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1 **Exploring alternative salting methods to reduce sodium content in blue-**
2 **veined cheeses**

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8 **ABSTRACT**

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

21

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

23 **1 Introduction**

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

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

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

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

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

59 The second strategy focuses on the total or partial replacement of NaCl by other salts.
60 In Cheddar, Mozzarella and Kefalograviera cheeses it has been shown that potassium chloride
61 (KCl) can be used successfully to achieve a large reduction in sodium without adverse effect
62 on the quality of cheeses (Chavhan et al., 2015; Grummer et al., 2012, 2013; Katsiari et al.,
63 1998). In contrast, other authors have reported that substitution of salt by KCl increases the
64 bitterness of Cheddar cheese and disrupt the biochemical reactions of the cheese such as
65 lipolysis and proteolysis (Fitzgerald & Buckley, 1985; Lindsay et al., 1982; Rulikowska et al.,
66 2013). However, because of hyperkalemia risks, KCl-substituted products should be
67 consumed with caution by consumers with renal disease (Berthet, 2009). The substitution of
68 NaCl by MgCl₂ or CaCl₂ has been reported imparting off flavours (sour, bitter, metallic, and
69 soapy) and decreases Cheddar cheese acceptability (Fitzgerald & Buckley, 1985; Grummer et
70 al., 2012). In addition, from a nutritional standpoint MgCl₂ and CaCl₂ are acid-forming

71 compounds (Remer, 2001). Little research has been done on the salt substitution in blue-
72 veined cheeses. One recent study used organic calcium salts to reduce the acid-forming
73 potential of blue-veined cheeses (Gore et al., 2019). This research revealed that a partial NaCl
74 replacement by calcium lactate induced a reduction of Na content by 19% in Fourme
75 d'Ambert. Except for Gore et al. study (2019), calcium lactate has been used so far in calcium
76 fortification of milk (39%), cottage cheese and yogurt (36%) (Reykdal & Lee, 1993; Singh &
77 Muthukumarappan, 2008; Yonis et al., 2013). Therefore, this substitute could be of nutritional
78 interest.

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

88 **2 Materials and methods**

89 **2.1 Cheese Making**

90 Cheesemaking of a blue-veined cheese 'Bleu d'Auvergne' was carried out according
91 to the PDO specifications and under industrial conditions (Figure S 1) (INAO, 2021). Raw
92 cow milk (about 1000 L) was pasteurised (72 °C, 30 s) and standardised for fat content and
93 calcium chloride. Milk then was matured (36 °C, 45 min) and added with a classical starter
94 culture (*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Streptococcus*
95 *thermophilus*) (Danisco, Danemark), hétérofermentative bacteria (*Leuconostoc mesenteroides*

96 *subsp. Mesenteroides*) (Lallemand, France) and *Penicillium roqueforti* (SAS LIP, France). A
97 quantity (30mL/100L) of liquid rennet extract (Caglificio Clerici, Italy) was also introduced
98 shortly thereafter at 34,6°C. After that, the coagulum was cut vertically and horizontally and
99 then stirred to form 'the cap' of the coagulum grains (3 cm³). The syneresis was carried out in
100 moulds to obtain drained curds. After the moulding, the cheese curds were dry-salted
101 according to the various salting treatments tested. Six days after the manufacture, the cheeses
102 were pricked. This step aims to create air channels in the core of the cheese to enable the
103 *Penicillium roqueforti* to grow and colonize it. The ripening was carried out at 8 °C and 95%
104 RH over a period of 28 days.

105 **2.2 Salting Treatments and Sampling**

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

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

127 **2.3 *Physicochemical and Biochemical Analyses***

128 The physicochemical composition of the cheeses was analysed in compliance with
129 ISO standards. Cheese pH was measured using a penetration pH electrode CG 840 (Schott,
130 Mainz, Germany). Dry matter (DM) was analysed by desiccation. In compliance with the
131 standard NF ISO 5534:2004, a weighed test portion mixed with sand was dried by heating it
132 for 24 hours at 102 °C. The dried test portion was then weighed to determine the mass loss.
133 Fat content was determined using the HEISS method according to the procedure described in
134 standard NF V 04-287:2019. In a cheese butyrometer, the proteins were first dissolved with a
135 combination of acetic acid and perchloric acid and then separated from the fat by
136 centrifugation. The fat content was read directly on the butyrometric scale with correction
137 equation. Water activity (a_w) was measured in ≈ 5 g grated cheese samples at 20 °C, as per the
138 manufacturer's instructions (HYGROLAB C1, Rotronic, Bassersdorf, Schweiz). Ratio of fat
139 in dry matter (FDM) was calculated. Total nitrogen matter (TNM) was determined by the
140 Kjeldahl method in compliance with the standard NF ISO 8968-1:2014. Total Nitrogen
141 content was converted to crude protein by a factor of 6.38. Calcium (Ca) and sodium (Na)
142 contents were measured by inductively coupled plasma optical emission spectroscopy
143 according to NF EN 16943:2017. The samples were first digested with nitric acid and

144 hydrochloric acid. After nebulisation, the aerosol was directed to a high frequency induced
145 argon plasma, in which the elements were atomised and excited for irradiation. Sodium
146 content was converted into salt percent. All measurements were performed in triplicate.

147 **2.4 Microbiological analyses**

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

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

168 deepest taxonomic rank (Meola et al., 2019; Quast et al., 2013). The filtered ASVs count table
169 was used to perform statistical analyses.

170 **2.5 Sensory Analyses**

171 A panel composed of 8 assessors from the sensory analysis laboratory of higher
172 education and research institute VetAgro Sup was selected according to standard ISO 8586-
173 1:2012 guidelines. These panellists had previous experience in the evaluation of dairy
174 products. In order to identify the differences between cheeses and to classify the product
175 groups in a sensory space, a flash profile was conducted. It is a fast descriptive method, based
176 on the selection of a combination of free choice terms and a comparative ranking evaluation
177 (Dairou & Sieffermann, 2002; Delarue & Lawlor, 2014). It's a method to study the degree of
178 similarity between samples and provides a relative sensory positioning (mapping) of the
179 samples.

180 The evaluation was conducted in two sessions. A slice of cheese (50 g) was presented
181 on plates coded with random 3-digit numbers. All cheeses were served at 20 ± 1 °C, presented
182 monadically and distributed according to a Williams Latin Square designed to take into
183 account the first effect of order and carry-over based on the simultaneous presentation of the
184 whole samples set. During the first session (2.5-hr), subjects selected their own terms to
185 describe and evaluate 9 blue-cheeses simultaneously: Eight cheeses salted with various
186 processes presented in Table 1 and one commercial blue cheese as a standard. Between each
187 sample, panellists were asked to rinse their mouth successively with unsalted crackers and
188 tepid water to remove fatty residual. At the end of this session, a discussion with panellists
189 allowed to finalise the list of descriptors generated by each panellist. During the second
190 session, all samples were presented again to the assessors who were asked to rank each
191 product on a ranking scale (rank 1 = least intense, rank 9 = most intense). For the final
192 session, assessors evaluated the cheeses again and ranked them, using the generated list of

193 descriptors. All evaluation sessions were led in computerised booth according to ISO standard
194 8589:2010. Data was collected using Tastel software® (version 2011; ABT Informatique,
195 Rouvroy-sur-Marne, France).

196 **2.6 *Texture Measurement***

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

206 **2.7 *Statistical Analyses***

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

217 **3 Results and Discussion**

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

225 **3.1 *Effect of Reducing Salt Content on Physicochemical and Biochemical Composition***

226 The physico-chemical and biochemical composition of blue-veined cheese cores salted
227 by different methods is presented in Table 2. Applied salt reduction methods have impacted all
228 parameters of the ripened cheeses ($P < 0.05$), except for mean fat and total nitrogen content of
229 28 and 19 g/100g respectively (data not shown).

230 The pH obtained for all the cheeses in this study was comparable to the pH found in
231 Bleu d'Auvergne (Bord et al., 2016; Duval et al., 2016; Ferroukhi et al., 2022). pH values did
232 not greatly change in the coarse salted cheeses. In contrast, Cf and S30 fine salted cheeses had
233 a higher pH than others with pH of 6.08 and 6.28, respectively. Among the tested salting
234 processes, a significant difference was observed in the a_w values. Cf cheese showed the
235 lowest a_w (0.954) compared to the salt-reduced and the coarse-salted cheeses. A high a_w was
236 also observed in salt-reduced Cheddar cheese (McCarthy et al., 2015; Rulikowska et al.,
237 2013). The dry matter of the studied cheeses did not vary with salt reduction or granulometry.
238 However, it was observed that SRc cheese had a low dry matter content (51.89%), related to
239 high a_w . However, the commercial cheese Std showed a higher dry matter than other cheeses
240 and a low a_w , probably related to a high salt content.

241 Regarding salt content and salt/moisture, there was no significant difference between
242 Cc (1.70% of NaCl) and Cf (1.68% of NaCl) control cheeses salted with two different salt
243 grain sizes. The opposite observations have been made in Fourme d'Ambert cheese, where
244 cheeses salted with fine salt had a higher salt content than cheeses salted with coarse salt
245 (Gore et al., 2019). This reflects that the change in salt granulometry did not induce a change
246 in sodium content in the cheese core, i.e. coarse and fine salt penetrated the cheese in the
247 same way. Nevertheless, there was more variability in cheeses salted with coarse salt than in
248 cheeses salted with fine salt. This suggests the difficulty of controlling dry surface salting
249 with coarse salt. The commercial cheese (Std) contained a high salt content (3.5% in salt and
250 6.4% in salt/moisture) compared to other cheeses. This elevated salt value is probably due to a
251 double dry-salting process which is a common practice in Bleu d'Auvergne cheese making,
252 explaining the difference reported in salt content. In order to assess the impact of new applied
253 salting methods and limit the variability, cheeses from this research were salted only once.

254 In coarse salted cheeses, tray salting (STc) and rubbing surface salting (SRc) processes
255 induced a high reduction of sodium contents (28 and 67%, respectively) compared to control
256 (Cc). Rubbing off the excess salt on the surface produced a significant reduction in salt,
257 particularly with coarse salt SRc, indicating that coarse salt is greatly removed if it is rubbed
258 off. However, it has been observed that rubbing the wet surface of the cheese may deteriorate
259 the rind and heterogeneously remove a significant salt quantity. For cheeses salted with fine
260 salt, STf and SRf cheeses were also reduced by 21 and 17% salt compared to control cheese
261 Cf. The tray salting method (STc and STf), which aimed to control deposition of salt on each
262 side of cheese, gave a close reduction of salt for both granulometries. The disadvantage of this
263 technique (STc or STf) was the time required to salt the full cheese surface. The objective was
264 to find solutions for the cheese industry, this method could control and reduce the amount of
265 salt but may be difficult for a large-scale production. The sprinkling treatment also led to a

266 significant salt reduction (-45% of salt) compared to control (Cf). Calcium lactate substitution
267 reduced sodium content by 10% ($P < 0.0001$). A significant decrease (-19%) in sodium has
268 been reported by Gore et al. (2019) in Fourme d'Ambert cheese upon the substitution of salt
269 with 75% calcium lactate. For the same substitution ratio of salt, other authors obtained a
270 higher reduction using KCl in Coalho and Akkawi cheeses (Costa et al., 2018; Kamleh et al.,
271 2015). In the dry-salted Feta cheese, the substitution of NaCl by KCl (3:1 and 1:1) did not
272 affect the salt content (Katsiari et al., 1997). We hypothesised that the low salt reduction
273 resulting from calcium lactate substitution in this study is probably due to the heterogeneity of
274 the cheese. The second salting process, as commonly used in the salting of blue cheese, could
275 probably increase the salt reduction level. Nevertheless, only one study previously tested salt
276 replacement in dry salting on the surface of blue-veined cheeses (Gore et al., 2019).

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

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

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

298 **3.2 Effect of Reducing Salt Content on the Microbial Communities**

299
300 Table 3 shows the counts of total microorganisms, lactic acid bacteria (LAB), yeasts
301 and moulds for cheeses salted with different salting methods. The microbial composition of
302 cheeses was significantly impacted by the salting processes ($P < 0.05$) (Table 3).

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

315 Yeasts and moulds (YM) were also affected by salting methods. In the coarse-salted
316 cheeses, the salt-reduced cheeses contained a lower YM counts than control cheeses which
317 had 9.00 log cfu/g. Cheeses salted with fine salt showed a lower YM counts than coarse salted
318 cheeses except for S30 which had the highest level of YM (9.26 log cfu/g). A large proportion
319 of cheeses contained a lower YM count than found in Bleu d’Auvergne cheese (Duval et al.,
320 2016). Moreover, opposite observations were reported on Halloumi and Cheddar cheeses
321 substituted with up to 50% KCl, which had no impact on yeast and mould counts (Kamleh et
322 al., 2012; Reddy & Marth, 1995). The development of the distinctive flavours of blue cheese
323 is dominated by metabolism of moulds during ripening. Salt in the moisture and opening of
324 the matrix by *Leuconostocs*, which produces CO₂ through the citrate metabolism, lead to the
325 growth of *Penicilium roqueforti* in the cheese (Marth & Steele, 2001). The low salt levels
326 obtained in this study could be responsible for the low growth of yeasts and moulds.

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

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

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

358 **3.3 Effect of Reducing Salt Content on Rheological and Sensory Parameters**

359 Table 4 shows the means for the TPA parameters studied in Bleu d'Auvergne cheese at
360 28 days of ripening. Hardness, cohesiveness and gumminess parameters were not significantly
361 affected by the salting methods. This finding is consistent with other salt reduction
362 investigations in Cheddar and Feta cheeses (Fitzgerald & Buckley, 1985; Katsiari et al.,
363 1997). However, SRc and STf cheeses tended to have a lower hardness value. The variability

364 in the matrix of blue-veined cheeses could account for the non-significant effect on the
365 hardness of cheeses. Adhesiveness was significantly lower in control cheeses Cc and Cf than
366 in salt-reduced cheeses. STc, SRc and STf cheeses had the highest adhesiveness (-0.539, -
367 0.472 and -0.501, respectively) SP and S30 had a lower adhesiveness value than the other
368 salt-reduced cheeses. Similarly, an increase in adhesiveness due to salt reduction has been
369 reported by Henneberry et al. (2015) in Mozzarella cheese. These authors explained that the
370 increase in adhesiveness during ripening is related to the increased hydration of caseins which
371 should reduce the cohesive (attractive) forces with the calcium phosphate *para*-casein
372 network.

373 The sensory analysis was carried out using a flash profile in order to assess the sensory
374 differences between different salt reductions applied to blue cheeses compared to control
375 cheeses and a standard commercial cheese. The panellist used different terms to characterise
376 the cheeses according to appearance, texture, aroma, and taste. The number of attributes
377 varied between 8 and 12, with an average of 10 attributes for each participant and a total of 74
378 terms of differentiation (Figure S 2). Most of the sensory attributes obtained in this study were
379 also generated in other blue-veined cheeses (Gkatzionis et al., 2013) and Camembert cheese
380 (Galli et al., 2019) analysed by flash profile. The consensus index (Rc) was 0.639 (63.9%)
381 with a high residual percentage (>100%). Std cheese had the lowest residual percentage
382 (83.4%), this could suggest that the commercial cheese had a complex descriptors from other
383 cheeses and a less homogeneous consensus among the panel for this product. This was
384 different from the consensus and higher total variance reported by (Gkatzionis et al., 2013).
385 The low consensus among the testers demonstrated in the test may be due to the difficulty in
386 ranking of the product caused by the high number of cheeses and the variability of their
387 matrix.

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

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

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

411 Bitterness was found in the S30 and both the Cc and Cf cheeses controls but this was
412 not representative of many judges. Bitterness in cheese was often related to salt levels. Mistry
413 and Kasperson (1998) showed a decrease in bitterness with increasing salt in Cheddar cheese.
414 However, the bitterness found in our study was not necessarily associated with salt (-10% of
415 sodium (g/100g) in S30) but instead, with a higher pH in these cheeses, especially in S30
416 (6.28). Lee and Warthesen (1996) have reported a high pH in the bitterest cheeses.
417 Furthermore, a significant level of calcium (such as in S30 cheese) contributes to bitterness
418 (Engel et al., 2000). Contrary to other salt substitutions with $MgCl_2$ or $CaCl_2$ used in the
419 salting of Cheddar cheese (Grummer et al., 2012), the impact of the calcium lactate salt
420 substitution on off-flavour was not found in this study.

421 During this investigation, salty taste was generated only by five panelists and did not
422 characterise the commercial cheese Std. It has been reported that food structures impacts the
423 sensory perception of salt, through changes in salt release rate and availability (Busch et al.,
424 2013; Tournier et al., 2014). An important relationship between food texture and masticatory
425 behaviour of subjects was also established (Kohyama et al., 2005). For example, the quantity
426 of salt released from a soft cheese is higher than that released from a hard cheese (Phan et al.,
427 2008).

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

434 **4 Conclusion**

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

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682 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

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

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

686 ^{a-e} Means in a row sharing common superscripts are similar as tested by Conover-Iman test ($P < 0.05$).687 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
688 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
689 industrial operator.690 ¹ Estimated by sodium measurement.

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

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

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702 ^{a-e} Means in a row sharing common superscripts are similar as tested by Conover-Iman test ($P < 0.05$).703 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
704 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).

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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}
<i>P-Value</i>	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).

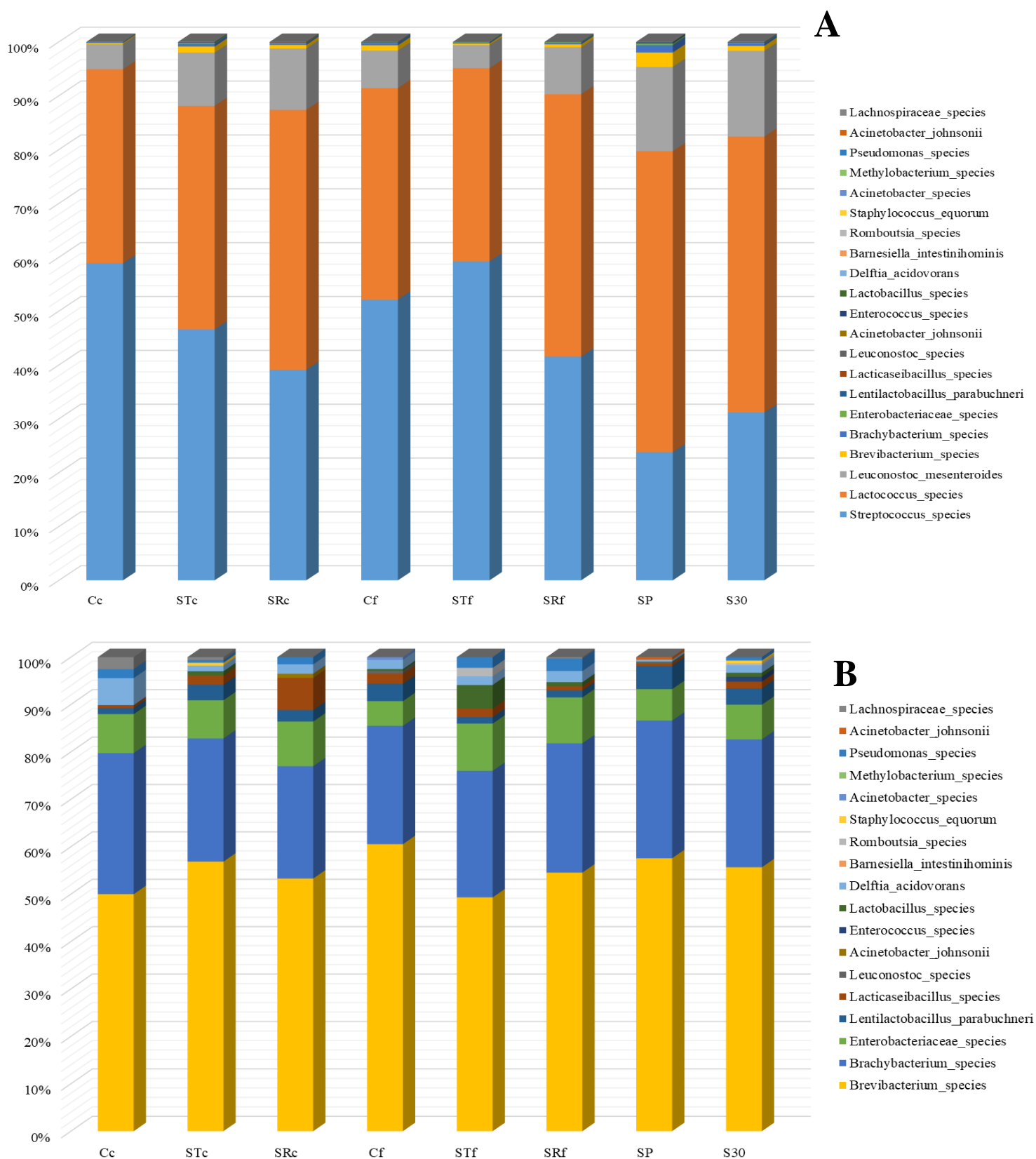
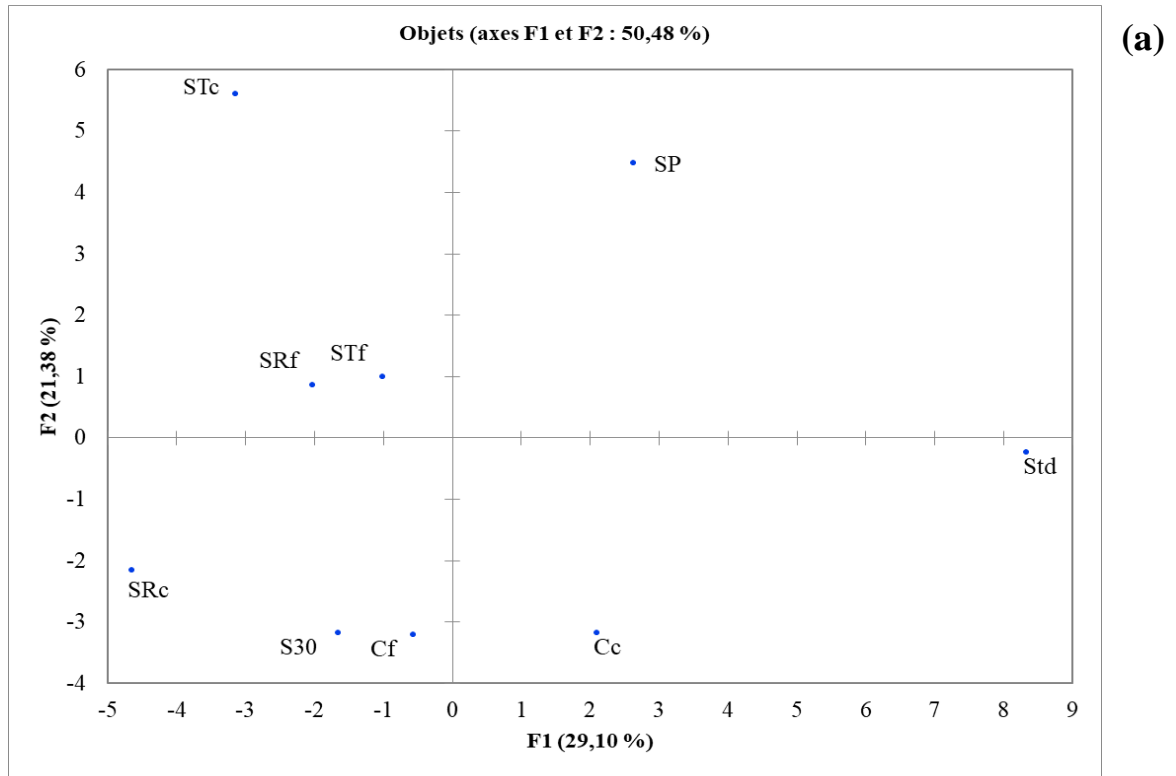


Figure 1. Microbial composition of produced cheeses. The vertical bars indicate the percentage of relative abundance of the corresponding bacterial sequences at species level. A: Total bacterial composition in each cheese, B: Bacterial composition of the subdominant population (without *Streptococcus*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

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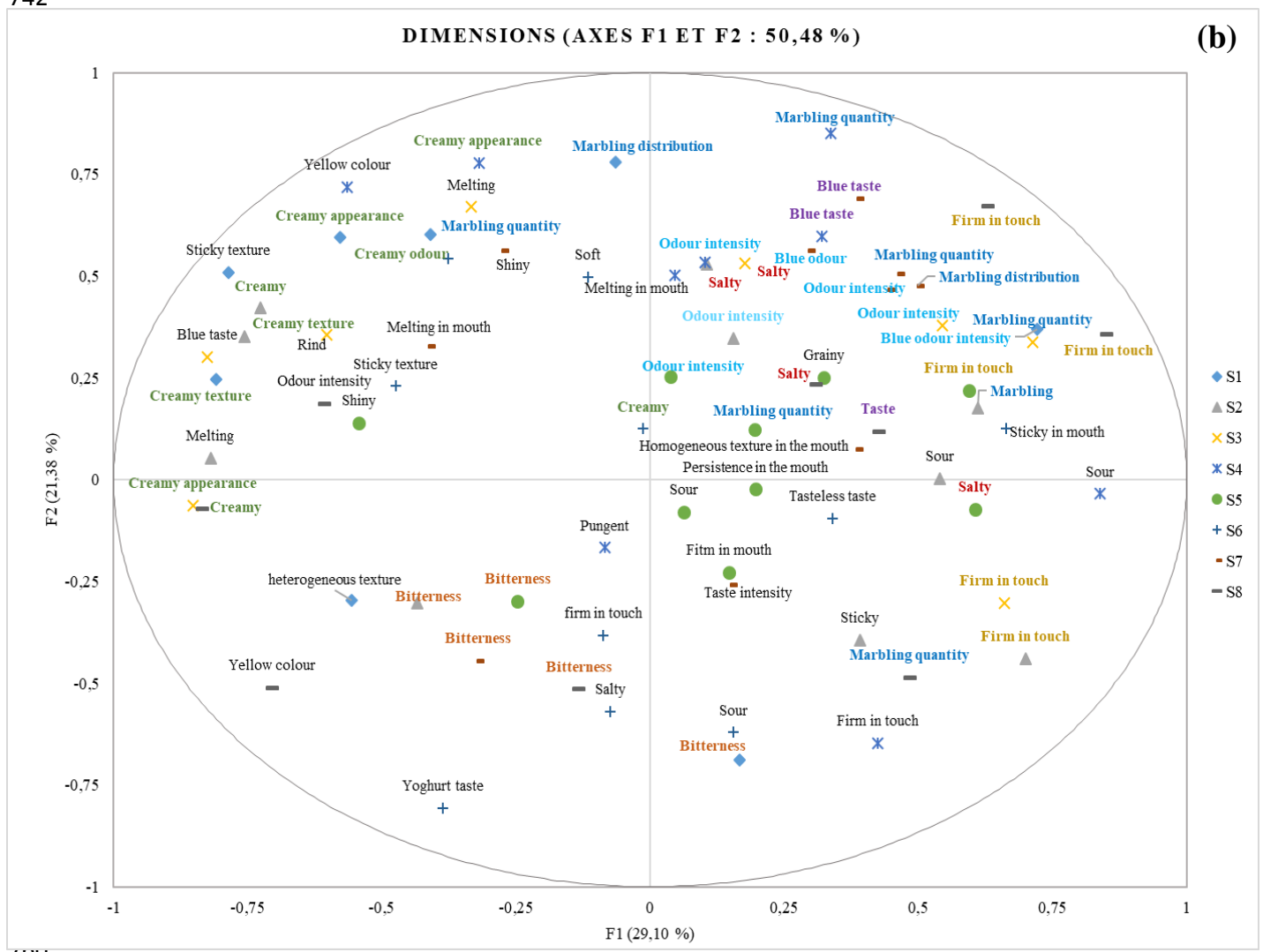


Figure 2. Map of the two principal axes of a generated Procustes Analysis (GPA) conducted on the flash profile data: (a) Cheese samples configuration, (b) Variables plot representing the distribution of subjects (S) according to the generated descriptors.

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762 **Supplementary data**

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

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

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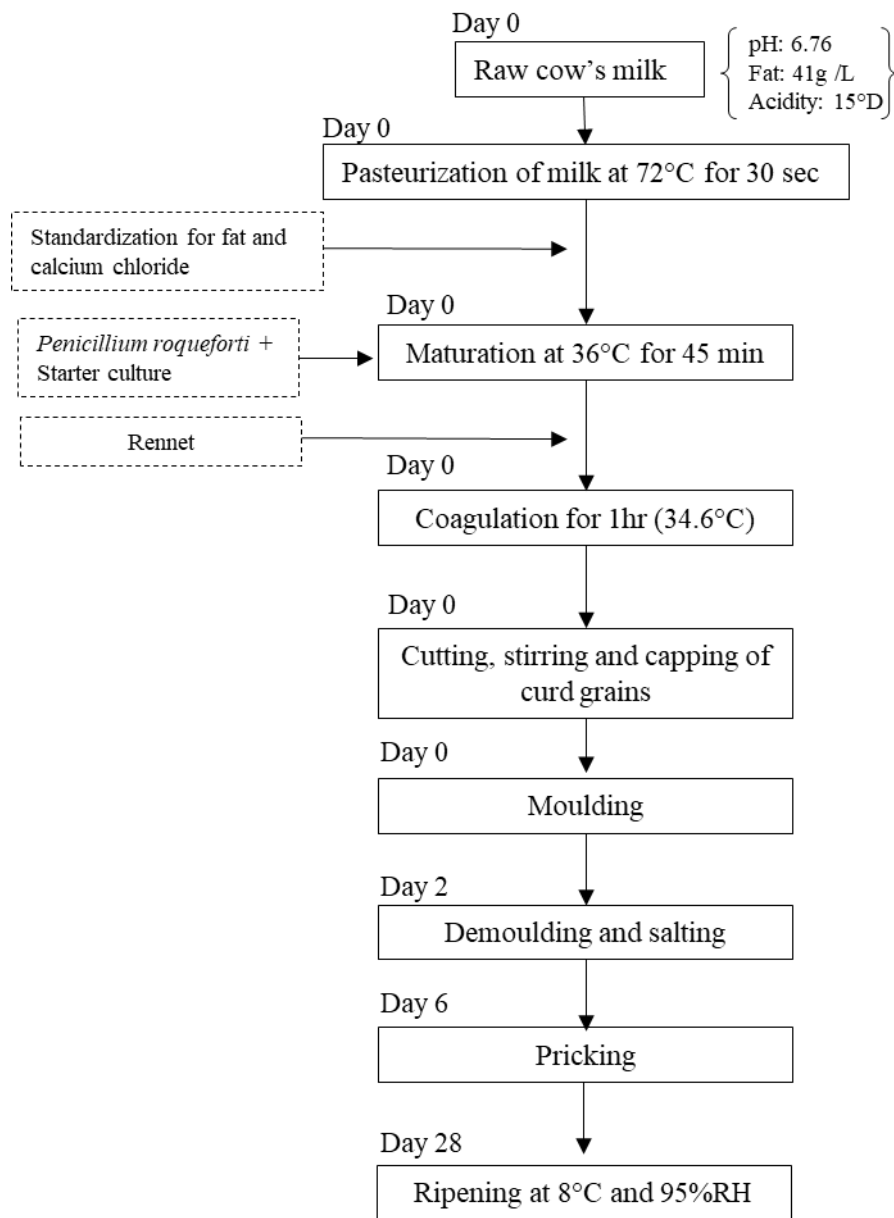
767 Table S 2 Number of reads and diversity indices obtained from blue-cheese cores.

	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

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770 **Figures**



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

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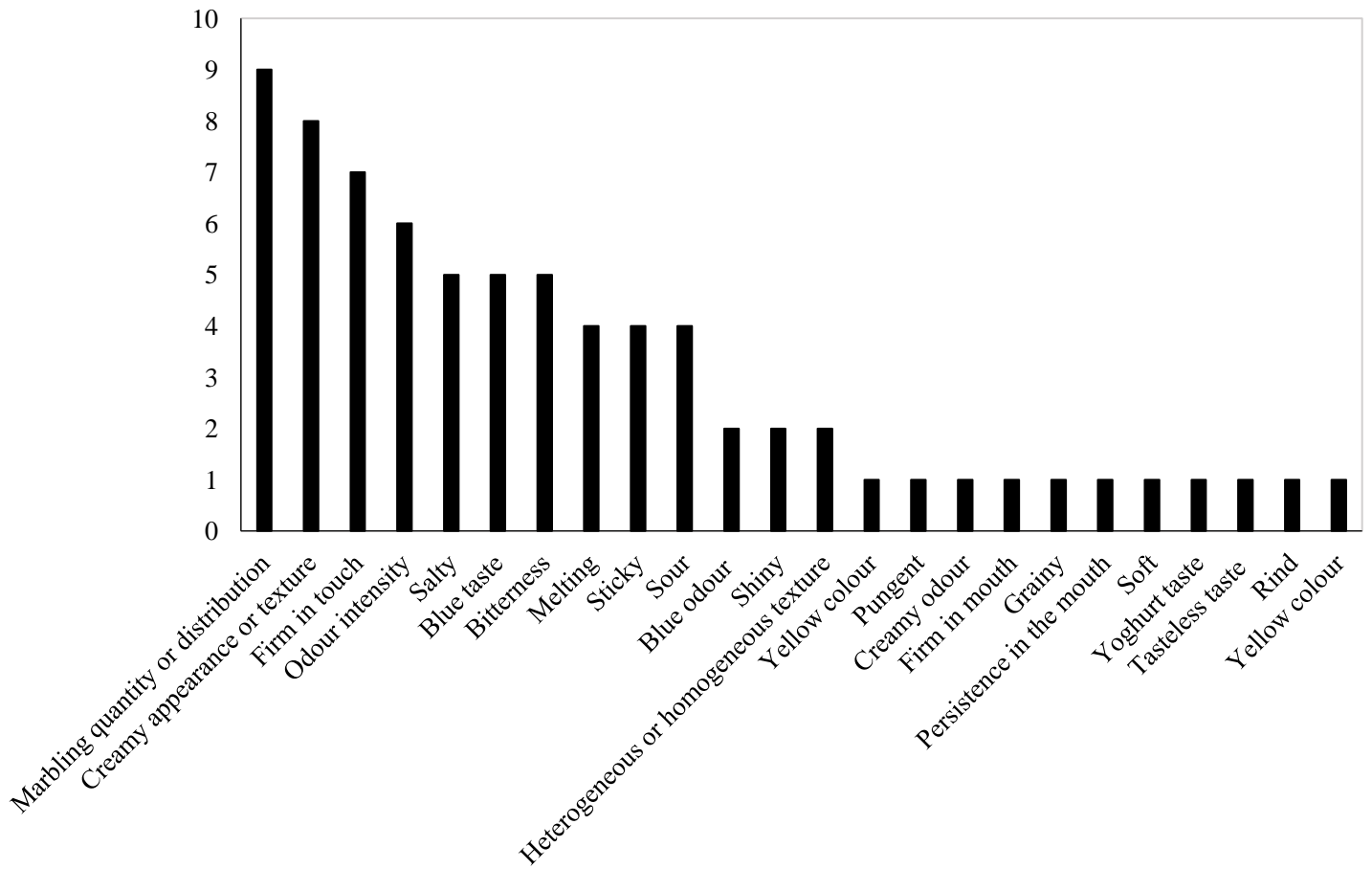


Figure S 2. Number of sensory descriptors generated by the judges

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