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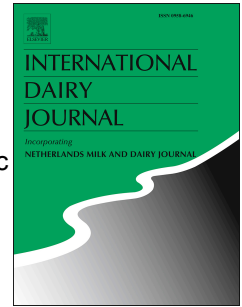
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Credit authorship contribution statement

Abirami Baleswaran: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Review & Editing, Visualization.

Christel Couderc: Methodology, Writing – Review & Editing, Supervision.

Jean Dayde: Writing – Review & Editing, Supervision.

Marine Reyrolle: Formal analysis, Writing – Review & Editing

Mickael Lebecqec: Formal analysis, Writing – Review & Editing

Hélène Tormo: Conceptualization, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Gwenaëlle Jard: Conceptualization, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

1 **Elaboration and characterisation of a miniature soft lactic goat cheese model to mimic a**
2 **factory cheese.**

3

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24

25

26 ABSTRACT

27

28 Although soft lactic goat cheeses are widely produced in France, there is little information
29 available in the literature about them and no miniature model for this kind of cheese has yet been
30 proposed. In this study, a miniature soft lactic goat cheese model was compared with equivalent
31 factory cheese to use it as a reliable model for this cheese family. The composition of the cheeses
32 was compared at four key stages of the production process using microbiological,
33 physicochemical and Sift-MS volatilome analysis. The compositions of the model and factory
34 cheeses were found to be similar until the ripening step, but some differences were observed on
35 ripened cheeses. Moreover, under carefully monitored laboratory conditions, the variability
36 across batches of the model cheese was reduced. This miniature soft lactic goat cheese could
37 therefore be used as a reliable model to study the aspects of the same cheese type.

38

39

40

41 1. Introduction

42

43 Pilot-scale cheesemaking experiments under controlled conditions are costly and time-
44 consuming in contrast to experiments at the laboratory scale (Shakeel-Ur-Rehman, Fox,
45 McSweeney, Madkor, & Farkye, 2001).

46 Protocols for the preparation of miniature cheese models, produced in laboratory-
47 controlled conditions, where key properties of the miniature cheese models resemble those of
48 conventionally manufactured cheeses, have been proposed for fresh cheeses (Hynes, Ogier, &
49 Delacroix-Buchet, 2000), soft cheeses, semi-hard cheeses (Shakeel-Ur-Rehman, McSweeney, &
50 Fox, 1998) and hard cheeses (Antonsson, Ardö, Nilsson, & Molin, 2002; Vélez, Perotti, Rebechi,
51 & Hynes, 2015). However, despite the importance of soft lactic cheeses (simultaneous
52 acidification and rennet coagulation), no comparable procedure has yet been described for them.
53 The microbiological, physicochemical, and sensorial characteristics of a few factory soft lactic
54 goat cheeses have been described (Couderc et al., 2022; Lucas et al., 2006), but not their volatile
55 profiles. Gas chromatography-mass spectrometry (GC-MS) is generally used to identify and
56 quantify volatile compounds, although this approach is costly and time-consuming. Selected ion
57 flow tube-mass spectrometry (Sift-MS), a recently developed alternative approach, allows faster
58 analysis and has already shown promising results for cheese aroma characterisation (Langford,
59 Padayachee, McEwan, & Barringer, 2019; Reyrolle et al., 2022).

60 The purpose of this study was to establish a reliable model for the soft lactic goat cheese
61 family and compare it with its factory equivalent. The compositions of the model and factory
62 cheeses were compared using microbiological, physicochemical and Sift-MS volatilome analysis.

63

64 2. Materials and methods

65

66 2.1. Production of factory and miniature model cheese

67

68 Three batches of raw goats' milk were collected from "Les Fermiers du Rocamadour"
69 (Alvignac, France) at different times of the year to make three batches of miniature models
70 (produced in laboratory-controlled conditions) and factory cheeses. Each batch of milk was split
71 into two parts; 180 L were used directly to factory cheese production at "Les Fermiers du
72 Rocamadour" and 2 L were transferred into 250 mL bottles and frozen at $-20\text{ }^{\circ}\text{C}$ for the
73 subsequent production of the miniature models.

74 Factory cheeses were produced in accordance with Rocamadour PDO cheese
75 specifications (Legifrance, 2014), as followed by Couderc et al. (2022). The process is outlined in
76 Fig. 1A.

77 Model cheese was produced under laboratory conditions based on the factory cheese
78 process (Fig. 1B). Frozen raw goats' milk was thawed for one day at $4\text{ }^{\circ}\text{C}$, pasteurised ($85\text{ }^{\circ}\text{C}$, 5
79 min) and cooled to $35\text{ }^{\circ}\text{C}$. The same concentrations of starter and ripening microorganisms were
80 used as in the factory process. CaCl_2 (Durcigel, Coquard, Villefranche-sur-Saône, France) was
81 added at 0.4 mL L^{-1} and a short maturation period of 45 min at $25\text{ }^{\circ}\text{C}$ was done. Once the pH had
82 decreased by 0.2 units, rennet ($100\text{ mL } 100\text{ L}^{-1}$ of 520 mg L^{-1} chymosin; Coquard) was added.
83 The milk was then distributed into 50 mL tubes and incubated at $25\text{ }^{\circ}\text{C}$ for 22 h.

84 As described in the protocols of previously produced model cheeses, centrifugation was
85 used to separate the curd from the whey (Hynes et al., 2000; Shakeel-Ur-Rehman et al., 1998;
86 Van Tassell, Ibarra-Sánchez, Takhar, Amaya-Llano, & Miller, 2015). To be in accordance with
87 Rocamadour PDO cheese specifications (minimum 31% of dry matter and 0.6–0.8% of salt

88 content; Legifrance, 2014), three conditions of centrifugation and three conditions of salting had
89 previously been tested to select the following parameters (data not shown). The tube was
90 centrifuged for 3 h 30 at 25 °C at 4430 rpm, the whey then discarded and the curd salted with a
91 185 mg mL⁻¹ NaCl brine solution. The curd was placed in a custom-built mould (diameter, 38
92 mm; height, 10 mm, Verres Vagner, Toulouse, France), 3.5 times smaller than those used for the
93 factory cheeses but with the same diameter/height ratio (Fig. 2A).

94 The resulting cheese was then placed in a miniature (175 cm³) ripening cellar with two air
95 inlets to insure proper ventilation (Fig. 2B). The mini cellar was placed in a temperature-
96 controlled refrigerated container. A Petri dish with sterile cotton wool soaked in sterile water was
97 placed at the bottom of the cellar to regulate relative humidity (RH). Temperature and RH were
98 monitored every 2 h during the ripening process using a LogTag recorder (Trix8, LogTag,
99 France) placed in the cellar.

100 All the equipment used were autoclaved or disinfected and UV-treated. Fig. 1B illustrates
101 the two stages of the ripening process: (i) one day of ripening at 13 ± 0.2 °C and 92.8 ± 0.8 % RH
102 and (ii) 6 days at 10 ± 0.1 °C and 94.5 ± 0.5 % RH. The cheeses were turned once a day
103 throughout the ripening process.

104 Samples of the model and factory cheeses were collected at four key stages of the
105 production process: before salting, after salting, on the first day of ripening and at the end of the
106 ripening stage (indicated with asterisks in Fig. 1).

107

108 2.2. *Cheese composition analysis*

109

110 2.2.1. *Physicochemical analysis*

111 Physicochemical analyses were performed on three factory cheeses and three model
112 cheeses stored at 4 °C at all four stages of all three production batches. Dry matter (DM), fat (FC)
113 and salt (S) contents were analysed according to NF EN ISO 5534, NF V04-287 and NF EN ISO
114 5943 standards, respectively.

115 The salt-in-moisture (S/M) and moisture on a fat-free basis (MFFB) contents were
116 calculated as follows:

$$117 \quad S/M = (\%S) / (100 - \%DM)$$

$$118 \quad MFFB = [(100 - \%DM) / (100 - \%FC)] \times 100$$

119 All analyses for the factory cheeses and the fat and salt content measurements for the
120 model cheeses were performed by Agrolabs (Auch, France). The DM and pH measurements for
121 the model cheeses were performed internally. The pH values were measured using a HQ11d pH
122 meter (Hach, France).

123

124 2.2.2. *Microbiological analysis*

125 Microbiological analyses were performed on three ripened factory cheeses and three
126 ripened model cheeses stored at 4 °C of three production batches. Aerobic mesophilic bacteria
127 levels were enumerated on plate count agar (PCA; Biokar, France) supplemented with skimmed
128 milk powder and, yeasts and moulds counts were enumerated on oxytetracycline glucose agar
129 (OGA; Biokar). Serial decimal dilutions in buffered peptone (Biomérieux, France) were prepared
130 and 0.1 mL samples of appropriate dilutions were plated on appropriate media. The PCA plates
131 were incubated at 30 °C for 48 h and the OGA plates were incubated at 25 °C for 5 days. The
132 same analyses were performed in Agrolabs (Auch, France) for the factory cheeses and internally
133 for the model cheeses.

134

135 2.2.3. *Sift-MS analysis*

136 Sift-MS analysis was performed on three ripened factory cheeses and three ripened
137 miniature cheeses from a single production batch, that were vacuum-packed and stored at $-20\text{ }^{\circ}\text{C}$.
138 The sample preparation protocol was inspired by Taylor, Wick, Castada, Kent, and Harper
139 (2013). Five grams ($\pm 0.3\text{ g}$) of ripened cheese was placed in a 1 L bottle and sealed with a
140 Teflon cap. The bottle was then heated at $40\text{ }^{\circ}\text{C}$ for 1 h before Sift-MS analysis to allow the
141 headspace to equilibrate.

142 The VOCs released by the cheese samples were analysed using a Sift-MS Voice 200 Ultra
143 device (Syft Technologies Ltd., New Zealand) using positive soft ionising precursor ions (H_3O^+ ,
144 NO^+ and O_2^+). After each analysis, a blank test was performed using an empty bottle.
145 Immediately after incubation, the headspace of the sample was introduced via a calibrated
146 capillary at a sampling flowrate of 20 mL min^{-1} into the reaction chamber (a flow tube at 120
147 $^{\circ}\text{C}$ and 0.07 kPa) with nitrogen (180 mL min^{-1}) as the carrier gas. The measurements were
148 performed in full scan mode from 15 to 250 m/z with count rates (signal intensity in counts per
149 second) calculated for each m/z unit.

150 The Sift-MS data were acquired and analysed using LabSyft v.1.6.2. (Syft Technologies,
151 New Zealand). Four full mass scans were recorded for each sample but only the last three were
152 analysed.

153 The data were co-added and pre-processed as described by Reyrolle et al. (2022). The
154 VOC profiles of the model and factory cheese samples were compared based on the reagent ion–
155 product ion combinations of 36 VOCs previously identified in goat cheeses (Supplementary
156 material Table S1) (Bontinis, Mallatou, Pappa, Massouras, & Alichanidis, 2012; Delgado,
157 González-Crespo, Cava, & Ramírez, 2011; Hayaloglu, Yasar, Tolu, & Sahingil, 2013).

158 The volatile fingerprints of the model and factory cheeses were compared using the mixOmics R
159 package (Rohart, Singh, Gautier, & Cao, 2017).

160

161 2.3. *Statistical analysis*

162

163 Gross cheese compositions were expressed as means and standard deviations over the three
164 production runs and four sampling stages and compared between the factory and model cheeses
165 using Kruskal-Wallis tests.

166 VOC product ion intensities were compared between the factory and the model cheeses
167 with Wilcoxon signed-rank tests. Homogeneity of variance was verified using Levene's test. The
168 threshold for statistical significance were set at $p < 0.05$.

169

170 **3. Results and discussion**

171

172 Regarding physicochemical parameters (Table 1), fat and salt contents of the model
173 cheeses were similar to those of the factory cheeses at all stages. For the other parameters, they
174 were similar until the first day of ripening. Indeed, during ripening, few differences were
175 recorded between the two types of cheeses concerning the dry matter contents, the moisture on a
176 fat-free basis and the pH values. The lower values of the moisture on a fat-free basis (higher dry
177 matter content) of the model cheeses could be explained by lower relative humidity during
178 ripening. At the end of ripening, the pH value of the model cheeses was also significantly higher
179 than those of the factory cheeses, showing more intense deacidifying microbial activity in the
180 model cheeses. In fact, increases in pH value during ripening can be due to the production of
181 ammonia and various amino acids by proteolysis, and by the consumption of the lactic acid by

182 moulds and yeasts (which are the main deacidifying microorganisms in cheeses) (Bonaiti,
183 Latrille, & Corrieu, 2004; Fox, 1989). This was confirmed by the fact that after ripening, the
184 factory cheeses had substantially lower levels of yeasts, such as *Debaryomyces hansenii*, than the
185 model cheeses (6.60 ± 0.47 versus $2 \log_{10} \text{cfu g}^{-1}$, respectively, Table 2). Nonetheless, the model
186 cheeses' levels of aerobic mesophilic bacteria and *Geotrichum candidum* were comparable with
187 those of the factory cheeses. The different microbiological compositions of cheeses could explain
188 the rise of the pH value that was more important in the model cheeses during ripening (+0.7
189 versus +0.3 units of pH for the model and factory cheeses, respectively). Moreover, this
190 observation was not surprising since in the case of model cheeses, the microbial contamination of
191 milk and environment are almost negligible (pasteurised milk, sterile mini cellar for the ripening)
192 compared with factory production conditions. These microorganisms, and especially *G.*
193 *candidum* that could be present in the factory ripening room's environment, may play an
194 important role in the inoculation of the fresh cheese. *G. candidum*'s high and early presence in
195 factory cheeses may make it harder for other yeasts to develop during ripening. Indeed, it is
196 known that *G. candidum* could inhibit *D. hansenii* during ripening (Mounier et al., 2008).

197 The volatile fingerprints of the factory and model cheeses, given by the average intensities
198 of 119 product ions (associated with the 36 selected VOCs, Supplementary material Table S1),
199 are compared in Fig. 3. Only 21/119 product ions intensities differed significantly between the
200 factory and model cheeses ($p < 0.05$, Wilcoxon tests, indicated by asterisks in Fig. 3 and
201 Supplementary material Table S1), showing a relatively similar cheese VOCs composition.
202 Moreover, a significant heterogeneity of variance between samples was observed for 3/119 model
203 cheeses product ions and 9/119 factory cheeses product ions ($p < 0.05$, Levene test,
204 Supplementary material Table S2). Therefore, the level of repeatability is higher on model

205 cheeses than on factory cheeses, certainly due to pasteurised milk used to make the model
206 cheeses.

207 The gross composition of the ripened model cheese presented in our study is similar to that
208 of soft lactic cheeses previously characterised (Gaborit, Menard, & Morgan, 2001; Raynal-
209 Ijutovac, Pape, Gaborit, & Barrucand, 2011; Sablé, Portrait, Gautier, Letellier, & Cottenceau,
210 1997) and could therefore be used as a reliable model for this cheese type, for which a model has
211 never been described in literature.

212

213 **4. Conclusion**

214

215 In this study, a miniature soft lactic goat cheese model was elaborated and compared with
216 a factory cheese, based on microbiological, physicochemical and Sift-MS volatilome analysis.
217 The model cheese has a similar composition to the factory cheese until ripening and differed on
218 non-*Geotrichum candidum* yeasts, moisture on a fat-free basis levels and pH values for ripened
219 cheese. During ripening, less variability was recorded in the model cheese between production
220 batches compared with the factory cheese. This model should therefore facilitate future studies on
221 the impact of various factors on quality of soft lactic cheeses.

222

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224

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228

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Figure legends

Fig. 1. Production flow charts for (A) factory and (B) miniature model soft lactic goat cheeses; asterisks (*) indicate sample collection stages.

Fig. 2. Photographs of (A) a miniature model cheese and (B) the miniature cheese ripening cellar.

Fig. 3. Volatile fingerprints of factory (blue) and miniature model (orange) cheese. Radar plots of the Sift-MS intensities of 119 product ions associated with 36 selected goat cheese VOCs described in the literature (for better readability, only a subset of the ions are labelled). Peak intensities correspond to production ion abundance on a logarithmic scale. The dashed lines and shaded areas correspond to means (blue, factory; orange, model) and standard deviations, respectively. The asterisks correspond to 21 product ions differing significantly between factory and model cheese.

Table 1

Physicochemical characteristics of miniature model and factory cheese at four production stages. ^a

Parameter	Cheese type	Before salting	After salting	1 st day of ripening	End of ripening
Dry matter (%)	Factory	38.3 ± 2.5 ^a	36.9 ± 2.0 ^a	36.8 ± 1.7 ^a	38.2 ± 2.6 ^a
	Model	38.8 ± 2.2 ^a	39.6 ± 1.7 ^a	40.7 ± 1.1^b	42.1 ± 0.8^b
Fat content (%)	Factory	19.8 ± 1.5 ^a	18.6 ± 1.4 ^a	18.4 ± 1.6 ^a	19.8 ± 2.6 ^a
	Model	18.4 ± 2.7 ^a	18.8 ± 0.9 ^a	19.1 ± 0.6 ^a	21.0 ± 0.8 ^a
Salt content (%)	Factory	nd	0.9 ± 0.1 ^a	0.9 ± 0.1 ^a	1.0 ± 0 ^a
	Model	nd	0.9 ± 0.1 ^a	0.9 ± 0.1 ^a	1.1 ± 0.1 ^a
Salt-in-moisture (%)	Factory	nd	1.4 ± 0.2 ^a	1.3 ± 0.1 ^a	1.6 ± 0.1 ^a
	Model	nd	1.6 ± 0 ^a	1.6 ± 0^b	1.8 ± 0^b
Moisture on fat-free basis (%)	Factory	77.0 ± 1.8 ^a	77.5 ± 1.2 ^a	77.4 ± 0.8 ^a	77.0 ± 1.0 ^a
	Model	75.2 ± 1.4 ^a	74.3 ± 1.2^b	73.2 ± 0.9^b	73.3 ± 0.2^b
pH	Factory	4.4 ± 0 ^a	4.4 ± 0 ^a	4.4 ± 0 ^a	4.7 ± 0.3 ^a
	Model	4.4 ± 0.1 ^a	4.5 ± 0.1 ^a	4.5 ± 0 ^a	5.2 ± 0.1^b

^a Data are expressed as mean ± standard deviation (n = 3); different superscript letters indicate significant differences between factory and model samples ($p < 0.05$); values in bold for the model cheese are significantly different from those measured for the factory cheese at the same stage.

Table 2

Microbial counts in the miniature model and factory cheese at the end of the ripening stage.^a

Microorganism	Cheese type	Counts
Aerobic mesophilic bacteria level	Factory	8.92 ± 0.10 ^a
	Model	9.11 ± 0.45 ^a
<i>Geotrichum candidum</i>	Factory	8.99 ± 2.01 ^a
	Model	7.89 ± 0.03 ^a
Other yeasts	Factory	<2 ^a
	Model	6.60 ± 0.47^b

^a Data (in log₁₀ cfu g⁻¹) are expressed as mean ± standard deviation (n = 3); different superscript letters indicate significant differences between factory and model samples (*p* < 0.05); values in bold for the model cheese are significantly different from those measured for the factory cheese at the same stage.

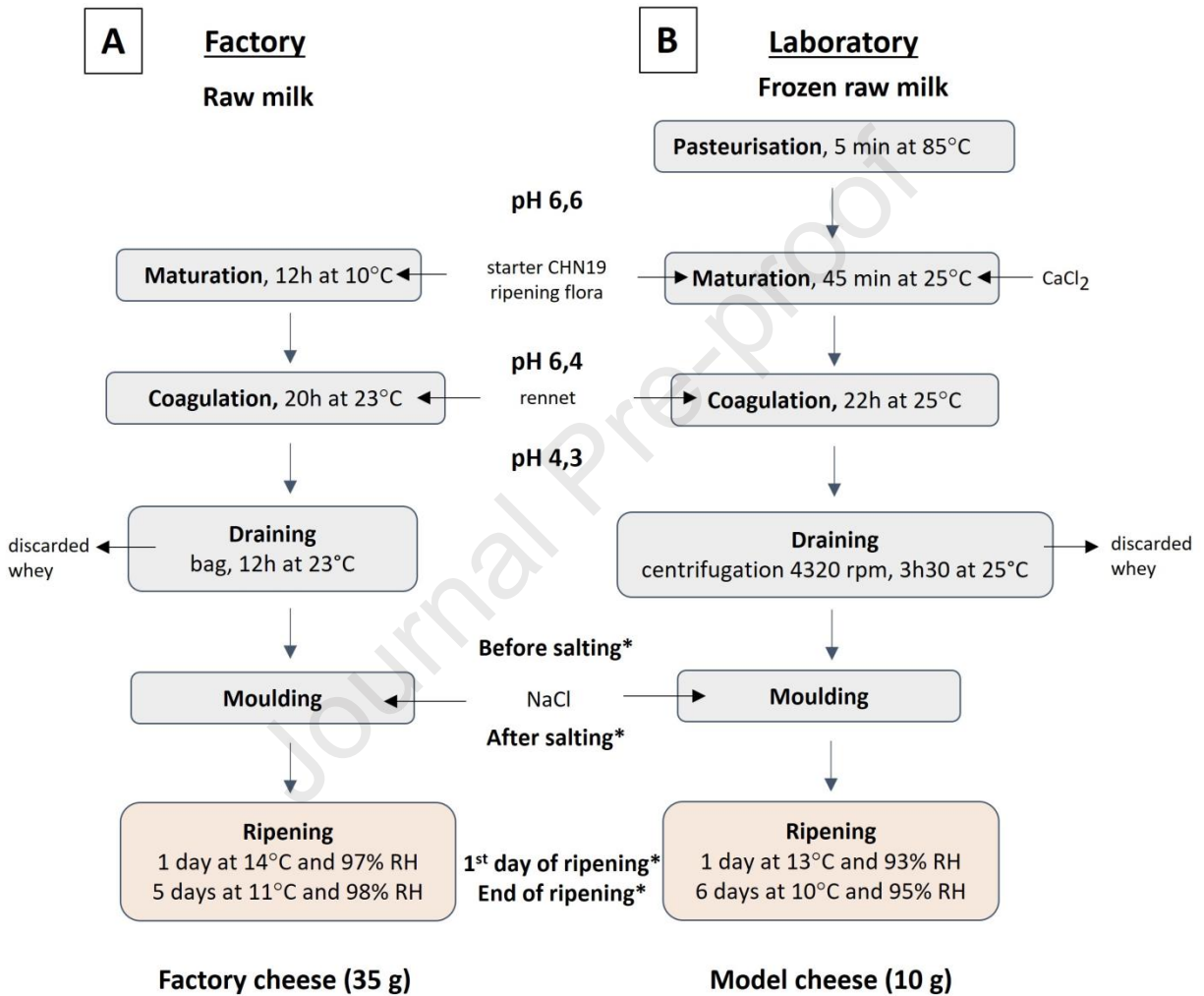


Figure 1

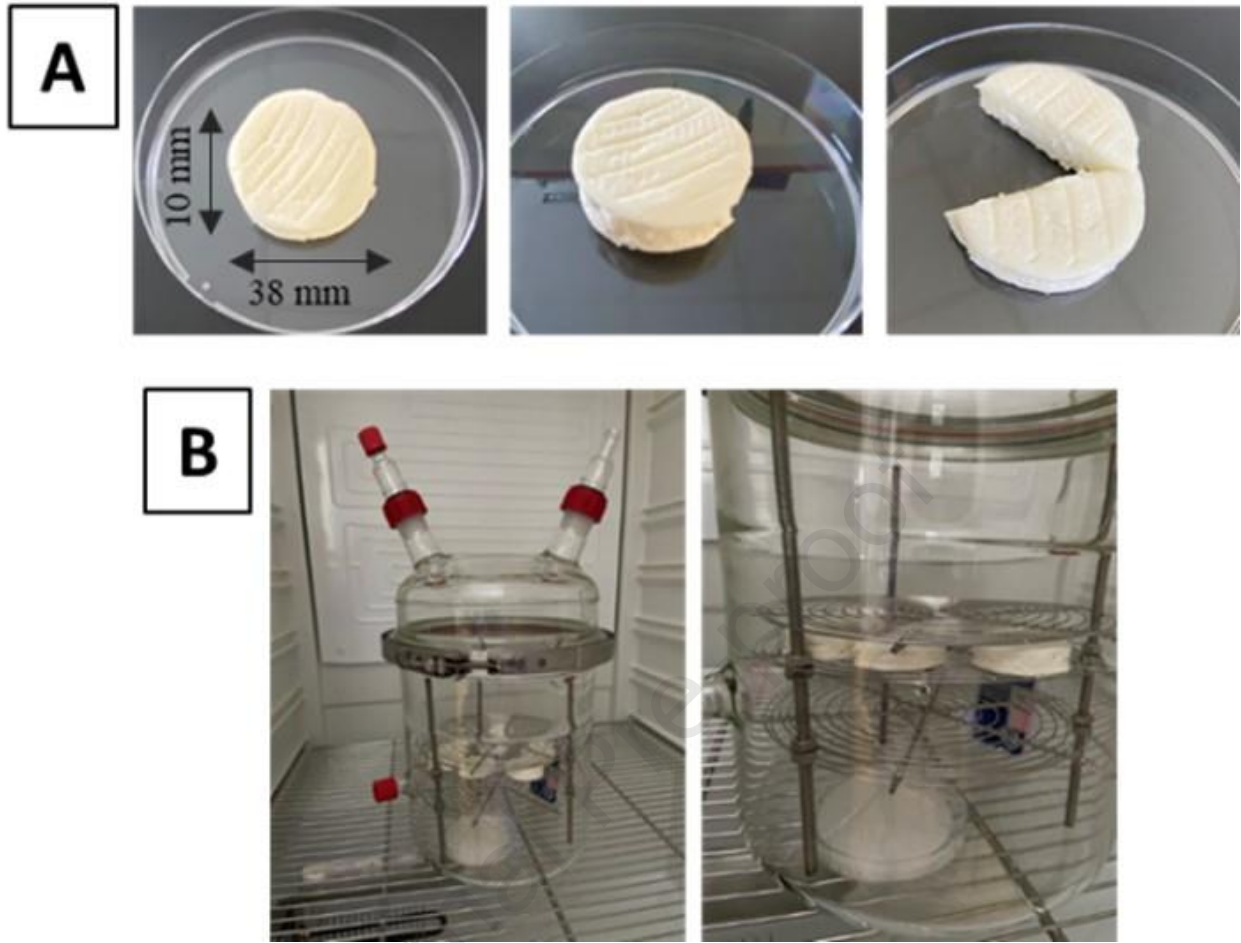


Figure 2

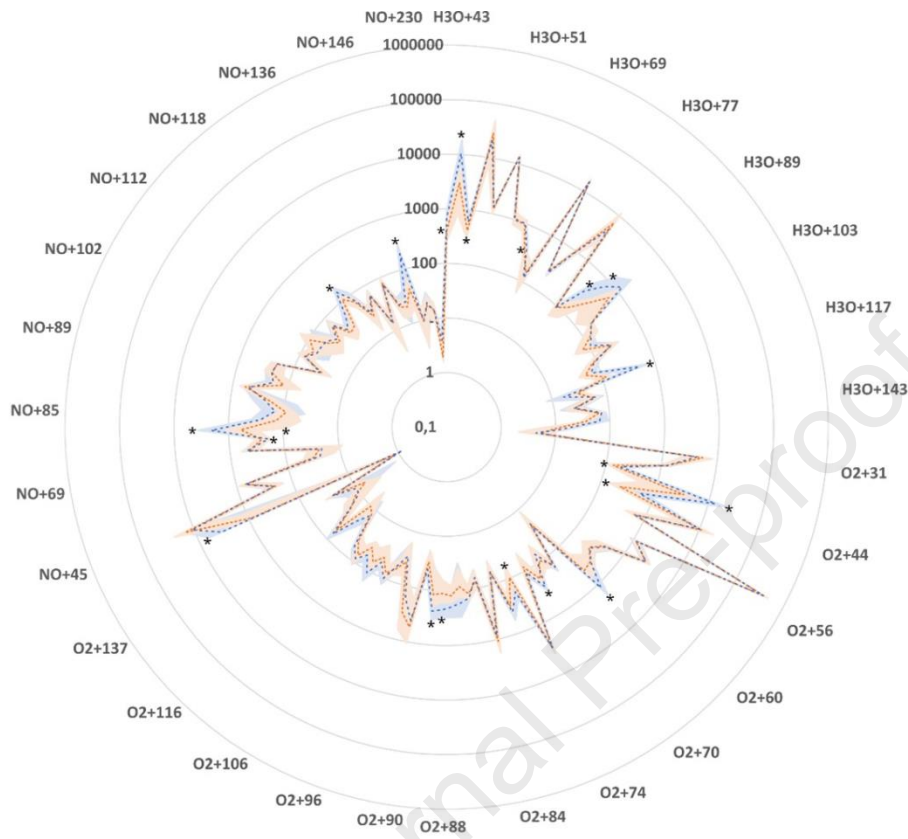


Figure 3

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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