



HAL
open science

Exploratory study of acid-forming potential of commercial cheeses: impact of cheese type

Ecaterina Gore, Julie Mardon, Delphine Guerinon, Annick Lebecque

► To cite this version:

Ecaterina Gore, Julie Mardon, Delphine Guerinon, Annick Lebecque. Exploratory study of acid-forming potential of commercial cheeses: impact of cheese type. *International Journal of Food Sciences and Nutrition*, 2016, 67 (4), pp.1465 - 3478. 10.3109/09637486.2016.1166188 . hal-02366018

HAL Id: hal-02366018

<https://hal.science/hal-02366018>

Submitted on 26 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Exploratory study of acid-forming potential of commercial cheeses:**
2 **impact of cheese type**

3

4

5

6

7

8 Ecatarina Gore; Julie Mardon*; Delphine Guerinon; Annick Lebecque

9

10

11

12

13

14 *Clermont University, VetAgro Sup, UPSP n°2011-03-100, CALITYSS, 89 avenue Europe, F-*
15 *63370 Lempdes, France*

16

17

18

19

20

21 *Corresponding author: Tel: (+3) 347-398-1339; fax: (+3) 347-398-1390

22 *Email address: julie.mardon@vetagro-sup.fr*

23 **Abstract**

24 Due to their composition, cheeses are suspected to induce an acid load to the body. To
25 better understand this nutritional feature, the acid-forming potential of five cheeses from
26 different cheese-making technologies and two milks was evaluated on the basis of their
27 Potential Renal Acid Load (PRAL) index (considering protein, P, Cl, Na, K, Mg and Ca
28 contents) and organic anions contents. PRAL index ranged from -0.8 mEq/100g edible
29 portion for fresh cheese to 25.3 mEq/100g hard cheese Cantal and 28 mEq/100g for blue-
30 veined cheese Fourme d'Ambert. PRAL values were greatly subjected to inter-batch
31 fluctuations. This work emphasized a great imbalance between acidifying elements of PRAL
32 calculation (Cl, P and proteins elements) and alkalinizing ones (Na and Ca). Particularly, Cl
33 followed by P elements had a strong impact on the PRAL value. Hard cheeses were rich in
34 lactate, thus, might be less acidifying than suspected by their PRAL values only.

35

36 **Keywords:** Potential Renal Acid Load (PRAL), Mineral composition, Organic anions,
37 PDO cheeses, Milk

38 **1. Introduction**

39 Cheese is consumed in significant quantities in western countries, with annual
40 consumptions of 25.7 kg, 22.2 kg, 22.2 kg, 20.5 kg, 16 kg per capita for France, Germany,
41 Switzerland, Italy and USA, respectively (Fox and Guinee, 2013). Although its composition
42 varies, cheese is recognized as a substantial source of general nutrients (McGill et al., 2008).
43 Numerous works demonstrated the beneficial effect of dairy products for bone health, mainly
44 attributed to their content in Ca, vitamin D and proteins (Demmer et al., 2014; Josse et al.,
45 2012; Rizzoli, 2014). Besides, cheese has been identified as one of the main food vectors of
46 salt (40 g of cheese account for 10% of the recommended daily salt intake) (Chekri et al.,
47 2012; Saint-Eve et al., 2009) and it has been clearly demonstrated that a high-salt diet leads to
48 health issues such as hypertension (Blaustein et al., 2012), a risk factor of cardiovascular
49 diseases (He et al., 2011).

50 A nutritional aspect less-studied till now is the acid-forming potential of cheeses. Indeed,
51 cheeses are classified as the most acidifying food group (Remer and Manz, 1995). It means
52 that their consumption and metabolism lead to the production of fixed acids resulting from
53 sulfate (generated from sulfur-containing amino acids), phosphate (generated from
54 phosphoproteins) and chloride, generated from salt (Oh, 2000; Poupin et al., 2012). When the
55 acid load generated by the diet exceeds the neutralization capacity of the homeostatic systems
56 of the body, the solicitation of bone minerals or muscular amino acids would occur (Cordain
57 et al., 2005). In the long term, this low-grade metabolic acidosis might result in deleterious
58 health consequences like renal stones (Frassetto et al., 2008, 2007), osteoporosis (Lemann et
59 al., 2003; Mardon et al., 2008; Pedone et al., 2010) or loss of muscle mass (Frassetto et al.,
60 2008).

61 The method proposed for estimating the acid-forming potential of food products is based
62 on the calculation of the PRAL (Potential Renal Acid Load) index (Remer and Manz, 1995).

63 The PRAL takes into account the protein and mineral composition of a foodstuff, the
64 intestinal absorption of these nutrients, the valence or ionic charge of the minerals, as well as
65 the amount of sulfate produced from metabolized proteins according to the equation:

$$\begin{aligned} 66 \quad \text{PRAL (mEq/100g edible portion)} &= 0.49 \times \text{protein (g/100g)} + 0.037 \times \text{P (mg/100g)} + \\ 67 \quad &0.027 \times \text{Cl (mg/100g)} - 0.041 \times \text{Na (mg/100g)} - 0.021 \times \text{K (mg/100g)} - 0.026 \times \text{Mg (mg/100g)} \\ 68 \quad &- 0.013 \times \text{Ca (mg/100g)} \end{aligned} \quad (1)$$

69 According to this calculation model, negative PRAL values indicate an excess of the base-
70 forming potential of foods, whereas positive PRAL values indicate an excess of the acid-
71 forming potential of foods.

72 Organic anions content of food products may also impact the acid-base balance of the
73 organism (Demigné et al., 2004; Oh, 2000). Certainly, the most important organic sources of
74 alkalis in the diet are organic anions associated with cations, coming mostly from fruits and
75 vegetables (citrate, malate...). In cheeses, organic anions are the result of biochemical
76 changes during processing and ripening (metabolism of residual lactose and of lactate and
77 citrate) by selected cultures of lactic acid bacteria (Fox, 2004; McSweeney, 2004). Citrate is
78 the most abundant organic acid present in raw milk (1233 mg/100g dry matter) and lactate is
79 the most abundant in yoghurt (14 509 mg/100g dry matter) and cheese (14 601 mg/100g dry
80 matter) (Tormo and Izco, 2004). Given the high level of consumption of dairy products in
81 western Europe (Fox and Guinee, 2013), their impact could be significant in the acid-base
82 balance of the diet. Indeed, ingested lactate and citrate salts lead to the production of
83 bicarbonate during their metabolism, a strong alkalinizing component in the body (Oh, 2000;
84 Poupin et al., 2012). Thus, despite organic anions are not taken into account in PRAL
85 calculation it would be more accurate to consider these compounds when evaluating the acid-
86 forming potential of dairy products.

87 Milk was reported to have a PRAL around zero (Remer and Manz, 1995), thus inducing a

88 negligible impact on the acid-base balance of the body. On the other hand, Camembert and
89 Parmesan are two cheese types showing positive but very different PRAL values, namely 14.6
90 and 34.2 mEq/100g, respectively (Remer and Manz, 1995). As suggested by Demigné et al.
91 (2004), it seems that cheese-making technologies would be responsible in the generation of
92 the acid-forming potential of cheeses. The production of all types of cheese involves a
93 generally similar process, including coagulation, stirring, salting and ripening, however, these
94 various steps are modified to give products with the desired characteristics and different
95 compositions (Lucas et al., 2006b). It is thus hypothesized that different cheese types would
96 have varying acidifying potentials.

97 To our knowledge, no work addressing the evaluation of the acidifying potential of
98 different cheese types is reported in the literature. Yet it could be potentially clinically useful
99 data for assessing the diet-dependent net acid load to the metabolic system or when studying
100 the nutritional impact of dairy products. Existing studies, focusing on the mineral content of
101 different dairy products (Gursoy et al., 2014; Lante et al., 2006; Moreno-Rojas et al., 1994)
102 rarely allow to get enough information to calculate a PRAL index. Insufficient data were
103 found from nutrition facts tables (ANSES and Santé Canada, 2007) as well to estimate
104 valuable acid-forming potential (due to incomplete data and heterogeneities in sources,
105 methods and confidence indexes) and often do not provide organic anions concentrations. We
106 propose to contribute to the improvement of this aspect.

107 As an exploratory step to study the impact of processing on acid-forming potential of
108 cheeses, the aim of this work was to characterize the acid-forming potential of different
109 cheese types chosen from various cheese-making technologies. Various Protected Designation
110 of Origin (PDO) cheeses, answering for rigorous specifications were selected to conduct the
111 study.

112

113 **2. Materials and methods**

114 **2.1 Sampling**

115 The study was carried out on commercial French cheeses selected for their different type:
116 fresh cheese Faisselle (FC), soft mould ripened cheese Camembert de Normandie (CN), blue
117 veined cheese Fourme d'Ambert (FA), two hard cheeses Cantal (CA) and Comte (CO). The
118 main characteristics of these cheeses are described in Table 1. Raw milk (RM) and
119 pasteurized milk (PM) were studied as well. All products were purchased in a same local
120 supermarket in Lempdes (Auvergne area, Center of France).

121 For each product three batches, obtained at one-week interval, were selected in order to
122 overcome the inter-batch compositional variations of the commercial products. Three samples
123 per batch (the same trademark) were chosen randomly to make a pool: 1 L of milk per
124 sample, 1 kg of FC per sample, 250 g of CN per sample, 1 kg of FA per sample, 1 kg of CA
125 per sample. For CO, only 2 batches and 2 samples (800 g) per batch were available. The
126 analysis were done only on the edible part of the cheeses: the rind of CN was kept and the one
127 of FA, CA, CO cheeses was removed. The products were then packed in plastic vials or
128 vacuum bags and frozen in the cooling cell (Tecnox, France) till -20 °C and kept in the freezer
129 (-20 °C). One day before analysis the samples were transferred in a fridge (4 °C).

130 **2.2 Basic chemical analysis**

131 Ground cheese samples were analyzed in triplicate for pH, dry matter, fat, ash, total
132 nitrogen and protein according to the ISO standards (AFNOR, 2013). The pH was measured
133 at 20 °C by using a pH meter (Schott, CG840, Paris, France) according to FD V04-035:2009.
134 The dry matter content was obtained by drying 3 g of product at 105 °C in oven for 24 h (NF
135 EN ISO 5534:2004). The fat content was assayed using a Gerber method (NF V04-210:2000)
136 and the ash content using a dry ash method (NF V04-208:1989). The total N was determined
137 by Kjeldahl nitrogen analysis, reference method for protein determination in dairy products

138 (ISO 8968-1:2013). The analysis was made using a Kjeldahl Unit (Büchi, Switzerland). The
139 amount of total N obtained was multiplied by a 6.38 factor to express the results in total
140 protein.

141 **2.3 Minerals determination (Calcium, Phosphorus, Potassium, Magnesium, Sodium)**

142 The mineralization phase of 1 g of product was realized in triplicate in a programmable
143 oven (Fours Nagat 44550, France), at 550 °C for 6 hours. The ashes obtained were dissolved
144 in 1 mL nitric acid (25%) and then added to 250 mL with demineralized water. After
145 appropriate dilution, the solution was injected in a Fast Sequential Atomic Absorption
146 Spectrometer (Varian, AA240FS) according to ISO 8070:2007 to assay Ca, K, Mg and Na
147 contents. The total P was assessed with the colorimetric nitro-vanado-molybdate method from
148 the same ash solution on a Spectrophotometer (Secomam, Domont, France), at 430 nm
149 wavelength.

150 **2.4 Analysis of chloride, citrate and lactate**

151 The Cl content in milk and cheese slurry was measured by potentiometric titration
152 (Titroline Easy Potentiometer) with silver nitrate 0.1 N (VWR International, France)
153 according to NF EN ISO 5943:2007.

154 The citrate and lactate contents were measured by enzymatic bio analysis (Enzytec Citric
155 acid, Enzytec Lactic acid, R-Biopharm AG, Darmstadt, Germany). Cheese samples (5 g) were
156 homogenized in 50 mL perchloric acid (1M) using an Ultra-Turax homogenizer. The
157 homogenate was then centrifuged at 18 000 x g for 10 min. The supernatant (20 mL) was
158 adjusted to pH 8-10 with approximately 4 mL KOH (5M) and placed for 15 min in the
159 refrigerator for the quantitative precipitation of the formed potassium perchlorate, and then
160 filtered. The amount of NADH oxidized in the reactions of the filtrate with the different
161 enzymes was determined by means of its light absorbance at 340 nm and was stoichiometric
162 to the amount of citrate and lactate. For each product the assay was performed in triplicate.

163 **2.5 Statistical analysis**

164 Statistical analysis was performed using XL STAT software package (Addinsoft, Paris,
165 France). Results are reported as means \pm SD. The Shapiro-Wilk ($P < 0.05$) statistic was
166 performed to check the normality of our data. A large segment of variables lacked normality
167 and therefore non-parametric (Kruskal-Wallis) test and post-hoc multiple comparison
168 (Conover-Iman procedure) were adopted to compare the concentrations of the studied
169 elements. Differences between mean values were considered significant at $P < 0.05$.

170

171 **3. Results**

172 **3.1 Gross composition**

173 Average pH, dry matter, fat, ash, total N, protein composition of the products are
174 summarized in Table 2. Both milks (RM and PM) did not differ significantly in pH, dry
175 matter and protein content. Conversely, RM showed a higher fat content and a lower ash
176 content than PM. The lactic coagulated fresh cheese (FC) showed a slightly higher dry matter
177 content than RM. It was also characterized by the most acidic pH. Among ripened cheeses,
178 CA and CO had a lower pH than CN. FA showed the highest pH value. The dry matter was
179 significantly higher in hard cheeses CO and CA than in soft cheeses FA and CN. Cheeses fat
180 content ranged from 5.8 g/100g for FC to 37.7 g/100g for CO. The highest ash concentration
181 was observed in FA and CA compared to CO, CN and FC. Ripened cheeses (CN, FA, CA,
182 CO) had a higher protein content than milks and FC. Among them, the highest protein
183 concentration was observed in the hard cheese CO, followed by CA, CN and FA.

184 **3.2 Mineral content**

185 Table 3 shows the mineral content of the dairy products presently studied. The Ca content
186 did not differ in milks (RM, PM) and FC. Conversely, FC showed a lower P content than RM
187 and PM. The highest Ca and P concentrations were found in CO and CA cheeses, followed by

188 FA and CN. Both milks exhibited the lowest Mg contents among the products. The highest
189 Mg value was detected in CO cheese, followed by CA, then by soft cheeses (CN, FA) and
190 lactic coagulated FC. Regarding K content, the soft cheese CN and both milks (PM and RM)
191 contained the highest K levels compared to other products. Among milks and FC, the Na
192 levels ranged from 39.2 g/100g to 77.8 g/100g. The average Na concentrations measured in
193 blue veined cheese FA and hard cheese CA were significantly higher than those found in
194 bloomy-rind CN and hard cheese CO. It is noticeable that Na content widely varied among
195 different batches of FC, FA, CA and CO with, for example, ranging from 681.1 mg/100g to
196 791.9 mg/100g for FA. The unsalted products (milks and FC) showed quasi-similar Cl
197 contents (Table 3), while the salted cheeses ranged from 714.5 mg of Cl per 100 g of edible
198 portion to 1,665.1 mg of Cl per 100 g.

199 **3.3 Organic anions composition**

200 In cheeses, the highest concentration in lactate was found in CA followed by CO, FC, FA
201 and CN, ranging from 1,204.2 mg/100g to 105.2 mg/100g (Table 3). Both milks did not differ
202 in the lactate content, with about 3 mg/100g. Conversely, milks were found to be the richest in
203 citrate, with 94.0 mg/100g for PM and 82.1 mg/100g for RM. Among the 5 cheeses studied
204 CO cheese contained the highest amount of citrate (49.5 mg/100g), followed by CN (28.7
205 mg/100g) and FA (13.6 mg/100g). CA and FC was found to be the poorest in citrate, with
206 about 1 mg/100g.

207 **3.4 Potential Renal Acid Load**

208 PRAL values of the studied products ranged from a minimum of -0.8 mEq/100g for FC to
209 a maximum of 28 mEq/100g for blue-veined cheese FA (Figure 1). There was no significant
210 difference in the PRAL values of RM (0.6 mEq/100g) and PM (0.7 mEq/100g). The PRAL
211 soft cheese CN presented medium PRAL values of 16.5 mEq/100g. By contrast, soft cheese

212 FA and hard cheeses CA and CO showed the highest PRAL values (28 mEq/100g, 25.3
213 mEq/100g and 22.3 mEq/100g, respectively).

214 The values of the different elements (nutrient contents weighted with their conversion
215 factors as defined by Remer and Manz (1995)) of the PRAL calculation are shown in Figure
216 2. For salted and ripened cheeses, Cl was the acidifying element having the strongest impact
217 on the PRAL index, except for CO in which P element had a similar acidifying impact. For
218 CA, the P element also showed a high value but had a lower impact in soft cheeses PRAL
219 indexes. In hard cheeses, protein element had the lowest impact among the acidifying
220 elements, and an equivalent one to the P element in soft cheeses. Na element showed high
221 absolute values in salted and ripened cheeses was by far the strongest alkalinizing element.
222 The alkalinizing Ca element had a highest impact on PRAL indexes in hard cheeses than in
223 soft cheeses. Mg and K elements had little impact on PRAL indexes in ripened cheeses given
224 their low concentrations in these products.

225 All PRAL elements of milks and fresh cheese were globally low and exhibited a
226 neutralization between acidic and alkalinizing elements.

227

228 **4. Discussion**

229 Cheeses were previously reported as the food group with the highest PRAL values (Remer
230 and Manz, 1995), meaning that their intake potentially induces an acid load to the body. This
231 characteristic was suggested to be due to their composition, closely related to the cheese-
232 making technology applied (Demigné et al., 2004). To better characterize the link between
233 cheese type and their acid-forming potential, the present study investigated physicochemical
234 and compositional properties of two milks and five cheeses. The research provides a detailed
235 nutritional characterization of different dairy products. This work clearly demonstrates the
236 strong impact of cheese type on the acid-forming potential of end products. It particularly

237 highlights the major impact of Cl followed by P on the high PRAL value of ripened cheeses.
238 This acid load cannot be totally compensated by the main alkalinizing elements of the PRAL
239 in these products: Na and to a lesser extent Ca. Otherwise, the high lactate contents of hard
240 cheeses, not taken into account in the PRAL index, might contribute to moderate their
241 acidifying potential.

242 **4.1 Composition of milks and cheeses**

243 In the whole, basic biochemical characteristics reported in this work were in agreement
244 with different data collected in the literature (Table 2) (Abbas, 2012; ANSES, 2013; Berdague
245 et al., 1987; Chatelard-Chauvin et al., 2015; Heck et al., 2009; Le Graet and Brulé, 1988).
246 Regarding mineral compositions, the hard cheese CO was the richest in Ca, P and Mg
247 whereas the lactic coagulated FC exhibited the poorest concentrations in these elements
248 (Table 3). These differences are in agreement with the finding of Lucas et al. (2006b) who
249 reported similar differences between hard cheese Abondance and lactic coagulated
250 Rocamadour. Assayed Ca, P and Mg contents for all products were close to those reported in
251 the nutritional facts database of ANSES (2013) except for RM, not listed in the database and
252 for CO cheese which was characterized by lower contents in these elements. The same
253 nutritional database declares a P content of FA cheese three times greater than ours. This
254 discrepancy had already been highlighted by Abbas (2012) and Jacquot et al. (2015). Overall,
255 Ca, P and Mg contents in cheeses were positively correlated to the dry matter content. Indeed,
256 these minerals are largely captured in the curd and moisture content decreases as whey is
257 drained out.

258 It is noteworthy that important inter-batch variations were reported in Na and Cl contents
259 of several ripened cheeses, and particularly CO cheese (Table 3). In fact, Na and Cl contents
260 are dramatically linked to salting process which can widely vary among cheese types. Similar
261 inter-batch fluctuations were observed in Prato, CO, Cheddar and Beaufort cheeses and were

262 attributed to the lack of standardization in salt application (Chamba et al., 1994; Cichoski et
263 al., 2002; Morris et al., 1985).

264 Globally, cheeses composition clearly differed among cheese types. These variations
265 might be attributed to two major determinants: conditions of milk production and cheese-
266 making technology. The first one was reported to impact cheese composition in fatty acids,
267 carotenoids, α -tocopherol (Lucas et al., 2006a). The second one was rather found to strongly
268 impact mineral and folate contents in cheeses (Lucas et al., 2006b). Indeed, differences in
269 milk processing (acidification, curdling and dehydration), mainly influence the aqueous phase
270 composition of curd, and thus impact the final mineral concentrations in cheese (Le Graet and
271 Brulé, 1993). Afterwards, we assume that, in the present work, the differences reported in the
272 studied compounds are mainly influenced by the cheese-making technology. Moreover, in the
273 PDO products studied, milks used for manufacturing result in a mix of milks coming from
274 different producers, thus smoothing differences due to conditions of milk production.

275 ***4.2 Impact of cheese type on PRAL values***

276 The PRAL values were clearly different among cheese types (Figure 1). Milks (RM, PM)
277 showed PRAL values around zero, as described by Remer and Manz (1995) in whole
278 pasteurized milk. The FC showed the unique negative PRAL (-0.8 mEq/100g) among the
279 studied products. Mean PRAL values of ripened cheeses ranged from 16.5 mEq/100g for CN
280 to 28 mEq/100g for FA. It confirmed the high acid-forming potential of ripened cheeses but in
281 very different extent as a function of cheese type. It is noticeable that PRAL values of soft
282 cheeses (bloomy-rind CN and blue-veined cheese FA) showed wide inter-batch variations.
283 The CN PRAL index ranged from 14.7 to 20.0 mEq/100g among batches whereas PRAL
284 variations ranged from 21.7 to 37.5 mEq/100g for FA. In both cases, these variations were
285 mainly due to mineral content fluctuations, already reported in commercial soft cheeses, using
286 similar analytical methods (Abbas et al., 2012; Bord et al., 2015; Jacquot et al., 2015; Jaoude

287 et al., 2010; Moreno-Rojas et al., 2012). Such variations are common in cheeses involving
288 mould-growth and are probably due to their matrix heterogeneity. This heterogeneity would
289 be assigned to the development of microbial flora *Geotrichum candidum*, *Penicillium*
290 *camembert* and *P. roqueforti* (Abbas, 2012; Prieto et al., 2000) and to the mineral migrations
291 it may cause (Le Graet et al., 1983; McSweeney and Fox, 2004; McSweeney and Sousa,
292 2000). This result highlights the fact that PRAL values are strongly subjected to variations in
293 inter-batch mineral compositions.

294 The great mean PRAL value found for FA was attributed to high Cl contents and more
295 precisely to much higher Cl concentrations than Na concentrations (Figure 2). Similar
296 observations were made in Parmesan from the work of Remer and Manz (1995), in blue-
297 veined cheeses (Prieto et al., 2002, 2000), in Camembert (Boutrou et al., 1999) and in Cantal
298 cheese (De Freitas et al., 2007). This result emphasize that these two ions play a major role in
299 the acid-forming potential of cheeses. It is noticeable that, from 2003, Na and Cl were omitted
300 from the PRAL formula when estimating a diet-dependent net acid load (Frassetto et al.,
301 2007; Remer, 2003). Indeed, in several clinical studies, Na and Cl were found to fully
302 compensate when the urinary cation-anion difference was calculated (Remer and Manz,
303 1994). This changing allowed to overcome missing Cl data from food tables and assumed that
304 in the case of processed salted foods Na and Cl would always have a stoichiometric
305 relationship, with equal concentrations. However, in cheese-making technology, Cl ions in
306 milk, and later in cheese whey, are accompanied by either Na or other cations. Salt is added
307 during processing and a portion of Na and Cl ions are allowed to drain off into the whey.
308 Therefore, Na and Cl concentrations changes are unlikely to be stoichiometric. This work
309 demonstrates the interest of considering Na and Cl contents when calculating PRAL values of
310 dairy products. In the literature, very few works addressed the distribution of Cl and Na in
311 cheese matrix to understand this feature. The diffusion of salt was studied, but NaCl content

312 was mainly considered from the Cl determination (Floury et al., 2009; Hardy, 1983; Morris et
313 al., 1985). This point would deserve further research. It is hypothesized that the applied
314 technology would explain this difference. A following of ions concentrations throughout
315 cheese processing would allow to address this issue. It is also possible that the addition of
316 CaCl₂, widely used in cheese-making technology to improve yields, might be involved in this
317 phenomenon.

318 The hard cheeses CA and CO also showed high PRAL values, in agreement with Remer
319 and Manz (1995) who reported similar PRAL in Cheddar-type cheese. These characteristics
320 were the result of stronger acidifying elements than absolute values of alkalinizing ones: Cl, P
321 and protein elements could not be compensated by Na and Ca elements, whereas K and Mg
322 had little impact given their low concentrations in the ripened cheeses. A higher protein
323 content in hard cheeses is a well-known consequence of the scalding/cooking and the pressing
324 processes used in the manufacture of cheese (Fox and Guinee, 2013). It was therefore an
325 awaited result. The acidogenic effect of proteins for the human body (Alexy et al., 2005;
326 Barzel and Massey, 1998; Frassetto et al., 1998) and especially animal protein (Sellmeyer et
327 al., 2001) has already been highlighted in several studies. Nevertheless, it is difficult to
328 evaluate the bearing of protein hydrolysis occurring during ripening on the acidifying
329 potential of cheeses. Proteolysis occurs in an extent heavily dependent on cheese
330 manufacturing technology (McSweeney and Sousa, 2000). The sulphur-containing amino
331 acids concentration, which are mainly responsible for the acidogenic impact of dietary
332 protein, is not supposed to be modified and justifies the assay of total nitrogen. However, it is
333 difficult to know whether the rate of these different fractions (intact/part hydrolysed proteins,
334 free amino acid) during digestion impact the acid-forming potential of cheese. Beyond the
335 well-known acidogenic impact of protein, an original result of this work deserves to be

336 highlighted as it shows that the Cl and P elements have a stronger impact on the PRAL value
337 of ripened cheeses than proteins in hard cheeses. This point has never been reported before.

338 ***4.3 Impact of cheese type on organic anion contents***

339 The most important organic sources of alkalis in the diet are organic acids that are
340 ingested in the form of salts of organic anions (Demigné et al., 2004; Poupin et al., 2012).
341 Despite PRAL calculation takes into account the cations contents (K, Na, Ca and Mg) of
342 foodstuffs, organic anions associated with these cations might have a different impact on the
343 acid-base balance whether they are metabolisable (for instance citrate and lactate) or not (for
344 instance oxalate or tartrate) (Demigné et al., 2004; Oh, 2000; Poupin et al., 2012; Sabboh et
345 al., 2007). Those that are effectively absorbed in the small intestine and are metabolized,
346 mostly in the liver, lead to the synthesis of HCO_3^- , a powerful buffer system in the organism
347 (Poupin et al., 2012). In this sense, valuable contents in metabolisable organic anions are
348 assumed to concur to the acid/alkaline potential of a food product (Demigné et al., 2004;
349 Sabboh et al., 2007). The daily food supply of organic anions might range from 1 to 4 g/day
350 as a function of dietary patterns. In subjects consuming a high-meat and high dairy products
351 diet, organic anions ingested with animal foods was suggested to be greater than that from
352 plant foods (Demigné et al., 2004).

353 In agreement with the literature, among the dairy products studied, milks were found as
354 the richest products in citrate, whereas cheeses were the richest in lactate (Table 3) (Tormo
355 and Izco, 2004; Upreti et al., 2006). The citrate content of milk (82.1 - 94.0 mg/100g) was
356 close to the average citrate content of vegetables (139 mg/100g) but much lower than that of
357 fruits, with more than 550 mg/100g in average (Demigné, 2008). FC was the poorest product
358 in citrate. This may be due to the fact that citrate would be metabolized by cheese starters and
359 non-starter microflora (McSweeney, 2004). By contrast, FC showed a high content in lactate,

360 which is produced from lactose by the starter cultures (McSweeney and Fox, 2004). Thus, FC
361 might be considered to show an alkalinizing potential because of its lactate content.

362 All ripened cheeses exhibited low citrate contents. This was presumably due to nonstarter
363 lactic acid bacteria action, as suggested by (McSweeney, 2004) who reported a decline of
364 citrate content to trace elements during ripening in Cheddar cheese. Even if citrate
365 theoretically has a three-fold higher alkalinizing effect than lactate in the body due to its three
366 carboxyl groups, the low citrate content in ripened cheeses makes its base-forming potential
367 negligible compared to the lactate's one. CN contained the lowest concentration in lactate. In
368 fact, Camembert cheeses are known to have an extensive surface catabolism, resulting in a
369 fast metabolization of lactate by *Geotrichum candidum* and *Penicillium camemberti* in CO₂
370 and H₂O (McSweeney and Fox, 2004). Hard cheeses presently studied showed great amounts
371 of lactate. The lactate content of CA (1204 mg/100g) was similar to those found in farmer's
372 cheese and Cheddar (Izco et al., 2002) but also in uncooked pressed (hard) cheese (Delbès-
373 Paus et al., 2012). Given these lactate contents, we could hypothesize that hard cheeses CA
374 and CO acid-forming potential might be less important than suspected from their PRAL
375 values only.

376 The main limitation of this work is that the lack of knowledge regarding the respective
377 weight of PRAL and organic anions contents in the effective acid-forming potential of
378 cheeses unable to fully conclude. Despite we emphasize that lactate could impact the PRAL
379 measurement for cheeses, the way to use anion contents in the formula cannot be suggested
380 from this study only. This issue would deserve further research and certainly clinical
381 investigations.

382

383 **Conclusion**

384 As demonstrated in this work, there is a strong link between the cheese type and the acid-
385 forming potential of the cheese. Fresh cheese was characterized by a slightly negative PRAL
386 and ripened cheeses exhibited different acid-forming potentials. The soft bloomy-rind
387 Camembert cheese showed a moderate acid-forming potential, whereas hard cheeses and
388 blue-veined cheese exhibited the strongest acidogenic characters. The study of the respective
389 weight of PRAL elements emphasize a great imbalance between acidifying elements (Cl, P
390 and proteins elements) and alkalinizing ones (Na and Ca) to explain it. This work also
391 demonstrates that PRAL values were greatly subjected to inter-batch fluctuations encountered
392 in cheese industry. This exploratory work deserves to be continued to determine the evolution
393 of the acid-forming potential during cheese-manufacturing and to better understand its
394 generation. It is suggested that key steps of the process might influence the acidogenic
395 character of end-products, such as draining or salting.

396 Another noteworthy result is the high lactate content reported in hard cheeses which could
397 suggest that these products could be less acidogenic than suspected from their PRAL value
398 only.

399 At last, the present study focused on the acid-forming potential of cheeses as their level of
400 consumption in western countries is suspected to impact the diet-dependent net acid load.
401 However dairy products deserve to be considered with broader nutritional perspectives, since
402 they play several roles on human health and remain the first contributors to Ca intake.

403

404 **Declaration of conflicting interest**

405 The authors declared no potential conflicts of interest with respect to the research,
406 authorship, and/or publication of this article.

407

408 **Acknowledgements**

409 The research was co-financed by FEDER (European Regional Development Funds) in
410 Auvergne (France).

References

- Abbas, K. (2012). Effet de traitements thermiques sur les propriétés fonctionnelles de fromages traditionnels : le cas des pâtes persillées (These). Blaise Pascal University. 145 p.
- Abbas, K., Karoui, R., Aït-Kaddour, A. (2012). Application of synchronous fluorescence spectroscopy for the determination of some chemical parameters in PDO French blue cheeses. *Eur. Food Res. Technol.* 234:457–465.
- AFNOR (2013). Association française de normalisation. Chemical analysis [WWW Document]. URL <http://sagaweb.afnor.org/fr-FR/sw/Identification/AccueilAbonnement> (accessed 11.9.15).
- Alexy, U., Remer, T., Manz, F., Neu, C.M., Schoenau, E. (2005). Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am. J. Clin. Nutr.* 82:1107–1114.
- ANSES (2013). Table de composition nutritionnelle Ciqual [WWW Document]. URL <https://pro.anses.fr/TableCIQUAL/index.htm> (accessed 11.9.15).
- ANSES, Santé Canada (2007). Web platform of nutritional analysis on Food Composition [WWW Document]. Compos. Aliments. URL <http://www.composition-des-aliments.fr/> (accessed 10.15.15).
- Barzel, U.S., Massey, L.K. (1998). Excess Dietary Protein Can Adversely Affect Bone. *J. Nutr.* 128:1051–1053.
- Berdague, L., Jeunet, R., Grappin, R., Duboz, G. (1987). Affinage et qualité du gruyère de Comté. III. Fermentation lactique et teneur en acides gras volatils des fromages de Comté. *Lait* 67:249–263.
- Blaustein, M.P., Leenen, F.H.H., Chen, L., Golovina, V.A., Hamlyn, J.M., Pallone, T.L., Huysse, J.W.V., Zhang, J., Wier, W.G. (2012). How NaCl raises blood pressure: a new paradigm for the pathogenesis of salt-dependent hypertension. *Am. J. Physiol. - Heart Circ. Physiol.* 302:H1031–H1049.
- Bord, C., Guerinon, D., Lebecque, A. (2015). Impact of heating on sensory properties of French Protected Designation of Origin (PDO) blue cheeses. Relationships with physicochemical parameters. *Food Sci. Technol. Int.* 0:1-12.
- Boutrou, R., Gaucheron, F., Piot, M., Michel, F., Maubois, J.-L., Léonil, J. (1999). Changes in the composition of juice expressed from Camembert cheese during ripening. *Lait* 79:503–513.
- Chamba, J.-F., Delacroix-Buchet, A., Berdague, J.-L., Clement, J.-F. (1994). Une approche globale de la caractérisation des fromages : l'exemple du fromage de Beaufort, in: Sciences Des Aliments. Presented at the Journées Sciences des aliments, Lavoisier, pp. 581–590.
- Chatelard-Chauvin, C., Pelissier, F., Hulin, S., Montel, M.C. (2015). Behaviour of *Listeria monocytogenes* in raw milk Cantal type cheeses during cheese making, ripening and storage in different packaging conditions. *Food Control* 54:53–65.
- Chekri, R., Noël, L., Millour, S., Vastel, C., Kadar, A., Sirot, V., Leblanc, J.-C., Guérin, T. (2012). Calcium, magnesium, sodium and potassium levels in foodstuffs from the second French Total Diet Study. *J. Food Compos. Anal.* 25:97–107.
- Cichoski, A.J., Valduga, E., Valduga, A.T., Tornadijo, M.E., Fresno, J.M. (2002). Characterization of Prato cheese, a Brazilian semi-hard cow variety: evolution of physico-chemical parameters and mineral composition during ripening. *Food Control* 13:329–336.

- Cordain, L., Eaton, S.B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. a, O'Keefe, J.H., Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *Am. J. Clin. Nutr.* 81:341–54.
- De Freitas, I., Pinon, N., Thierry, A., Lopez, C. (2007). In depth dynamic characterisation of French PDO Cantal cheese made from raw milk. *Lait* 87:97–117.
- Delbès-Paus, C., Pochet, S., Helinck, S., Veisseire, P., Bord, C., Lebecque, A., Coton, M., Desmasures, N., Coton, E., Irlinger, F., Montel, M.-C. (2012). Impact of Gram-negative bacteria in interaction with a complex microbial consortium on biogenic amine content and sensory characteristics of an uncooked pressed cheese. *Food Microbiol.* 30:74–82.
- Demigné, C. (2008). Fonctionnalité alcalinisante, in: *Aliments Fonctionnels* (2e Ed). Lavoisier, pp. 541–558.
- Demigné, C., Sabboh, H., Puel, C., Rémésy, C., Coxam, V. (2004). Organic anions and potassium salts in nutrition and metabolism. *Nutr. Res. Rev.* 17:249–58.
- Demmer, E., Gertz, E., Rogers, T., Hillgonds, D., Garrod, M., Loan, M.V. (2014). Effect of dairy foods vs. calcium and vitamin-D supplements on bone metabolism: use of ⁴¹Ca tracer (1018.12). *FASEB J.* 28:1018.12.
- Floury, J., Rouaud, O., Le Poullennec, M., Famelart, M.-H. (2009). Reducing salt level in food: Part 2. Modelling salt diffusion in model cheese systems with regards to their composition. *LWT - Food Sci. Technol.* 42:1621–1628.
- Fox, P.F. (2004). *Cheese: General aspects*. Elsevier Academic Press. 656 p.
- Fox, P.F., Guinee, T.P. (2013). *Cheese Science and Technology*, in: *Milk and Dairy Products in Human Nutrition*. Park Young W. and Haein George F.W., pp. 357–389.
- Frassetto, Lanham-New, S., Macdonald, H.M., Remer, T., Sebastian, A., Tucker, K.L., Tyllavsky, F.A. (2007). Standardizing Terminology for Estimating the Diet-Dependent Net Acid Load to the Metabolic System. *J. Nutr.* 137:1491–1492.
- Frassetto, L., Todd, K.M., Morris, R.C., Sebastian, A. (1998). Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am. J. Clin. Nutr.* 68:576–583.
- Frassetto, Morris, R., Sellmeyer, D., Sebastian, A. (2008). Adverse effects of sodium chloride on bone in the aging human population resulting from habitual consumption of typical American diets. *J. Nutr.* 138:419S–422S.
- Gursoy, O., Gokce, R., Con, A.H., Kinik, O. (2014). Survival of *Bifidobacterium longum* and its effect on physicochemical properties and sensorial attributes of white brined cheese. *Int. J. Food Sci. Nutr.* 65:816–820.
- Hardy, J. (1983). Diffusion et distribution du chlorure de sodium dans les fromages. Influence sur l'activité de l'eau et les propriétés de sorption de l'eau. (These). Institut National Polytechnique de Lorraine, Nancy. 175 p.
- Heck, J.M.L., Van Valenberg, H.J.F., Dijkstra, J., Van Hooijdonk, A.C.M. (2009). Seasonal variation in the Dutch bovine raw milk composition. *J. Dairy Sci.* 92:4745–4755.
- He, F.J., Burnier, M., MacGregor, G.A. (2011). Nutrition in cardiovascular disease: salt in hypertension and heart failure. *Eur. Heart J.* 32:3073–3080.
- Izco, J.M., Tormo, M., Jiménez-Flores, R. (2002). Rapid Simultaneous Determination of Organic Acids, Free Amino Acids, and Lactose in Cheese by Capillary Electrophoresis. *J. Dairy Sci.* 85:2122–2129.
- Jacquot, S., Karoui, R., Abbas, K., Lebecque, A., Bord, C., Aït-Kaddour, A. (2015). Potential of Multispectral Imager to Characterize Anisotropic French PDO Cheeses: A Feasibility Study. *Int. J. Food Prop.* 18:213–230.
- Jaoude, D.A., Olabi, A., Najm, N.E.O., Malek, A., Saadeh, C., Baydoun, E., Toufeili, I. (2010). Chemical composition, mineral content and cholesterol levels of some regular

- and reduced-fat white brined cheeses and strained yogurt (Labneh). *Dairy Sci. Technol.* 90:699–706.
- Josse, A.R., Atkinson, S.A., Tarnopolsky, M.A., Phillips, S.M. (2012). Diets Higher in Dairy Foods and Dietary Protein Support Bone Health during Diet- and Exercise-Induced Weight Loss in Overweight and Obese Premenopausal Women. *J. Clin. Endocrinol. Metab.* 97:251–260.
- Lante, A., Lomolino, G., Cagnin, M., Spettoli, P. (2006). Content and characterisation of minerals in milk and in Crescenza and Squacquerone Italian fresh cheeses by ICP-OES. *Food Control* 17:229–233.
- Le Graet, Y., Brulé, G. (1993). Effects of pH and ionic strength on mineral balance of milk. *Lait* 73:51–60.
- Le Graet, Y., Brulé, G. (1988). Migration des macro et oligo-éléments dans un fromage à pâte molle de type Camembert. *Lait* 68:219–234.
- Le Graet, Y., Lepienne, A., Brulé, G., Ducruet, P. (1983). Migration du calcium et des phosphates inorganiques dans les fromages à pâte molle de type Camembert au cours de l'affinage. *Lait* 63:317–332.
- Lemann, J., Bushinsky, D. a, Hamm, L.L. (2003). Bone buffering of acid and base in humans. *Am. J. Physiol. Renal Physiol.* 285:F811–32.
- Lucas, A., Agabriel, C., Martin, B., Ferlay, A., Verdier-Metz, I., Coulon, J.-B., Rock, E. (2006a). Relationships between the conditions of cow's milk production and the contents of components of nutritional interest in raw milk farmhouse cheese. *Lait* 86:177–202.
- Lucas, A., Rock, E., Chamba, J., Verdier-Metz, I., Brachet, P., Coulon, J.B. (2006b). Respective effects of milk composition and the cheese-making process on cheese compositional variability in components of nutritional interest. *Lait* 86:21–41.
- Mardon, J., Habauzit, V., Trzeciakiewicz, A., Davicco, M.-J., Lebecque, P., Mercier, S., Tressol, J.-C., Horcajada, M.-N., Demigné, C., Coxam, V. (2008). Long-Term Intake of a High-Protein Diet with or without Potassium Citrate Modulates Acid-Base Metabolism, but Not Bone Status, in Male Rats. *J. Nutr.* 138:718–724.
- McGill, C.R., Fulgoni, V.L., DiRienzo, D., Huth, P.J., Kurilich, A.C., Miller, G.D. (2008). Contribution of dairy products to dietary potassium intake in the United States population. *J. Am. Coll. Nutr.* 27:44–50.
- McSweeney, P.L.H. (2004). Biochemistry of cheese ripening. *Int. J. Dairy Technol.* 57:127–144.
- McSweeney, P.L.H., Fox, P.F. (2004). Metabolism of residual lactose and of lactate and citrate, in: *Cheese: General Aspects*. Academic Press, pp. 361–371.
- McSweeney, P.L.H., Sousa, M.J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Lait* 80:293–324.
- Moreno-Rojas, R., Cámara-Martos, F., Sánchez-Segarra, P.J., Amaro-López, M.Á. (2012). Influence of manufacturing conditions and discrimination of Northern Spanish cheeses using multi-element analysis. *Int. J. Dairy Technol.* 65:594–602.
- Moreno-Rojas, R., Zurera-Cosano, G., Amaro-Lopez, M.A. (1994). Concentration and seasonal variation of calcium, magnesium, sodium and potassium in raw cow, ewe and goat milk. *Int. J. Food Sci. Nutr.* 45:99–105.
- Morris, H.A., Guinee, T.P., Fox, P.F. (1985). Salt Diffusion in Cheddar Cheese. *J. Dairy Sci.* 68:1851–1858.
- Oh, M.S. (2000). ACID-BASE IN RENAL FAILURE: New Perspectives on Acid-Base Balance. *Semin. Dial.* 13:212–219.

- Pedone, C., Napoli, N., Pozzilli, P., Lauretani, F., Bandinelli, S., Ferrucci, L., Antonelli-Incalzi, R. (2010). Quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. *Bone* 46:1063–7.
- Poupin, N., Calvez, J., Lassale, C., Chesneau, C., Tomé, D. (2012). Impact of the diet on net endogenous acid production and acid-base balance. *Clin. Nutr. Edinb. Scotl.* 31:313–21.
- Prieto, B., Franco, I., Fresno, J.M., Bernardo, A., Carballo, J. (2000). Picón Bejes-Tresviso blue cheese: an overall biochemical survey throughout the ripening process. *Int. Dairy J.* 10:159–167.
- Prieto, B., Franco, I., Prieto, J.G., Bernardo, A., Carballo, J. (2002). Compositional and Physico-chemical Modifications during the Manufacture and Ripening of León Raw Cow's Milk Cheese. *J. Food Compos. Anal.* 15:725–735.
- Remer, T., Dimitriou, T., Manz, F. (2003). Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am. J. Clin. Nutr.* 77:1255–60.
- Remer, T., Manz, F. (1994). Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am. J. Clin. Nutr.* 59:1356–61.
- Remer, T., Manz, F. (1995). Potential Renal Acid Load of Foods and its Influence on Urine pH. *J. Am. Diet. Assoc.* 95:791–797.
- Rizzoli, R. (2014). Dairy products, yogurts, and bone health. *Am. J. Clin. Nutr.* 99:1256S–1262S.
- Sabboh, H., Coxam, V., Horcajada, M.-N., Révész, C., Demigné, C. (2007). Effects of plant food potassium salts (citrate, galacturonate or tartrate) on acid-base status and digestive fermentations in rats. *Br. J. Nutr.* 98:72–7.
- Saint-Eve, A., Lauerjat, C., Magnan, C., Déléris, I., Souchon, I. (2009). Reducing salt and fat content: Impact of composition, texture and cognitive interactions on the perception of flavoured model cheeses. *Food Chem.* 116:167–175.
- Sellmeyer, D.E., Stone, K.L., Sebastian, A., Cummings, S.R. (2001). A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. *Am. J. Clin. Nutr.* 73:118–122.
- Tormo, M., Izco, J.M. (2004). Alternative reversed-phase high-performance liquid chromatography method to analyse organic acids in dairy products. *J. Chromatogr. A* 1033:305–310.
- Upreti, P., McKay, L.L., Metzger, L.E. (2006). Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on Cheddar cheese quality: changes in residual sugars and water-soluble organic acids during ripening. *J. Dairy Sci.* 89:429–443.

Figure captions

Figure 1. Box plots of the Potential Renal Acid Load (PRAL) index of the products studied RM, raw milk; PM, pasteurized milk; FC, lactic coagulated fresh cheese; CN, soft mould-ripened Camembert de Normandie; FA, blue-veined cheese Fourme d'Ambert; CA, hard cheese Cantal; CO, hard cheese Comte. The data represents the average \pm SD, expressed in mEq/100g of edible portions; n=9.

Figure 2. PRAL indexes and PRAL elements of the products studied weighted with their derived factors

RM, raw milk; PM, pasteurized milk; FC, lactic coagulated fresh cheese; CN, soft mould-ripened Camembert de Normandie; FA, blue-veined cheese Fourme d'Ambert; CA, hard cheese Cantal; CO, hard cheese Comte. The data represents the average \pm SD, expressed in mEq/100g of edible portions; n=9.

Figure 1:

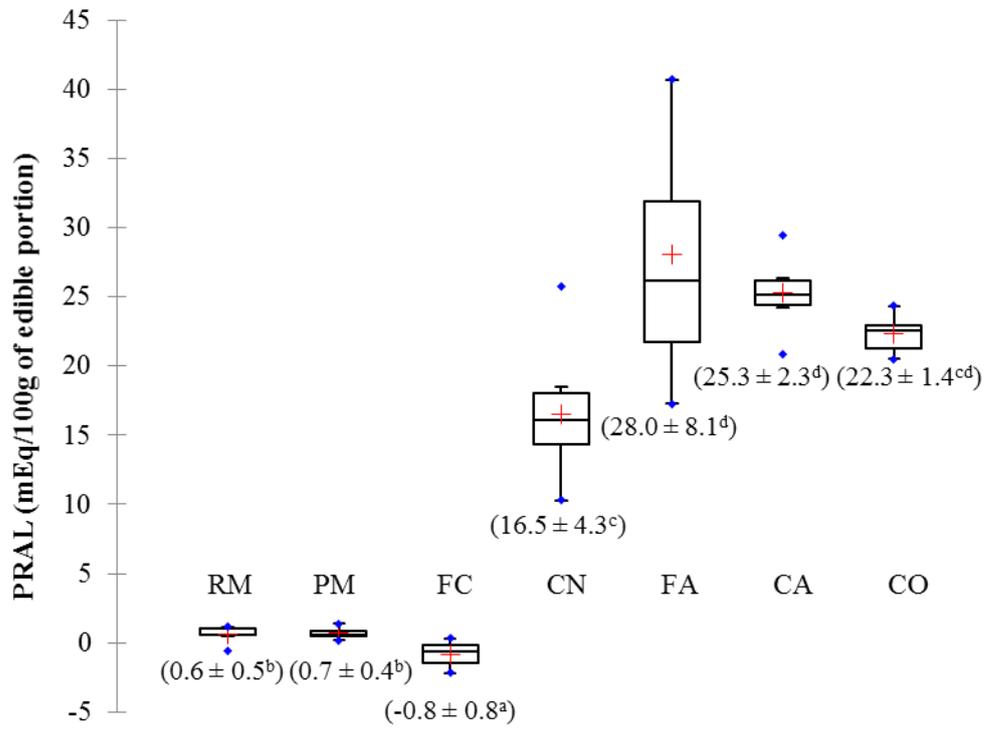


Figure 2:

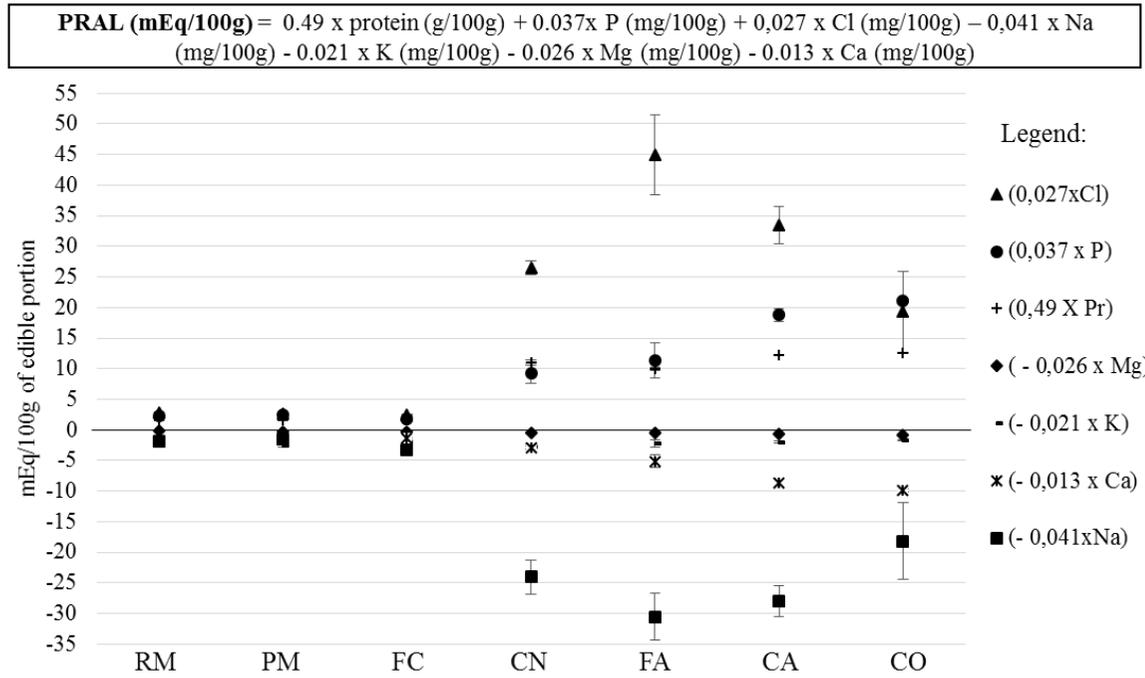


Table 1. Characteristics of the five cheese varieties studied

	Cheese-type				
	Fresh cheese	Soft cheeses		Hard cheeses	
Cheese name	Faisselle (FC)	Camembert de Normandie (CN)	Fourme d'Ambert (FA)	Cantal (CA)	Comte (CO)
Official quality sign ¹	-	PDO	PDO	PDO	PDO
Production area	-	Normandy	Auvergne	Auvergne	Franche-Comte
Milk nature	pasteurized	raw	pasteurized	pasteurized	raw
Species	cow	cow	cow	cow	cow
Cheese-making technology	lactic coagulation	surface mould-ripened	blue-veined	pressed uncooked	pressed cooked
Weight (kg)	1	0.25	2.2	35-45	35-45
Ripening time (d)	0	21	63	150	150

¹PDO – Protected Designation of Origin

Table 2. Basic chemical analysis (pH, dry matter content, fat, ash, protein and total nitrogen content) of milks and cheeses processed with different technologies (related to 100 g edible portion)

	Product type ^{1,2}							p-value
	RM	PM	FC	CN	FA	CA	CO	
pH	6.72 ± 0.00 ^c	6.72 ± 0.01 ^c	4.28 ± 0.03 ^a	6.77 ± 0.10 ^c	7.09 ± 0.03 ^d	5.70 ± 0.09 ^b	5.82 ± 0.02 ^b	<0.0001
Dry matter, g	12.7 ± 0.1 ^{ab}	12.6 ± 0.1 ^a	13.3 ± 1.5 ^b	47.2 ± 1.3 ^c	52.8 ± 2.6 ^d	59.9 ± 0.6 ^e	65.8 ± 0.3 ^e	<0.0001
Fat, g	4.0 ± 0.1 ^b	3.5 ± 0.0 ^a	5.8 ± 0.2 ^c	23.6 ± 0.8 ^d	28.7 ± 0.6 ^e	32.6 ± 2.1 ^f	37.7 ± 0.2 ^g	<0.0001
Ash, g	0.66 ± 0.03 ^a	0.81 ± 0.02 ^c	0.71 ± 0.02 ^b	2.42 ± 0.09 ^d	4.04 ± 0.24 ^f	4.10 ± 0.20 ^f	3.42 ± 0.39 ^e	<0.0001
Protein, g	3.3 ± 0.0 ^a	3.4 ± 0.0 ^a	3.9 ± 0.3 ^b	22.3 ± 0.9 ^d	20.3 ± 0.5 ^c	25.0 ± 0.2 ^e	25.6 ± 0.2 ^f	<0.0001
Total nitrogen, g	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.6 ± 0.0 ^b	3.5 ± 0.2 ^d	3.2 ± 0.1 ^c	3.9 ± 0.0 ^e	4.0 ± 0.0 ^f	<0.0001

¹ RM, raw milk; PM, pasteurized milk; FC, lactic coagulated fresh cheese; CN, soft mould-ripened Camembert de Normandie; FA, blue-veined cheese Fourme d'Ambert; CA, uncooked pressed cheese Cantal; CO, cooked pressed cheese Comte.

² Results are presented by means of replicates ± SD; n=9.

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

Table 3. Mineral and organic anions content of milks and cheeses processed with different technologies (related to 100 g edible portion)

	Nutrient contents ^{1,2} (mg/100g)							p-value
	RM	PM	FC	CN	FA	CA	CO	
Calcium	117.3 ± 15.0 ^a	113.8 ± 5.8 ^a	105.0 ± 12.3 ^a	222.5 ± 14.0 ^b	396.4 ± 78.9 ^c	668.0 ± 33.2 ^d	761.8 ± 23.7 ^d	<0.0001
Phosphorus	61.0 ± 4.3 ^b	65.0 ± 5.5 ^b	44.8 ± 9.4 ^a	250.4 ± 46.0 ^c	304.8 ± 78.0 ^c	508.7 ± 28.6 ^d	571.4 ± 8.1 ^d	<0.0001
Magnesium	7.8 ± 1.2 ^a	8.3 ± 0.7 ^a	11.5 ± 1.4 ^b	21.0 ± 5.3 ^c	20.6 ± 5.0 ^c	26.5 ± 1.9 ^d	31.1 ± 0.9 ^e	<0.0001
Potassium	114.6 ± 11.1 ^b	126.2 ± 9.3 ^c	94.1 ± 14.3 ^a	129.3 ± 12.3 ^c	93.3 ± 14.3 ^a	93.2 ± 8.0 ^a	83.8 ± 3.1 ^a	<0.0001
Sodium	46.6 ± 3.9 ^a	39.2 ± 4.2 ^a	77.6 ± 21.3 ^b	586.5 ± 67.6 ^c	743.9 ± 93.5 ^d	682.1 ± 62.9 ^d	443.3 ± 153.1 ^c	<0.0001
Chloride	104.0 ± 1.4 ^c	95.3 ± 3.0 ^b	90.2 ± 2.7 ^a	982.3 ± 43.3 ^e	1,665.1 ± 240.2 ^g	1,240.8 ± 113.7 ^f	714.5 ± 242.8 ^d	<0.0001
Lactate	3.3 ± 0.7 ^a	3.0 ± 0.6 ^a	682.7 ± 38.9 ^d	105.2 ± 25.7 ^b	399.9 ± 17.8 ^c	1,204.2 ± 56.8 ^f	873.6 ± 38.5 ^e	<0.0001
Citrate	82.1 ± 2.4 ^d	94.0 ± 3.3 ^e	1.0 ± 0.3 ^a	28.7 ± 4.0 ^c	13.6 ± 1.6 ^b	1.5 ± 0.6 ^a	49.5 ± 19.5 ^c	<0.0001

¹ RM, raw milk; PM, pasteurized milk; FC, lactic coagulated fresh cheese; CN, soft mould-ripened Camembert de Normandie; FA, blue-veined cheese Fourme d'Ambert; CA, hard cheese Cantal; CO, hard cheese Comte.

² Means of replicates ± SD; n=9.

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