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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 Lebechec: Formal analysis, Writing – Review & Editing

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

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

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

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

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

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2.1. Production of factory and miniature model cheese

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

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

Model cheese was produced under laboratory conditions based on the factory cheese process (Fig. 1B). Frozen raw goats' milk was thawed for one day at 4 °C, pasteurised (85 °C, 5 min) and cooled to 35 °C. The same concentrations of starter and ripening microorganisms were used as in the factory process. CaCl₂ (Durcigel, Coquard, Villefranche-sur-Saône, France) was added at 0.4 mL L⁻¹ and a short maturation period of 45 min at 25 °C was done. Once the pH had decreased by 0.2 units, rennet (100 mL 100 L⁻¹ of 520 mg L⁻¹ chymosin; Coquard) was added. The milk was then distributed into 50 mL tubes and incubated at 25 °C for 22 h.

As described in the protocols of previously produced model cheeses, centrifugation was used to separate the curd from the whey (Hynes et al., 2000; Shakeel-Ur-Rehman et al., 1998; Van Tassell, Ibarra-Sánchez, Takhar, Amaya-Llano, & Miller, 2015). To be in accordance with 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 cm ³) 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 \pm 0.2 $^{\circ}C$ and 92.8 \pm 0.8 % RH
102	and (ii) 6 days at 10 \pm 0.1 °C and 94.5 \pm 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).
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108	2.2. Cheese composition analysis
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110	2.2.1. Physicochemical analysis

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

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

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

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

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

2.2.2. Microbiological analysis

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

2.2.3. Sift-MS analysis

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Sift-MS analysis was performed on three ripened factory cheeses and three ripened miniature cheeses from a single production batch, that were vacuum-packed and stored at -20 °C. The sample preparation protocol was inspired by Taylor, Wick, Castada, Kent, and Harper (2013). Five grams (± 0.3 g) of ripened cheese was placed in a 1 L bottle and sealed with a Teflon cap. The bottle was then heated at 40 °C for 1 h before Sift-MS analysis to allow the headspace to equilibrate. The VOCs released by the cheese samples were analysed using a Sift-MS Voice 200 Ultra device (Syft Technologies Ltd., New Zealand) using positive soft ionising precursor ions (H₃O⁺, NO⁺ and O₂⁺). After each analysis, a blank test was performed using an empty bottle. Immediately after incubation, the headspace of the sample was introduced via a calibrated capillary at a sampling flowrate of 20 nmL min⁻¹ into the reaction chamber (a flow tube at 120 °C and 0.07 kPa) with nitrogen (180 nmL min⁻¹) as the carrier gas. The measurements were performed in full scan mode from 15 to 250 m/z with count rates (signal intensity in counts per second) calculated for each m/z unit. The Sift-MS data were acquired and analysed using LabSyft v.1.6.2. (Syft Technologies, New Zealand). Four full mass scans were recorded for each sample but only the last three were analysed. The data were co-added and pre-processed as described by Reyrolle et al. (2022). The VOC profiles of the model and factory cheese samples were compared based on the reagent ion product ion combinations of 36 VOCs previously identified in goat cheeses (Supplementary material Table S1) (Bontinis, Mallatou, Pappa, Massouras, & Alichanidis, 2012; Delgado, González-Crespo, Caya, & Ramírez, 2011; Hayaloglu, Yasar, Tolu, & Sahingil, 2013).

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

2.3. Statistical analysis

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

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

3. Results and discussion

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

moulds and yeasts (which are the main deacidifying microorganisms in cheeses) (Bonaiti,
Latrille, & Corrieu, 2004; Fox, 1989). This was confirmed by the fact that after ripening, the
factory cheeses had substantially lower levels of yeasts, such as Debaryomyces hansenii, than the
model cheeses (6.60 ± 0.47 versus 2 log_{10} cfu $g^{\text{-}1}$, respectively, Table 2). Nonetheless, the model
cheeses' levels of aerobic mesophilic bacteria and Geotrichum candidum were comparable with
those of the factory cheeses. The different microbiological compositions of cheeses could explain
the rise of the pH value that was more important in the model cheeses during ripening (+0.7
versus +0.3 units of pH for the model and factory cheeses, respectively). Moreover, this
observation was not surprising since in the case of model cheeses, the microbial contamination of
milk and environment are almost negligible (pasteurised milk, sterile mini cellar for the ripening)
compared with factory production conditions. These microorganisms, and especially G .
candidum that could be present in the factory ripening room's environment, may play an
important role in the inoculation of the fresh cheese. G. candidum's high and early presence in
factory cheeses may make it harder for other yeasts to develop during ripening. Indeed, it is
known that G. candidum could inhibit D. hansenii during ripening (Mounier et al., 2008).
The volatile fingerprints of the factory and model cheeses, given by the average intensities
of 119 product ions (associated with the 36 selected VOCs, Supplementary material Table S1),
are compared in Fig. 3. Only 21/119 product ions intensities differed significantly between the
factory and model cheeses (p <0.05, Wilcoxon tests, indicated by asterisks in Fig. 3 and
Supplementary material Table S1), showing a relatively similar cheese VOCs composition.
Moreover, a significant heterogeneity of variance between samples was observed for 3/119 model
cheeses product ions and $9/119$ factory cheeses product ions ($p < 0.05$, Levene test,
Supplementary material Table S2). Therefore, the level of repeatability is higher on model

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

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

4. Conclusion

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

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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 1Physicochemical 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 Model	38.3 ± 2.5^{a} 38.8 ± 2.2^{a}	36.9 ± 2.0^{a} 39.6 ± 1.7^{a}	36.8 ± 1.7^{a} 40.7 ± 1.1 ^b	38.2 ± 2.6^{a} 42.1 ± 0.8 ^b
Fat content (%)	Factory Model	19.8 ± 1.5^{a} 18.4 ± 2.7^{a}	$18.6 \pm 1.4^{a} \\ 18.8 \pm 0.9^{a}$	18.4 ± 1.6^{a} 19.1 ± 0.6^{a}	$19.8 \pm 2.6^{a} \\ 21.0 \pm 0.8^{a}$
Salt content (%)	Factory Model	nd nd	$0.9 \pm 0.1^{a} \ 0.9 \pm 0.1^{a}$	$0.9 \pm 0.1^{a} \ 0.9 \pm 0.1^{a}$	1.0 ± 0^{a} 1.1 ± 0.1^{a}
Salt-in-moisture (%)	Factory Model	nd nd	1.4 ± 0.2^{a} 1.6 ± 0^{a}	1.3 ± 0.1^{a} 1.6 ± 0^{b}	1.6 ± 0.1^{a} 1.8 ± 0^{b}
Moisture on fat-free basis (%)	Factory Model	$77.0 \pm 1.8^{a} 75.2 \pm 1.4^{a}$	77.5 ± 1.2^{a} 74.3 ± 1.2^{b}	77.4 ± 0.8^{a} 73.2 ± 0.9^{b}	77.0 ± 1.0^{a} 73.3 ± 0.2^{b}
pН	Factory Model	4.4 ± 0^{a} 4.4 ± 0.1^{a}	4.4 ± 0^{a} 4.5 ± 0.1^{a}	$4.4 \pm 0^{a} \\ 4.5 \pm 0^{a}$	4.7 ± 0.3^{a} 5.2 ± 0.1 ^b

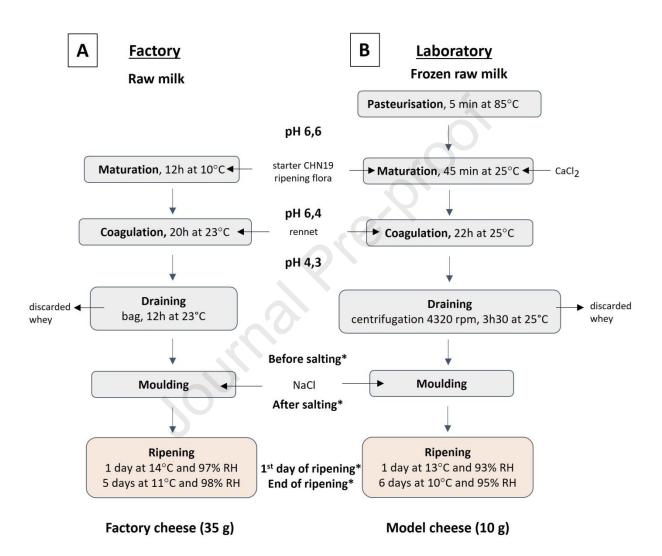
^a Data are expressed as mean \pm standard deviation (n = 3); different superscript letters indicate significant differences between factory and model samples (p <0.05); values in bold for the model cheese are significantly different from those measured for the factory cheese at the same stage.

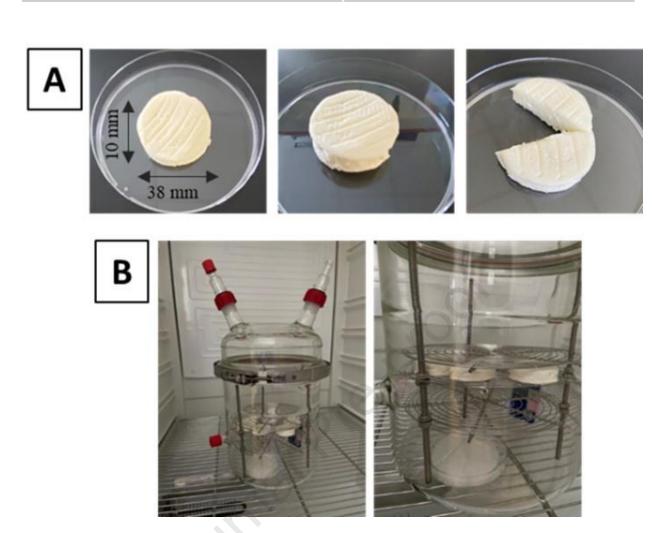
Table 2

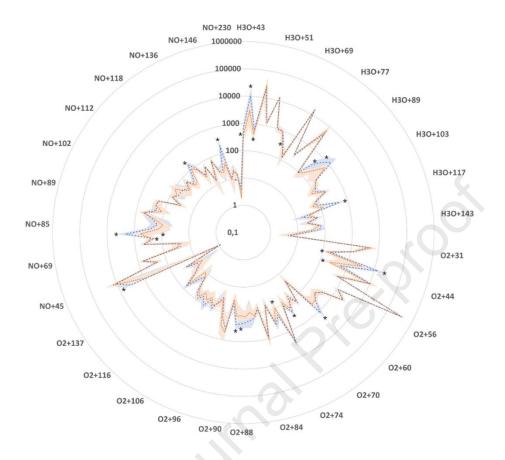
Microbial counts in the miniature model and factory cheese at the end of the ripening stage.^a

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

^a Data (in \log_{10} cfu g⁻¹) are expressed as mean \pm 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.







Declaration of interests

oxtimes 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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