2 R package to produce amplicon sequence variants (ASVs) as taxonomic units. A decontamination step was carried out based on prevalence of contaminant ASVs, as identified in the blank samples, and on DNA concentration, as described by the decontam package included in the rANOMALY pipeline. Taxonomic assignment of bacterial sequences was based on two databases, i.e. DAIRYdb v2.0 and SILVA 138, keeping the assignment with the highest confidence or the deepest taxonomic rank (Meola et al., 2019; Quast et al., 2013). The filtered ASVs count table was used to perform statistical analyses.

2.5 *Sensory Analyses*

A panel composed of 8 assessors from the sensory analysis laboratory of higher education and research institute VetAgro Sup was selected according to standard ISO 8586-1:2012 guidelines. These panellists had previous experience in the evaluation of dairy products. In order to identify the differences between cheeses and to classify the product groups in a sensory space, a flash profile was conducted. It is a fast descriptive method, based on the selection of a combination of free choice terms and a comparative ranking evaluation (Dairou & Sieffermann, 2002; Delarue & Lawlor, 2014). It's a method to study the degree of similarity between samples and provides a relative sensory positioning (mapping) of the samples.

The evaluation was conducted in two sessions. A slice of cheese (50 g) was presented on plates coded with random 3-digit numbers. 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 based on the simultaneous presentation of the whole samples set. During the first session (2.5-hr), subjects selected their own terms to describe and evaluate 9 blue-cheeses simultaneously: Eight cheeses salted with various processes presented in Table 1 and one commercial blue cheese as a standard. Between each sample, panellists were asked to rinse their mouth successively with unsalted crackers and tepid water to remove fatty residual. At the end of this session, a discussion with panellists allowed to finalise the list of descriptors generated by each panellist. During the second session, all samples were presented again to the assessors who were asked to rank each product on a ranking scale (rank 1 = least intense, rank 9 = most intense). For the final session, assessors evaluated the cheeses again and ranked them, using the generated list of descriptors. All evaluation sessions were led in computerised booth according to ISO standard 8589:2010. Data was collected using Tastel software® (version 2011; ABT Informatique, Rouvroy-sur-Marne, France).

2.6 *Texture Measurement*

The texture parameters Hardness (N), adhesiveness (N.s), cohesiveness and gumminess were determined using a rheometer (Kinexus pro+, rSpace for Kinexus 1.61 Software, Malvern Instruments, Malvern, UK), by carrying out the texture profile analysis (TPA) method described by (Nishinari et al., 2013). Three cylindrical samples (2 cm of diameter) were randomly collected from each cheese. Each sample was held at 20 °C for 1 h before analysis and was maintained at 20 °C during measurement with the Peltier heating element. Samples were compressed to 20% of their original height, using two compression cycles at a constant crosshead velocity of 50 mm/s². The texture parameters were calculated according to Henneberry et al. (2015).

2.7 *Statistical Analyses*

Statistical analysis of all data was performed using XLSTAT software 2020 (Addinsoft, Paris, France). Results are reported as a means ± standard deviation. Shapiro-Wilk test ($p < 0.05$) was used to check the normality of the data. A non-parametric test (Kruskal-Wallis) and a post-hoc comparison (Conover-Iman procedure) were applied to all data (Except for sensory data) as it did not have a normal distribution. Differences between mean values were considered significant at $P < 0.05$. Regarding sensory data, the Generalised Procrustes Analysis (GPA) was used for the configuration of the consensus between the judges' sensory maps. GPA calculates a consensus from data matrices of an experiment in sensory profiling and allows comparing the proximity between the attributes that are generated by panellists. For the Flash profile, one data matrix corresponded to each judge.

3 Results and Discussion

As salt intake is a major public health problem, it is essential to implement actions to reduce salt in foods (Bansal & Mishra, 2020). Blue-veined cheeses represent an important source of salt due to their specific salting methods usually performed in an excess of salt. For the first time, this work investigates the relevance of different surface dry salting methods to reduce salt content in blue-veined cheeses. In other words, this research was done in order to screen a high number of salting methods to identify the most relevant alternatives that will be considered in blue-veined cheese technology.

3.1 *Effect of Reducing Salt Content on Physicochemical and Biochemical Composition*

The physico-chemical and biochemical composition of blue-veined cheese cores salted by different methods is presented in Table 2. Applied salt reduction methods have impacted all parameters of the ripened cheeses ($P < 0.05$), except for mean fat and total nitrogen content of 28 and 19 g/100g respectively (data not shown).

The pH obtained for all the cheeses in this study was comparable to the pH found in Bleu d'Auvergne (Bord et al., 2016; Duval et al., 2016; Ferroukhi et al., 2022). pH values did not greatly change in the coarse salted cheeses. In contrast, Cf and S30 fine salted cheeses had a higher pH than others with pH of 6.08 and 6.28, respectively. Among the tested salting processes, a significant difference was observed in the a_w values. Cf cheese showed the lowest a_w (0.954) compared to the salt-reduced and the coarse-salted cheeses. A high a_w was also observed in salt-reduced Cheddar cheese (McCarthy et al., 2015; Rulikowska et al., 2013). The dry matter of the studied cheeses did not vary with salt reduction or granulometry. However, it was observed that SRc cheese had a low dry matter content (51.89%), related to high a_w. However, the commercial cheese Std showed a higher dry matter than other cheeses and a low a_w, probably related to a high salt content.

Regarding salt content and salt/moisture, there was no significant difference between Cc (1.70% of NaCl) and Cf (1.68% of NaCl) control cheeses salted with two different salt grain sizes. The opposite observations have been made in Fourme d'Ambert cheese, where cheeses salted with fine salt had a higher salt content than cheeses salted with coarse salt (Gore et al., 2019). This reflects that the change in salt granulometry did not induce a change in sodium content in the cheese core, i.e. coarse and fine salt penetrated the cheese in the same way. Nevertheless, there was more variability in cheeses salted with coarse salt than in cheeses salted with fine salt. This suggests the difficulty of controlling dry surface salting with coarse salt. The commercial cheese (Std) contained a high salt content (3.5% in salt and 6.4% in salt/moisture) compared to other cheeses. This elevated salt value is probably due to a double dry-salting process which is a common practice in Bleu d'Auvergne cheese making, explaining the difference reported in salt content. In order to assess the impact of new applied salting methods and limit the variability, cheeses from this research were salted only once.

In coarse salted cheeses, tray salting (STc) and rubbing surface salting (SRc) processes induced a high reduction of sodium contents (28 and 67%, respectively) compared to control (Cc). Rubbing off the excess salt on the surface produced a significant reduction in salt, particularly with coarse salt SRc, indicating that coarse salt is greatly removed if it is rubbed off. However, it has been observed that rubbing the wet surface of the cheese may deteriorate the rind and heterogeneously remove a significant salt quantity. For cheeses salted with fine salt, STf and SRf cheeses were also reduced by 21 and 17% salt compared to control cheese Cf. The tray salting method (STc and STf), which aimed to control deposition of salt on each side of cheese, gave a close reduction of salt for both granulometries. The disadvantage of this technique (STc or STf) was the time required to salt the full cheese surface. The objective was to find solutions for the cheese industry, this method could control and reduce the amount of salt but may be difficult for a large-scale production. The sprinkling treatment also led to a significant salt reduction (-45% of salt) compared to control (Cf). Calcium lactate substitution reduced sodium content by 10% ($P<0.0001$). A significant decrease (-19%) in sodium has been reported by Gore et al. (2019) in Fourme d'Ambert cheese upon the substitution of salt with 75% calcium lactate. For the same substitution ratio of salt, other authors obtained a higher reduction using KCl in Coalho and Akkawi cheeses (Costa et al., 2018; Kamleh et al., 2015). In the dry-salted Feta cheese, the substitution of NaCl by KCl (3:1 and 1:1) did not affect the salt content (Katsiari et al., 1997). We hypothesised that the low salt reduction resulting from calcium lactate substitution in this study is probably due to the heterogeneity of the cheese. The second salting process, as commonly used in the salting of blue cheese, could probably increase the salt reduction level. Nevertheless, only one study previously tested salt replacement in dry salting on the surface of blue-veined cheeses (Gore et al., 2019).

Salt reductions influenced Ca content of cheese cores ($P<0.0001$). Cheeses salted with fine salt had a relatively higher amount of calcium than cheeses salted with coarse salt. Blue-veined cheeses are a category with a complex matrix resulting in the variability of composition parameters such as salt (Ferroukhi et al., 2022; Gkatzionis et al., 2014). Difference in Ca content might be related to the heterogeneity of the matrix which causes variable mineral migration during ripening.

The calcium lactate-substituted cheese had the highest calcium content (606.6 mg/kg) and this was 21 and 13% higher than the control Cf and the standard Std cheese. Considering the Ca level brought by S30 treatment, a 40g serving of cheese would attain 25.5% of recommended daily calcium intake (ANSES, 2021). This salt has already been studied to fortify Cottage cheese and yoghurt with calcium (Reykdal & Lee, 1993; Wongkhalaung & Boonyaratanakornkit, 2000; Yonis et al., 2013). This may highlight the nutritional interest of this salt in cheese making.

The level of calcium in the cheese depends on the Ca content of the milk but also on mineral exchange after salting. It was reported that the salt on the surface enters inside the cheese, causing a counterflow of whey which exists towards the surface loaded with minerals such as calcium (Y. Le Graet & Brulé, 1988; Y. L. Le Graet et al., 1983). However, salt diffusion into the cheese matrix (and conversely Na and Cl migration) is limited by some cheese characteristics, such as free water, viscosity of the aqueous phase, tortuosity and porosity (Guinee, 2004; Guinee & Fox, 1983) This might be the reason for the difference in calcium content in obtained cheeses.

3.2 *Effect of Reducing Salt Content on the Microbial Communities*

Table 3 shows the counts of total microorganisms, lactic acid bacteria (LAB), yeasts and moulds for cheeses salted with different salting methods. The microbial composition of cheeses was significantly impacted by the salting processes ($P<0.05$) (Table 3).

Total counts were not different in the salt-reduced cheeses. A comparable effect was observed in a dry salted cheese (São João cheese) reduced in salt content (Soares et al., 2015). As opposed to other studies of salt reduction in Cheddar (Rulikowska et al., 2013; Schroeder et al., 1988), there were no marked effects of salt treatment among all experimental Bleu d'Auvergne cheeses in the total bacterial count. Aerobic mesophilic bacteria should mainly correspond to lactic acid bacteria (LAB) added after milk pasteurisation, as expected levels of total aerobic mesophilic bacteria were found in experimental and control cheeses. Nevertheless, the *Lactococcus* counts were lower in the Cf and STf cheeses. This might be due to test or sample variability during the analysis. In this study, *Enterobacteriaceae* were not found (below the required count) suggesting that the salt reductions implemented had no impact on the sanitary aspect of the cheeses. A similar result has been reported in salt-reduced cheese (Soares et al., 2015).

Yeasts and moulds (YM) were also affected by salting methods. In the coarse-salted cheeses, the salt-reduced cheeses contained a lower YM counts than control cheeses which had 9.00 log cfu/g. Cheeses salted with fine salt showed a lower YM counts than coarse salted cheeses except for S30 which had the highest level of YM (9.26 log cfu/g). A large proportion of cheeses contained a lower YM count than found in Bleu d'Auvergne cheese (Duval et al., 2016). Moreover, opposite observations were reported on Halloumi and Cheddar cheeses substituted with up to 50% KCl, which had no impact on yeast and mould counts (Kamleh et al., 2012; Reddy & Marth, 1995). The development of the distinctive flavours of blue cheese is dominated by metabolism of moulds during ripening. Salt in the moisture and opening of the matrix by *Leuconostocs*, which produces CO_2 through the citrate metabolism, lead to the growth of *Penicilium roqueforti* in the cheese (Marth & Steele, 2001). The low salt levels obtained in this study could be responsible for the low growth of yeasts and moulds.

A total of 354620 16S rRNA (V3-V4 regions) sequencing reads were generated from the 8 cheese samples, covering different salting methods. The reads were assigned to 36 ASVs for which taxonomic assignment was possible down to species level (or group of species sharing almost identical sequences) in most cases. These ASVs belonged to 21 different bacterial species (Figure 1) and four different bacterial phyla (*Firmicutes, Actinobacteriota, Proteobacteria and Bacteroidetes*). In all cheeses analysed, *Streptococcus, Lactococcus* and *Leuconostoc mesenteroides* species were dominant with a raw abundance sum of 123966, 127126 and 27923 reads, respectively. In a previous study, a dominance of these starter culture species was observed in Bleu d'Auvergne cheese (Ferroukhi et al., 2022) and other blue-cheeses (Caron et al., 2021; Flórez & Mayo, 2006). This bacterial dominance could explain why the bacterial abundance of non-starter LAB strains was extremely low, as these bacteria occur at higher levels only in later stages of ripening (Blaya et al., 2018).

Also species of *Brevibacterium* and *Brachybacterium* genus were present with a relative abundance compared to the others of 0.1 and 3%, respectively (Table S *1*). *Enterobacteriaceae* species were detected in all samples with a relative abundance of less than 0.5%. In line with the microbial count, the low percentage of *Enterobacteriaceae* indicates that the different salting methods did not negatively impact the sanitary quality of the products. *Lactococcus* and *Leuconostoc* species showed a higher percentage in the salt-reduced cheeses, especially in the SP and S30 cheeses. This is supported by the results obtained with microbial enumeration. In contrast, *Streptococcus* was lower in the salt-reduced cheeses. Another *Leuconostoc* species was detected only in SP cheese with 0.011% relative abundance.

In Table S 2, community richness and diversity were estimated for each cheese using four alpha diversity parameters (Observed, Chao1, Shannon and Simpson). The values of richness indicators were higher in the salt-reduced cheeses. SP and S30 cheeses had Observed and Chao1 indices of 27 and 28, respectively, for both indicators ($P<0.05$). The same observations were made on bacterial diversity with Shannon and Simpson's index (Table S *2*). The control cheeses had the same richness and diversity values. Species dissimilarity between cheeses (Beta-diversity) was assessed using the Bray-Curtis method. The results showed that species present in the cheeses were similar ($p>0.05$) and the salt reduction did not affect the type of species present in the cheese.

3.3 *Effect of Reducing Salt Content on Rheological and Sensory Parameters*

Table 4 shows the means for the TPA parameters studied in Bleu d'Auvergne cheese at 28 days of ripening. Hardness, cohesiveness and gumminess parameters were not significantly affected by the salting methods. This finding is consistent with other salt reduction investigations in Cheddar and Feta cheeses (Fitzgerald & Buckley, 1985; Katsiari et al., 1997). However, SRc and STf cheeses tended to have a lower hardness value. The variability in the matrix of blue-veined cheeses could account for the non-significant effect on the hardness of cheeses. Adhesiveness was significantly lower in control cheeses Cc and Cf than in salt-reduced cheeses. STc, SRc and STf cheeses had the highest adhesiveness (-0.539, -0.472 and -0.501, respectively) SP and S30 had a lower adhesiveness value than the other salt-reduced cheeses. Similarly, an increase in adhesiveness due to salt reduction has been reported by Henneberry et al. (2015) in Mozzarella cheese. These authors explained that the increase in adhesiveness during ripening is related to the increased hydration of caseins which should reduce the cohesive (attractive) forces with the calcium phosphate *para*-casein network.

The sensory analysis was carried out using a flash profile in order to assess the sensory differences between different salt reductions applied to blue cheeses compared to control cheeses and a standard commercial cheese. The panellist used different terms to characterise the cheeses according to appearance, texture, aroma, and taste. The number of attributes varied between 8 and 12, with an average of 10 attributes for each participant and a total of 74 terms of differentiation (Figure S 2). Most of the sensory attributes obtained in this study were also generated in other blue-veined cheeses (Gkatzionis et al., 2013) and Camembert cheese (Galli et al., 2019) analysed by flash profile. The consensus index (Rc) was 0.639 (63.9%) with a high residual percentage (>100%). Std cheese had the lowest residual percentage (83.4%), this could suggest that the commercial cheese had a complex descriptors from other cheeses and a less homogeneous consensus among the panel for this product. This was different from the consensus and higher total variance reported by (Gkatzionis et al., 2013). The low consensus among the testers demonstrated in the test may be due to the difficulty in ranking of the product caused by the high number of cheeses and the variability of their matrix.

The Generalised Procrustes Analysis (GPA) analysis of the Flash profile data provided the relative positioning of the samples based on generated attributes. The plots defined by the first two factors of the GPA analysis (Figure 2) explained a medium percentage of the total variance (50.48%; 29.10% and 21.38% for F1 and F2 respectively). The distribution of products in the Factorial Map was heterogeneous (Figure 2 (a)). Generally, good discrimination between cheeses was observed. The first axis discriminated the coarse control cheese Cc and commercial cheese Std with sprinkled cheese SP on one side and the fine salt control cheese Cf, the substituted cheese S30 and rubbed cheese SRc with other reduced salt cheeses (STc, SRf and STf) on the other side.

Looking at the attribute map (Figure 2 (b)), among the 74 descriptors generated, the attributes of creamy, marbling and odour intensity were found by many judges and were grouped on the biplot. Bitterness, salty and firmness also appeared in some panellists.

The results showed that texture was impacted by salt reductions. The control cheese Cc and the standard commercial cheese Std were characterised by a firm texture whereas the salt reduced cheeses STc, STf and SRf were characterised by a creamy texture. This result was in agreement with the hardness and adhesiveness index previously found in the texture of these cheeses. A number of studies have demonstrated the impact of salt on the structure and texture of cheeses (Pastorino et al., 2003; Schroeder et al., 1988). Regarding the marbling and odour intensity descriptors, it seemed that this character was more present in SP and Std cheeses. This indicated that marbling quantity was impacted by salt reduction (in agreement with microbiological results), but also related to the heterogeneity of cheese slices distributed to panellists. A within-product variability, due to blue-veined heterogeneity distribution, was already observed during the sensory analysis of this type of cheese (Bord et al., 2016).

Bitterness was found in the S30 and both the Cc and Cf cheeses controls but this was not representative of many judges. Bitterness in cheese was often related to salt levels. Mistry and Kasperson (1998) showed a decrease in bitterness with increasing salt in Cheddar cheese. However, the bitterness found in our study was not necessarily associated with salt (-10% of sodium (g/100g) in S30) but instead, with a higher pH in these cheeses, especially in S30 (6.28). Lee and Warthesen (1996) have reported a high pH in the bitterest cheeses. Furthermore, a significant level of calcium (such as in S30 cheese) contributes to bitterness (Engel et al., 2000). Contrary to other salt substitutions with $MgCl_2$ or $CaCl_2$ used in the salting of Cheddar cheese (Grummer et al., 2012), the impact of the calcium lactate salt substitution on off-flavour was not found in this study.

During this investigation, salty taste was generated only by five panelists and did not characterise the commercial cheese Std. It has been reported that food structures impacts the sensory perception of salt, through changes in salt release rate and availability (Busch et al., 2013; Tournier et al., 2014). An important relationship between food texture and masticatory behaviour of subjects was also established (Kohyama et al., 2005). For example, the quantity of salt released from a soft cheese is higher than that released from a hard cheese (Phan et al., 2008).

Distribution of other sensory attributes was relatively variable and heterogeneous between judges, suggesting that salting methods have impacted sensory quality of products (notably texture and marbling development) and this effect was differentially perceived between panellists. In summary, in this study, the salting methods had a clear effect on cheese texture with a higher adhesiveness index and a creamy appearance but had little effect on other sensory aspects.

4 Conclusion

We investigated for the first time the relevance of different alternative salting processes to reduce the salt level in a surface dry-salted blue cheese. All the salting methods applied significantly reduced the salt content without altering the sanitary quality of the cheeses. However, several tested methods showed their technical non feasibility, especially the application of coarse salt which increases variability in blue cheeses. Calcium lactate appeared to be a good salt substitute with a salt reduction. Calcium lactate has proven to be a substitute for salt with a 10% reduction in salt and a 21% increase in calcium. Given the variability and heterogeneity of blue-veined cheeses, it would be interesting to validate the effect of calcium lactate as a salt substitute on the quality of these cheeses. Regarding the sensory characteristics, a good discrimination between cheese samples and the identification of the most salient sensory attributes of all samples was achieved. Texture, appearance and certain sensory characteristics of the cheese, such as odour, were highly influenced compared to the salty perception. The bitterness was not representative and there was no off-flavour in this result. This exploratory study clearly demonstrated the technical feasibility of various salt reduction methods and their impact on the quality of blue cheese. Sprinkling and calcium lactate substitution methods are a practicable technique that could be improved and applied at large scale. Finally, this research has screened a large number of salting methods in order to identify the most relevant alternatives that can be further investigated in the salting of blue cheeses.

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Tables

Table 1. Salting methods

Treatments	salt	Granulometry	Salting methods	
			Code name	Description
Net reduction	NaCl	coarse salt	Cg : Control	Salting with standard method of rubbing the cheese surface with excess of coarse salt
	NaCl	coarse salt	STc : Salting on a tray	A layer of of coarse salt was spread on a horizontal tray. The cheese was salted by rolling the around and both sides in coarse salt without rubbing or pressing.
	NaCl	coarse salt	SRc : Surface rubbing	After salting in an excess of coarse salt , the surface of the cheese was scrubbed to remove the excess of salt
	NaCl	Fine salt	Cf : Control	Salting with standard method of rubbing the cheese surface with excess of fine salt
	NaCl	Fine salt	STf : Salting on a tray	A layer of of fine salt was spread on a horizontal tray. The cheese was salted by rolling the around and both sides in fine salt without rubbing or pressing.
	NaCl	Fine salt	SRf : Surface rubbing	After salting in an excess of fine salt, the surface of the cheese was scrubbed to remove the excess of salt
	NaCl	Fine salt	SP : Sprinkling	A fixed quantity of fine salt (100 g) was sprinkled all over the cheese surface
Partial substitution	NaCl+ calcium lactate	Fine salt	S30 : 30% Calcium lactate+70% NaCl	Salting with the standard method in an excess of salt mixture

Table 2. Gross composition of cheese cores salted by different methods (mean ± standard deviation)

	Composition						
	Ca (mg/100g)	Na (mg/100g)	NaCl (%) [1]	Salt/Moisture (%)	pH	DM (%)	a_w
Cc	410.5 ± 0.1 ^{b}	683.7 ± 22.2 ^{f}	1.7 ± 0.1 ^{f}	3.3 ± 0.1 ^{f}	5.7 ± 0.0 ^{cd}	52.1 ± 0.0 ^{a}	0.969 ± 0.001 ^{c}
STc	439.9 ± 0.7 ^{c}	491.3 ± 11.1 ^{c}	1.2 ± 0.0 ^{c}	2.4 ± 0.0 ^{c}	5.5 ± 0.1 ^{b}	52.0 ± 0.2 ^{a}	0.967 ± 0.002 ^{c}
SRc	381.8 ± 1.0 ^{a}	223.5 ± 16.9 ^{a}	0.6 ± 0.0 ^{a}	1.1 ± 0.1 ^{a}	5.7 ± 0.0 ^{c}	51.9 ± 0.6 ^{a}	0.981 ± 0.001 ^{d}
Cf	477.7 ± 3.7 ^{d}	675.0 ± 12.4 ^{f}	1.7 ± 0.0 ^{f}	3.2 ± 0.1 ^{f}	6.1 ± 0.0 ^{g}	53.4 ± 1.3 ^{a}	0.954 ± 0.001 ^{b}
STf	517.5 ± 1.5 ^{e}	535.9 ± 5.5 ^{d}	1.3 ± 0.0 ^{d}	2.5 ± 0.1 ^{cd}	5.4 ± 0.0 ^{a}	53.1 ± 0.6 ^{a}	0.966 ± 0.003 ^{c}
SRf	484.5 ± 2.3 ^{d}	559.9 ± 5.3 ^{d}	1.4 ± 0.0 ^{d}	2.7 ± 0.0 ^{d}	5.8 ± 0.0 ^{de}	52.5 ± 0.0 ^{a}	0.968 ± 0.003 ^{c}
SP	467.1 ± 2.5 ^{cd}	369.5 ± 1.6 ^{b}	0.9 ± 0.0 ^{b}	1.8 ± 0.0 ^{b}	5.9 ± 0.0 ^{f}	52.4 ± 0.1 ^{a}	0.964 ± 0.006 ^{c}
S30	606.6 ± 3.5 ^{f}	609.5 ± 25.8 ^{e}	1.5 ± 0.1 ^{e}	2.9 ± 0.1 ^{e}	6.3 ± 0.0 ^{h}	52.7 ± 0.3 ^{a}	0.980 ± 0.001 ^{d}
Std	523.5 ± 28.5 ^{e}	1415.0 ± 15.0 ^{g}	3.5 ± 0.0 ^{g}	6.4 ± 0.0 ^{g}	5.8 ± 0.0 ^{ef}	55.1 ± 0.6 ^{b}	0.867 ± 0.007 ^{a}
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001

$^{a-e}$ Means in a row sharing common superscripts are similar as tested by Conover-Iman test ($P<0.05$).

Cc= Control with coarse salt; STc= Salting on a tray with coarse salt; SRc= Surface rubbing with coarse salt; Cf= Control with fine salt; STf= Salting on a tray with fine salt; SRf= Surface rubbing with fine salt; SP= Sprinkling with fine salt; S30= Cheese salted with a mix of 70% of calcium lactate and 30% of fine salt (NaCl); Std= Commercial cheese salted with coarse salt by industrial operator.

[1] Estimated by sodium measurement.

Table 3. Microbial populations (log cfu/g) in cheese cores at 28 days of ripening (mean ± standard deviation)

	Cc	STc	SRc	Cf	STf	SRf	SP	S30	P-Value
Lactococcus	9.00 ± 0.09 ^{cd}	8.84 ± 0.02 ^{bc}	9.23 ± 0.02 ^{d}	6.74 ± 0.07 ^{a}	6.97 ± 0.04 ^{ab}	8.97 ± 0.04 ^{cd}	8.96 ± 0.06 ^{cd}	8.49 ± 0.06 ^{ab}	0.00
Lactobacillus	8.84 ± 0.02 ^{bc}	8.93 ± 0.03 ^{cd}	9.04 ± 0.05 ^{d}	8.75 ± 0.04 ^{ab}	8.86 ± 0.05 ^{c}	8.85 ± 0.02 ^{bc}	8.65 ± 0.05 ^{a}	8.66 ± 0.11 ^{a}	0.00
Leuconostocs	8.35 ± 0.13 ^{ab}	8.25 ± 0.04 ^{ab}	8.30 ± 0.08 ^{b}	7.98 ± 0.16 ^{ab}	7.97 ± 0.14 ^{ab}	7.98 ± 0.07 ^{a}	8.07 ± 0.17 ^{ab}	8.09 ± 0.10 ^{ab}	0.03
Yeasts and Moulds	9.00 ± 0.05 ^{de}	7.16 ± 0.03 ^{ab}	7.23 ± 0.05 ^{bc}	7.27 ± 0.03 ^{cd}	7.24 ± 0.04 ^{bc}	7.28 ± 0.01 ^{cde}	6.83 ± 0.08 ^{a}	9.26 ± 0.02 ^{e}	0.00
Total microbiota	8.58± 0.12 ^{a}	8.79 ± 0.05 ^{abc}	8.75 ± 0.04 ^{ab}	8.95 ± 0.05 ^{bcd}	9.04 ± 0.05 ^{d}	9.01 ± 0.08 ^{cd}	8.95 ± 0.08 ^{bcd}	8.90 ± 0.12 ^{abcd}	0.01
Enterobacteriaceae	ND	ND	ND	ND	ND	ND	ND	ND	

$^{a-e}$ Means in a row sharing common superscripts are similar as tested by Conover-Iman test ($P<0.05$).

Cc= Control with coarse salt; STc= Salting on a tray with coarse salt; SRc= Surface rubbing with coarse salt; Cf= Control with fine salt; STf= Salting on a tray with fine salt; SRf= Surface rubbing with fine salt; SP= Sprinkling with fine salt; S30= Cheese salted with a mix of 70% of calcium lactate and 30% of fine salt (NaCl).

Table 4. Texture profile analysis parameters for cheeses at 28 days of ripening

	Hardness	Cohesiveness	Gumminess (N)	Adhesiveness (J)
Cc	1.41 ± 0.07 [a]	0.59 ± 0.15 [a]	0.82 ± 0.17 [a]	-0.07 ± 0.00 [bc]
STc	1.13 ± 0.06 [a]	0.47 ± 0.00 [a]	0.53 ± 0.03 [a]	-0.54 ± 0.00 [a]
SRc	0.67 ± 0.42 [a]	0.76 ± 0.17 [a]	0.47 ± 0.21 [a]	-0.472 ± 0.01 [a]
Cf	1.49 ± 0.27 [a]	0.66 ± 0.14 [a]	1.00 ± 0.39 [a]	-0.01 ± 0.00 [c]
STf	0.78 ± 0.24 [a]	0.45 ± 0.02 [a]	0.35 ± 0.09 [a]	-0.50 ± 0.01 [a]
SRf	1.44 ± 0.40 [a]	0.43 ± 0.12 [a]	0.59 ± 0.00 [a]	-0.25 ± 0.01 [abc]
SP	1.03 ± 0.00 [a]	0.58 ± 0.03 [a]	0.59 ± 0.04 [a]	-0.22 ± 0.01 [abc]
S30	1.38 ± 0.40 [a]	0.42 ± 0.23 [a]	0.57 ± 0.30 [a]	-0.37 ± 0.24 [ab]
P-Value	0.05	0.22	0.17	0.00

Cc= Control with coarse salt; STc= Salting on a tray with coarse salt; SRc= Surface rubbing with coarse salt; Cf= Control with fine salt; STf= Salting on a tray with fine salt; SRf= Surface rubbing with fine salt; SP= Sprinkling with fine salt; S30= Cheese salted with a mix of 70% of calcium lactate and 30% of fine salt (NaCl).

Figures

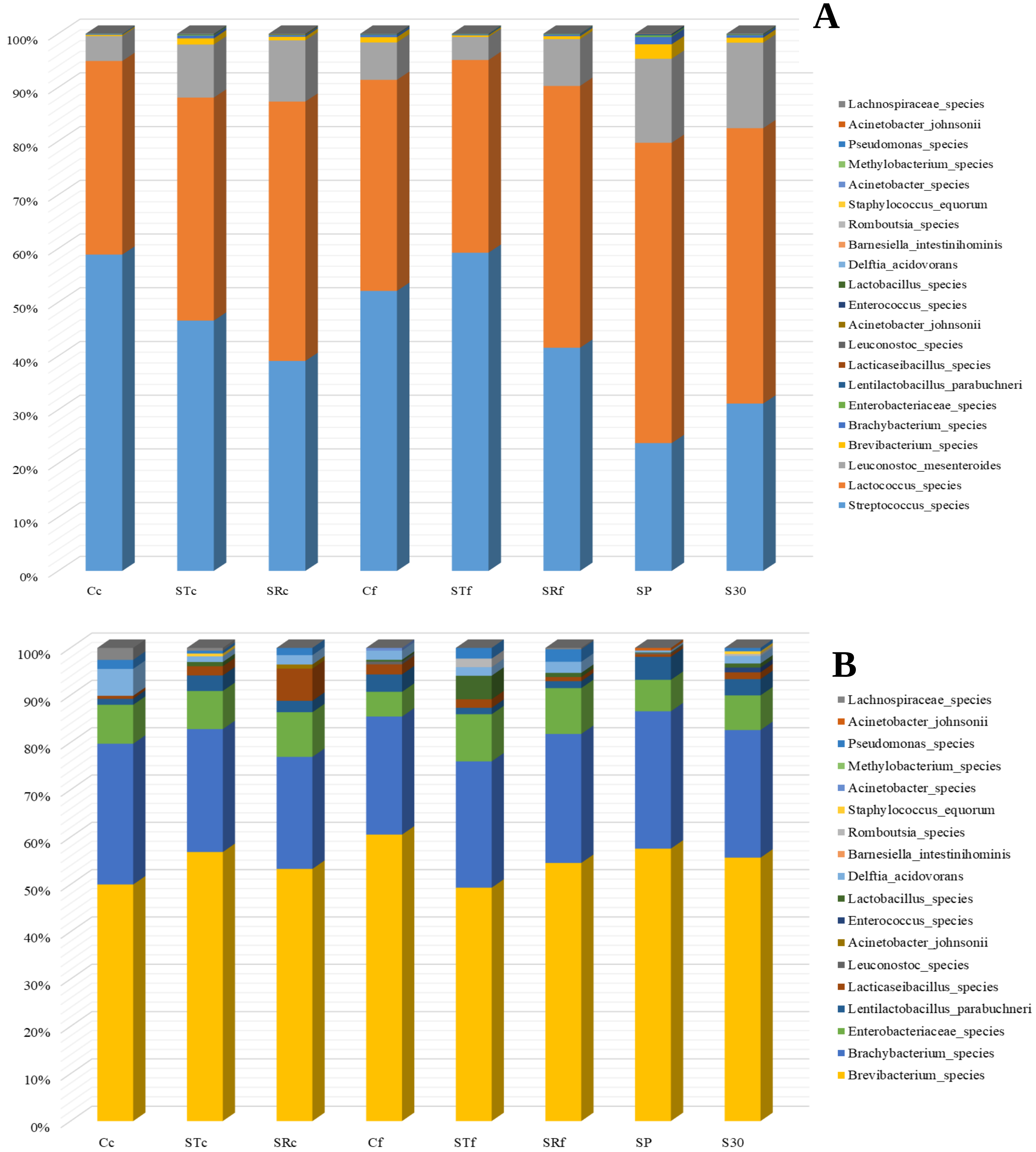

Figure 1. Microbial composition of produced cheeses. The vertical bars indicate the percentage of relative abundance of the corresponding bacterial sequences at species level. A: Total bacterial composition in each cheese, B: Bacterial composition of the subdominant population (without *Streptococcus* species, *Lactococcus* species and *Leuconostoc mesenteroides*) in each cheese.

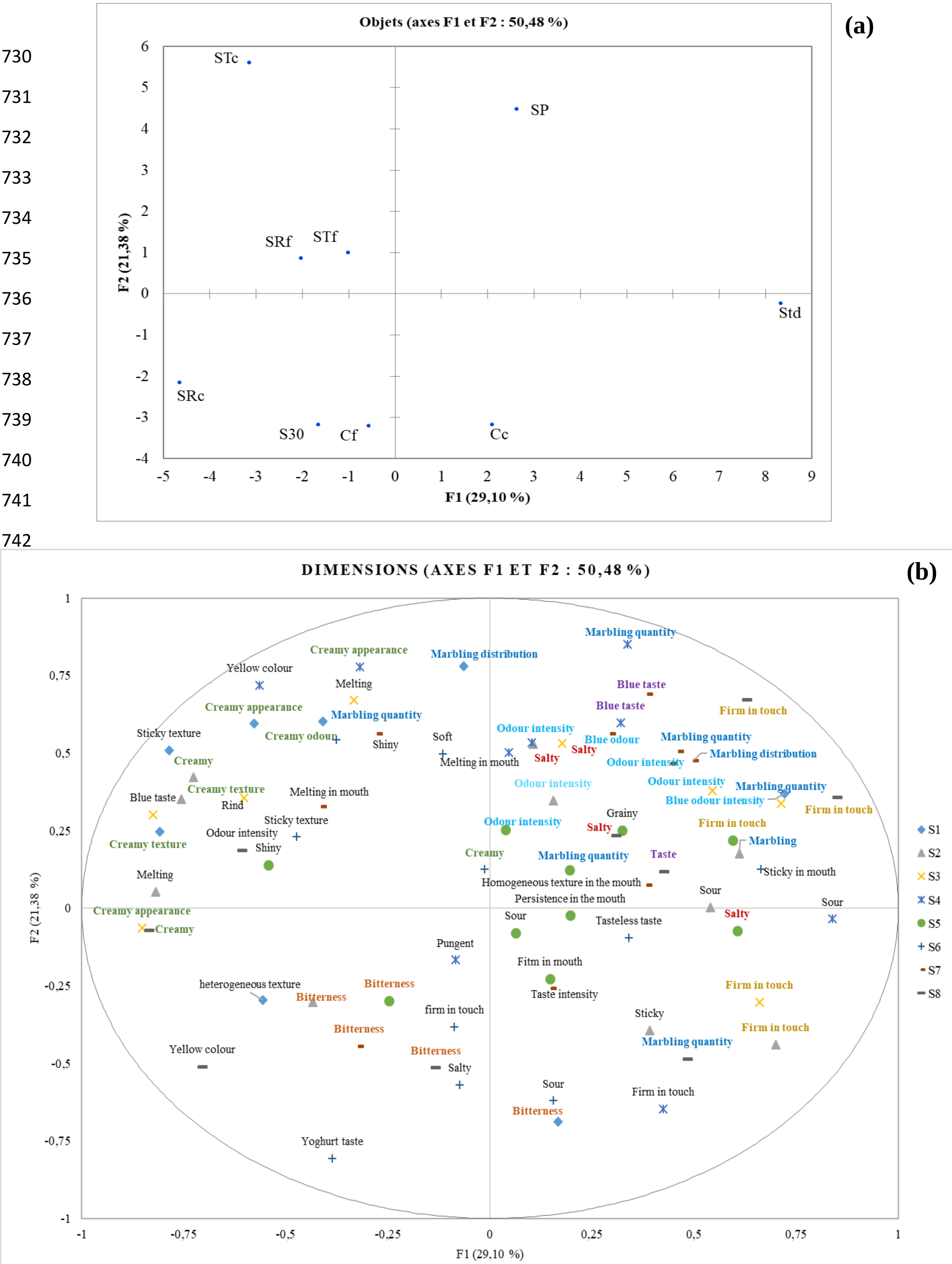

Figure 2. Map of the two principal axes of a generated Procustes Analysis (GPA) conducted on the flash profile data: (a) Cheese samples configuration, (b) Variables plot representing the distribution of subjects (S) according to the generated descriptors.

Supplementary data

Table S 1 Sum of relative abundance for each species in produced blue-cheese cores.

	Cc	STc	SRc	Cf	STf	SRf	SP	S30
Streptococcus_species	58.88	46.60	39.08	52.12	59.23	41.55	23.79	31.15
Lactococcus_species	36.04	41.51	48.29	39.29	35.87	48.73	55.90	51.26
Leuconostoc_mesenteroides	4.65	9.88	11.39	6.96	4.27	8.71	15.65	15.91
Brevibacterium_species	0.22	1.14	0.66	0.99	0.31	0.55	2.69	0.93
Brachybacterium_species	0.13	0.52	0.29	0.41	0.17	0.28	1.35	0.45
Enterobacteriaceae_species	0.04	0.16	0.12	0.09	0.06	0.10	0.31	0.12
Lentilactobacillus_parabuchneri	0.01	0.07	0.03	0.06	0.01	0.02	0.23	0.06
Lacticaseibacillus_species	0.00	0.04	0.08	0.04	0.01	0.01	0.03	0.02
Leuconostoc_species	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
Acinetobacter_johnsonii	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Enterococcus_species	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Lactobacillus_species	0.00	0.02	0.00	0.01	0.03	0.01	0.01	0.01
Delftia_acidovorans	0.03	0.02	0.02	0.03	0.01	0.02	0.02	0.03
Barnesiella_intestinihominis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Romboutsia_species	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01
Staphylococcus_equorum	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Acinetobacter_species	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Methylobacterium_species	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas_species	0.01	0.01	0.02	0.00	0.01	0.03	0.00	0.01
Acinetobacter_johnsonii	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
Lachnospiraceae_species	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00

Table S 2 Number of reads and diversity indices obtained from blue-cheese cores.

	Cc	STc	SRc	Cf	STf	SRf	SP	S30
Reads	36011	33303	36738	35558	31761	33554	35227	41566
Observed ASV's	20	24	22	22	22	23	27	28
Chao1	20	24	22	22	22	23	27	28
Shannon	1.09	1.28	1.25	1.09	1.2	1.21	1.37	1.3
Simpson	0.57	0.64	0.64	0.57	0.61	0.62	0.64	0.65
P-Value	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Figures

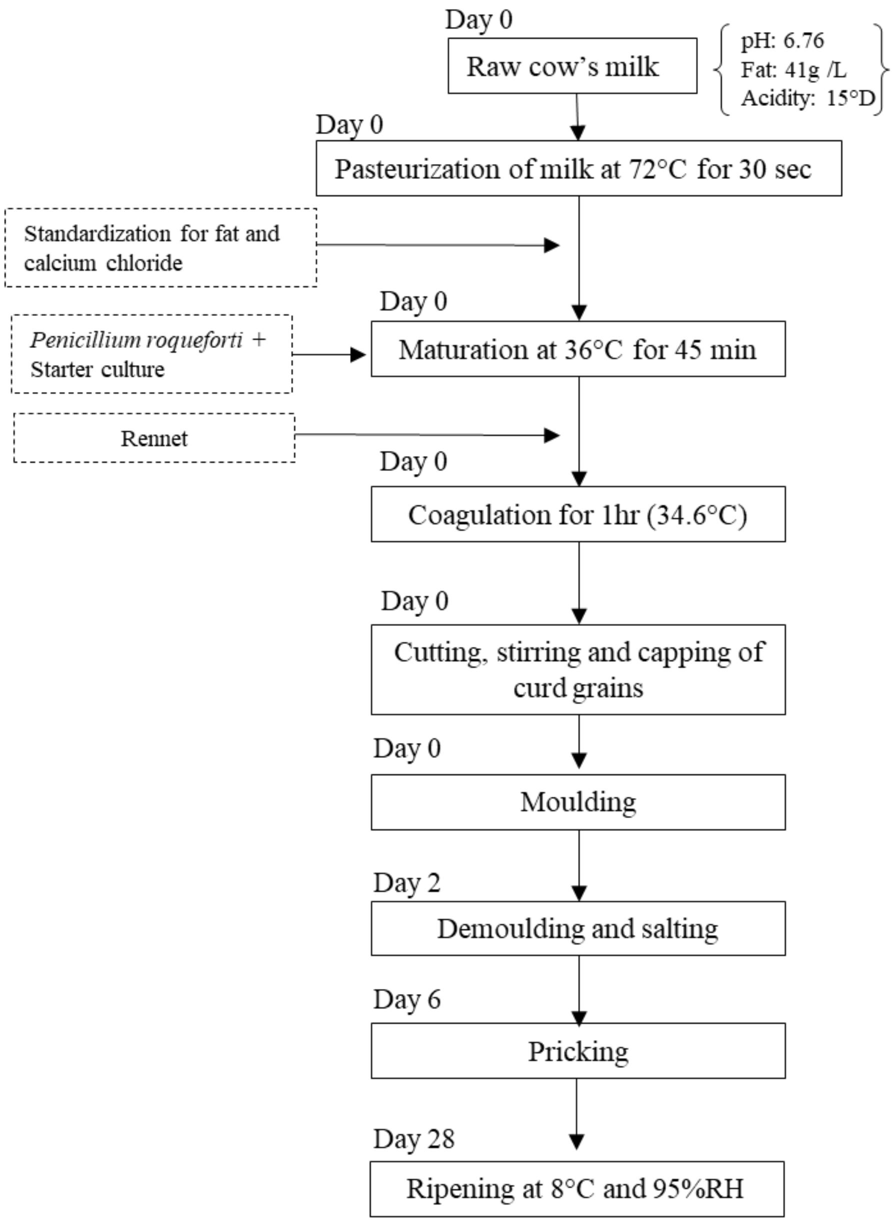

Figure S 1. Flowchart of Bleu d'Auvergne-type cheese manufacture.

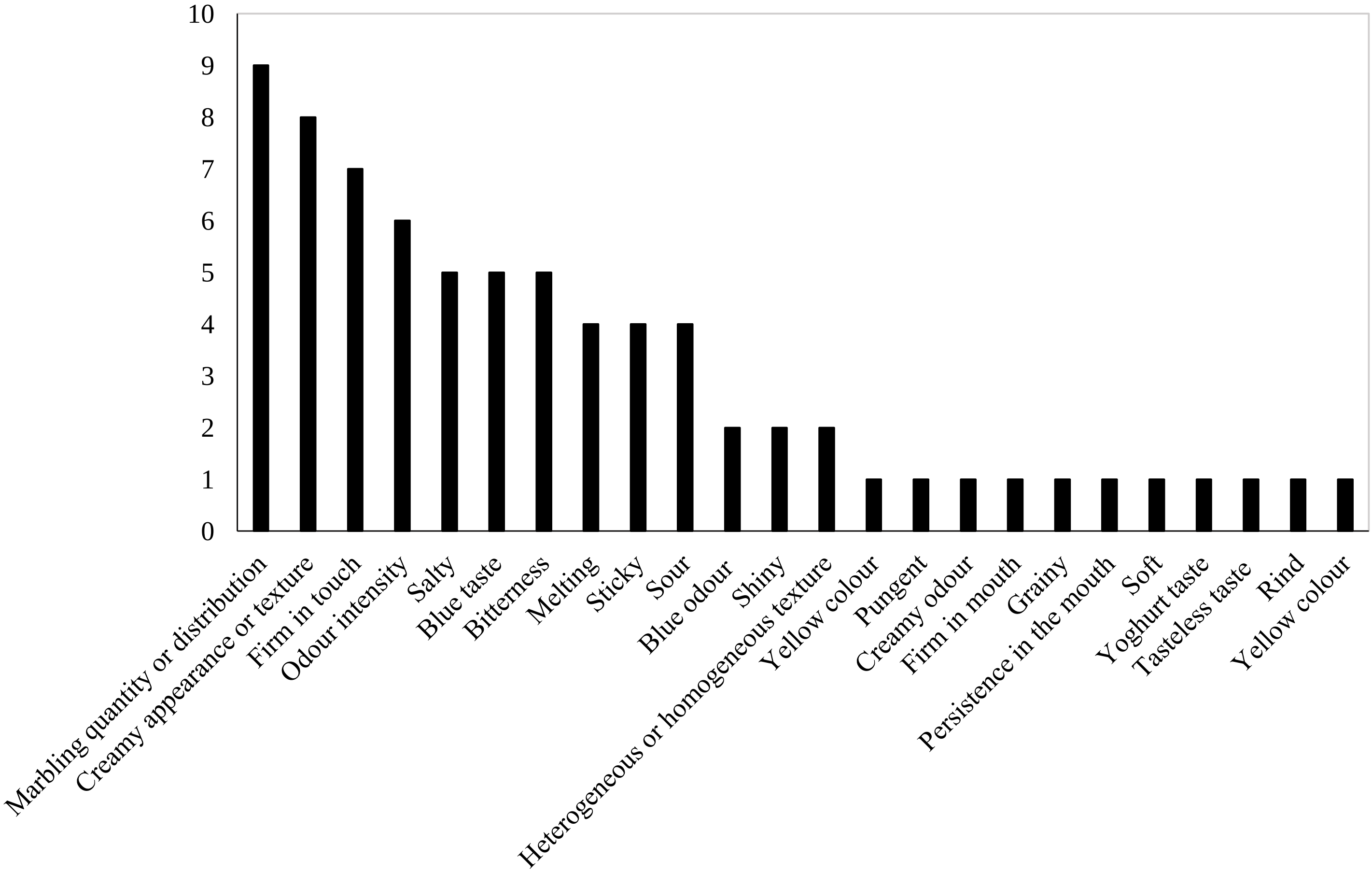

Figure S 2. Number of sensory descriptors generated by the judges