Impact of environmental conditions on the ripening of Queijo de Évora PDO cheese

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Abstract ‘‘Queijo de Évora" is a traditional Portuguese cheese from raw ewe's milk and granted with PDO label. It is ripened traditionally in rooms with empirical control of temperature and humidity. Nowadays, almost all cheese factories use rooms with temperature and humidity control, but still a significant heterogeneity among cheeses is acknowledged due to unequal distribution of environmental conditions. This paper discusses the influence of the environmental conditions on the ripening of Queijo de Évora, including the application of computational fluid dynamics in steady state conditions. Experimental data was obtained in cheeses ripened along the traditional ripening cycle, in different locations. A significant influence of environmental conditions was observed, especially air velocity and humidity, affecting physical-chemical, microbiological and sensory characteristics. Locations with higher air velocity, presented cheeses with lower moisture content, higher mesophilic bacteria count, darker appearance and higher number of holes. Locations with higher humidity presented cheeses with lower scores on some sensorial parameters like appearance, firmness and intensity of odor. The results of computational fluid dynamics made possible the identification of areas in and around the cheese stacks were the air distribution is less than adequate or uneven, which may influence the evolution of cheese during ripening.

Keywords Cheese • Ripening • Queijo de Évora • CFD • Sensorial

Introduction

‘‘Queijo de Évora" is a traditional Portuguese cheese granted with Protected Designation of Origin (PDO) label (Council Regulation (EEC) No. 1107/1996) and characterised by a semi hard texture, yellowish-coloured paste, intense flavour and a peculiar slightly bitter taste. It is produced in the center of Alentejo region (south Portugal) from raw ewes' milk, using an aqueous infusion of the dried flowers from *Cynara cardunculus* L. as coagulant. The cheese making process involves filtration of whole ewe's raw milk through a fine cloth, followed by heating to 30-31 ^{O}C in open steel vats. Later, salt and C. *cardunculus* aqueous infusion is added for the coagulation (Gomes et al. 2019). After coagulation for ca. 40 min at 32 ^{O}C, the coagulum is cut and left for 15 min until the syneresis process is completed. The whey is then drained and the curd is transferred to microperforated plastic moulds, which have been previously lined with a cloth. The subsequent pressing stage extends for 45 min, after which cheeses are ripened for a minimum of 30 or 90 days (Despacho No. 29/1994). Actually, a large heterogeneity is observed inside the ripening room of such kind of traditional cheeses but there is few published information about

this effect over the quality of cheese and, even today, the regulation of atmospheric composition of ripening chambers remains mostly empirical (Picque et al. 2006).

Since mid-1980s, the development of more accessible, more powerful computers and user-friendly codes has taken computational fluid dynamics (CFD) from the development stage to its actual status as a viable alternative in all industries. Previous studies of CFD applied to cheese ripening rooms included the study of the blowing duct on the ventilation profile (Mirade et al. 2006), prediction of air velocity and circulation of CO_2 (Mirade and Daudin 2006) and impact of sequential ventilation on energy consumption (Picque et al. 2009). In all these works, CFD technique has presented itself as reliable and well-suited tool for testing low cost new technical solutions designed for improving food processes, while saving time in relation to an experimental approach (Mirade et al. 2006; Mirade and Daudin 2006).

The aim of this study was to evaluate the impact of local environmental conditions on physical, chemical, microbiological and sensorial properties of ‘‘Queijo de Évora" PDO cheese. After the installation of the sensors inside a ripening room, experimental investigations were performed during ripening time, combined with a CFD simulation to assess the air flow pattern inside the ripening room and, thus, evualuate the impact of environmental conditions over cheese.

Materials and methods

Cheesemaking

A batch of 100 cheeses was produced using 84 L of ewe's raw milk, curdled with 50 mL of an aquous solution of 2 g thistle flower (Cynara cardunculus L.). The coagulation was made at 28 °C for 60 min. After, coagulum was cut randomly, whey was drained gently and the curd was placed inside plastic molds. The average dimensions of each cheese were 7 cm diameter, 5 cm height and with an average weight around 120 g. Cheeses were ripened for 25 days inside a ripening room with the dimensions 4.85 m length, 3.16 m width and 2.30 m height and placed in metallic stacks (1.05 m x 0.74 m x 1.96 m), with 14 racks each (Fig. 1a). The locations for cheese ripening were selected based on instrumental measurement of temperature, humidity and air velocity inside the ripening room. After, four location were identified as ‘‘top right" (TR), ‘‘floor right" (FR), ‘‘top left" (TL) and ‘‘floor left" (FL), as presented in Table 1 and marked with (+) in Fig. 1b.

The refrigeration system was composed by an evaporator installed in one end of the room, then two metal ducts placed along ceiling blown air into the room through eight rectangular 45 cm 9 15 cm openings, marked with blue color in Fig. 1b. The suction of air from the room was made by twelve circular 16 cm 0 openings placed in a metal duct running along the centre of the room into the evaporator, marked with red color in Fig. 1b.

Instrumentation of the ripening chamber

The architecture of the system included four portable modules, placed in the locations identified in Table 1, and an aggregator module that collected the data recorded by the local modules every 30 min, transmitting it afterwards to a cloud-based system for recording and processing. Each local module included sensors for temperature and humidity (model DHT22, Aosong Electronics, Guangzhou, China) and air velocity (model F660, DegreeC, Milford, USA), connected to a computer hardware platform (model WiPy 3.0, PyCom, Guildford, UK). The aggregator module (model Pi 3+, Raspberry, Cambridge, UK) was remotely controlled using Raspbian operating system (Raspberry, Cambridge, UK). The MQTT protocol (Hillar 2017) was used for transmitting the data from local modules to a remote control system.

CFD modelling

From the geometrical configuration of the ripening room presented before, a hybrid mesh (i.e., including hexahedral and tetrahedral elements) was created using software Ansys Meshing (Ansys, Canonsburg, USA) and containing a total of 7,500,000 elements and 3,200,000 nodes. An hexaedral mesh was considered for the volume inside the stacks, while a tetrahedral mesh was considered for the remaining volume of the ripening room. During simulations, airflow was considered steady, incompressible and turbulent. The simulation used k-Ɛ model, as it is a multipurpose, typical model successfully employed in such cases, without added computational cost. The first-order upwind discretization scheme was selected because no false diffusion and convergence were expected in this type of model, while allowing for a good convergence time. The domain inside the stacks of cheese was considered as a porous medium, coupled with the Ergun equation, according to Mirade et al. (2006). Equation 1 was used for estimating viscous resistance (α), where D_p is the characteristic length of each cheese (considered 7 cm in this study) and evf is the void fraction (considered 0.564 in this study). Equation 2 was used for calculating inertial resistance (C_2), both equations used to set up the medium porosity configuration.

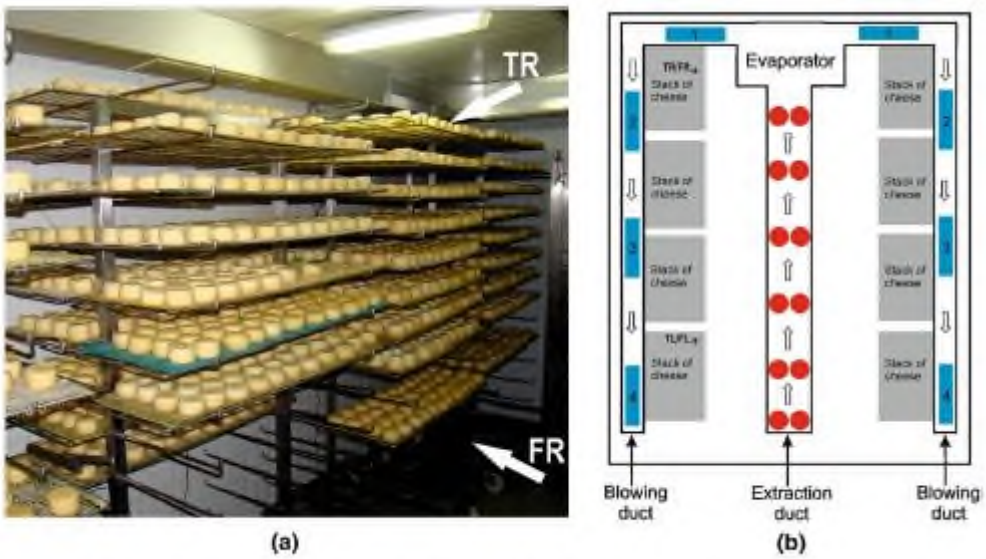

Fig. 1 Representation of ripening room according to: **a** inside view, and **b** top view

Table 1 Distances of selected test locations inside ripening room

Location	Distance to ceiling (m)	Distance to floor (m)	Pipe length to evaporator (m)	Temperature (°C)	Humidity (%)	Air velocity (m/s)
FL	1.93	0.23	3.75	12.9^{c}	76.5^{d}	0.37^{bc}
				(0.5)	(3.9)	(0.32)
TL	0.37	1.79	3.75	13.4^{d}	72.4^{c}	0.41^{c}
				(2.1)	(9.5)	(0.34)
FR	1.93	0.23	1.00	12.0^{a}	69.5^{b}	0.23^{ab}
				(0.7)	(4.7)	(0.22)
TR	0.37	1.79	1.00	12.7^{b}	47.5^{a}	0.14^{a}
				(1.1)	(4.6)	(0.02)

$^{a,b,c,...}$ Means in the same column marked with different letters are significantly different ($p < 0.05$, Scheffé test). Standard deviation is presented between parentheses

$$\alpha = \frac{D_p^2}{150}\frac{\varepsilon_{vf}^3}{\left(1-\varepsilon_{vf}\right)^2} \quad (1)$$

$$C_2 = \frac{3.5\left(1-\varepsilon_{vf}\right)}{D_p}\frac{}{\varepsilon_{vf}^3} \quad (2)$$

The velocities and temperatures of each inlet (Fig. 1) were measured instrumentally using a portable datalogger (model Ami 300 Multifunction, Kimo Instruments, France). The measured air velocity through the inlet openings (Fig. 1b) were 1.8 m/s (inlet 1), 2.4 m/s (inlet 2), 4.5 m/s (inlet 3) and 1.8 m/s (inlet 4). The measured temperature was 5 ± 0.1 ^{O}C in each inlet. The air direction was considered normal to boundary surface. The turbulent intensity for all inlets was 5% and turbulent ratio 10, as per default. The boundary conditions for the room walls were set to adiabatic. The generation of heat and humidity inside the domain of stacks was addressed using a dedicated domain with estimated porosity (56.4%), along with values for its inertial and viscous resistance. This domain was set with two source terms to generate humidity (3 kg/m^3 s) and heat (10 W/m^3) from cheeses, based on available literature (Mirade 2007). The numerical simulations were performed using software Fluent v17.1 (Ansys, Canonsburg, USA) and hardware Xeon E3-1241 3.50 GHz processor, 16 GB DDR3 RAM memory, GPU NVIDIA Quadro K2200 4 GB graphic card. The convergence residues for simulations was 0.001 concerning continuity, turbulent kinetic energy (k), energy dissipation (Ɛ) and velocity of the x, y and z vector components. Computation time was about 150 h.

Physical and chemical analysis

The moisture was performed according to ISO 5534 (2004), titrable acidity was performed according to AOAC official method 920.24 (AOAC 1997) and pH was evaluated with a penetration electrode at 20 ± 1 °C (model 691, Metrohm, Herisau, Switzerland). Small amplitude oscillatory measurement

was performed, at 20 ± 1 °C, using a controlled shear-strain rheometer (Malvern Kinexus lab+, England) according to Alvarenga et al. (2011), where storage modulus G′ at 1 Hz (in kPa) was measured. Texture analysis was performed using a texture analyser TA.XT Plus100 (Stable Micro Systems, Godalming, UK), according to Alvarenga et al. (2008), where hardness (in N) and adhesiveness (in - N.s) were measured. The digital image acquisition was made according to Dias et al. (2018). The percentage of the area occupied by gas holes (in %) was estimated based on the area of black holes and the total area of the slice of cheese, similar to Caccamo et al. (2004). The colour of rind and core was analysed with a portable colorimeter (model CR300, Minolta, Tokyo, Japan) according to Dias et al. (2018). Analysis were performed in triplicate. Whiteness index (WI) was calculated using Eq. 3 (Dias et al. 2018):

$$WI = 100 - \left[(100 - L)^2 + a^2 + b^2\right]^{0.5} \quad (3)$$

Microbiological analysis

For the microbial counts, cheese samples (10 g each) were transferred aseptically to sterile stomacher bags with 90 mL of sterile sodium citrate (GPR) solution (2% w/v) and homogenised in a stomacher instrument (model Lab Blender 400, Seward Lab, London, UK) for 120 s at room temperature. Each homogenate was serially diluted with Ringer solution and duplicate 1 mL or 0.1 mL samples of appropriate dilutions were poured or spreaded on total or selective agar plates. Unless otherwise stated, all media and supplements were Biokar Diagnostics (Pantin, France).

Total mesophilic bacteria were estimated on Plate Count Agar (PCA) after 48 h of incubation at 30 °C. The total mesophilic lactic acid bacteria (LAB) were counted on de Man, Rogosa and Sharpe (MRS) agar acidified to pH 5.6, after 72 h at 30 °C. Enterococci, typical pink or dark red colonies with a narrow whitish border, were counted on Slanetz and Bartley (SB) agar at 37 °C for 48 h, Enterobacterias rose-coloured colonies surrounded by a halo of purple precipitate (Gram-negative and oxidase negative), were estimated on Violet Red Bile Glucose Agar (VRBG) after 24 h at 37 °C. Yeasts were quantified in Rose Bengal Chloramphenicol Agar Plates (RBC), at 25 °C for 72 h. Analysis for each batch were performed in triplicate.

Sensory analysis

Sensory assessment of sample cheeses, with 25d ripening time, was performed by 20 trained panellists (13 females and 7 males), recruited and screened according to international standards ISO 8586-1 (1993) and ISO 8589 (1998). All members were regular consumers of cheese, presenting a high level of discrimination, sensitivity and consistency. Samples were wedge-shaped, without rind, placed in individual closed Petri dishes and randomly coded with a three-digit numbers. Panellists evaluated cheese samples on a 7-points continuous scale, from the locations identified in Table 1 for colour outside (from white to yellowish), colour inside (from white to yellowish), holes inside (from no holes to many holes), firmness (from soft to hard), unctuosity (from low to high), intensity of odor (from low to high) and intensity of flavour (from low to high). All cheese samples used in sensory analysis fulfilled the microbiological criteria of Commission Regulation (EC) No. 2073/2005.

Statistical analysis

The average, standard deviation, and 0.95 confidence interval values were determined. Experimental data was subjected to one-way ANOVA (pairwise comparison of means with Scheffé test) in order to compare the average values of different samples, using software Statistica 6.0 (StatSoft, Tulsa, USA).

Results

Environmental parameters

The measured temperature inside ripening room ranged from 12.0 to 13.4 °C (Table 1). Lower temperature values were observed in the profile along cross-section at 1.0 m from the evaporator ($p < 0.05$), due to the shorter pipe length (Table 1). The measured air velocity is represented in Table 1. The highest value was observed in location TL, while the lowest value was observed in location TR ($p < 0.05$). The inlet air was blown vertically downwards from the rectangular openings placed along the blowing duct (Fig. 1b) and, according to the model, the vertical profile of the air velocity presented higher velocity next to such openings, regardless the distance from the evaporator (Figs. 2 and 3).

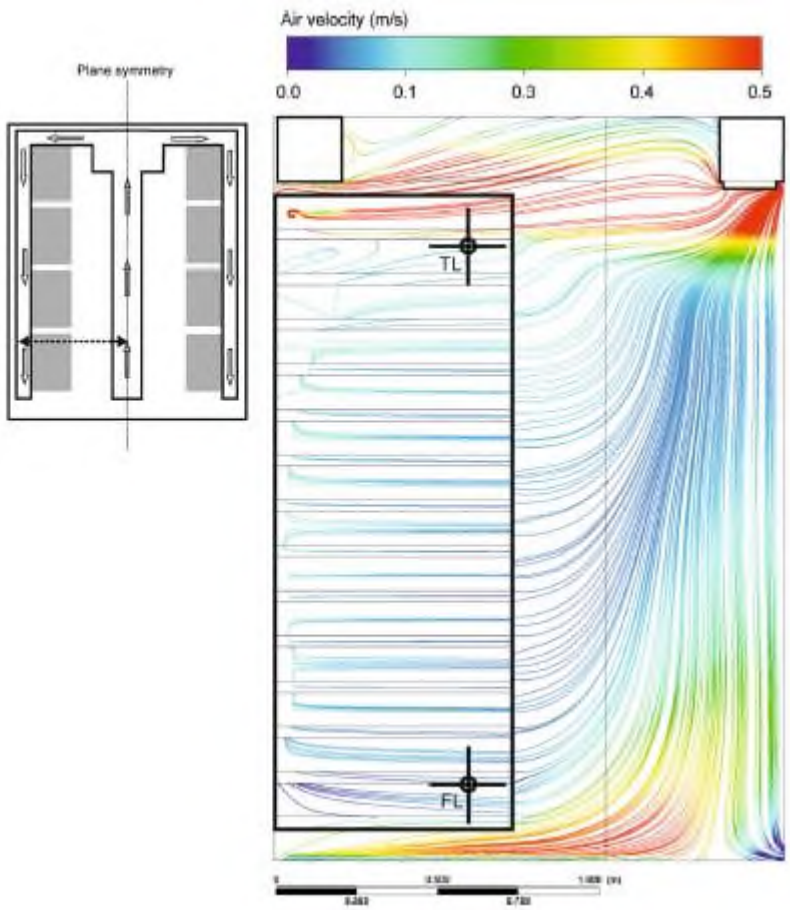

Fig. 2 Representation of streamlines on the vertical cross-section at 3.75 m from evaporator

In fact, predicted air velocity for locations TR and TL were quite similar (around 0.1-0.2 m/s), which can be due to low load losses inside blowing duct. At floor level, airstream curves into the central area of the room, presenting values around 0.4-0.5 m/s (Figs. 2 and 3), then air directs upwards into the extraction duct and into the evaporator (Fig. 2). Such air velocity field creates a swirl in the inner area of the stacks, especially visible in the cross-section at 1.0 m, with velocity around 0-0.2 m/s (Fig. 3). Similar profile was observed previously by Mirade et al. (2006), where blowing ducts with 20 mm diameter created a similar stagnation zone in the inner core of stacks.

The measured humidity presented a high range of values, from 47.5% (location TR) to 76.5% (location FL), as observed in Table 1. This uneven distribution may create extreme dry or damp areas and both situations are undesirable, as they may be potentially harmful to ripening process (Picque et al. 2009).

Physical-chemical results

The moisture of cheese at 0d was 63.9% (Table 2) and decreased during ripening time in all tested locations, caused by the natural loss of moisture and affected by the air velocity (Leclercq-Perlat et al. 2019; Delgado et al. 2009). This effect is governed by the difference between the partial vapour pressure at the surface of the cheese and in the air, depending on the relative humidity inside the ripening room and the water activity (a_w) at the surface of the cheese (Riahi et al. 2007). At 25d, moisture ranged from 19.4 to 31.5%. The lowest moisture values, at 25d, were observed at location TR ($p < 0.05$), while the highest values were observed in location FL (Table 2). This difference is directly related with the distribution of humidity inside ripening room, affecting the mass loss of cheeses. Similar effect has been observed previously in Picodon cheese (Leclercq-Perlat et al. 2019). The wide range of moisture values observed at 25d is an indicator of the heterogeneity of the environmental conditions inside ripening room, also stated in previous works and claimed to be a cause of quality losses (Mirade et al. 2006).

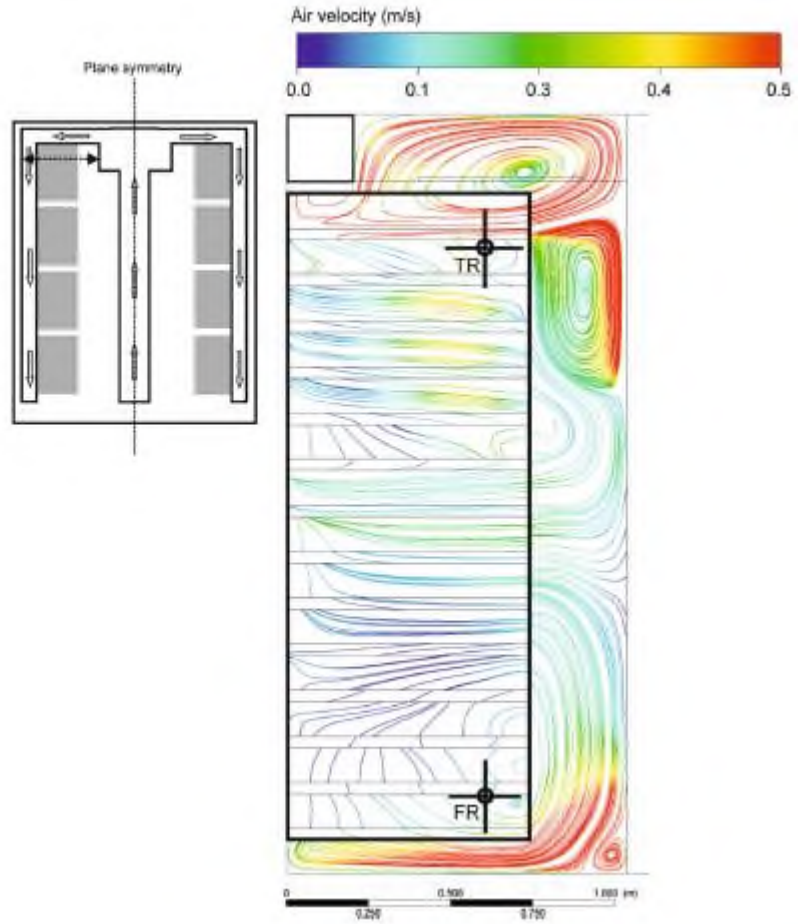

Fig. 3 Representation of streamlines on the vertical cross-section at 1.00 m from evaporator

The pH presented initial values close to 6.6, similar to other Portuguese cheeses using ewe's milk (Macedo et al. 1996). Until 10d, the pH values decreased to values around 5.09-5.24, result of the activity of lactic acid bacterias (Alvarenga et al. 2008; Fox et al. 2017). From 10 to 25d, the pH values increased considerably especially in the locations TR and FR, consequence of the metabolic activity of micro-organisms, like yeasts, using lactic acid as a source of carbon (Delgado et al. 2009) and are responsible for the production of alkaline metabolites (Santos et al. 2017; Pereira-Dias et al. 2000). The evolution of titrable acidity during ripening was coherent with the evolution of pH, as larger acidity values matched lower pH values (Table 2).

According to literature, the texture of cheese is affected by several factors such as milk composition, moisture and fat content, salt, pH and degree of proteolysis (Alvarenga et al. 2008), but also by environmental conditions (Picque et al. 2009; Mirade and Daudin 2006). The rheological analysis of the cheeses included the evaluation of hardness, adhesiveness and $G'_{1\,Hz}$ (Table 2). The hardness presented an initial value around 2.6 N and increased to values between 31.7 and 58.6 N at 25d ($p < 0.05$), as a result of the drying process during ripening (Alvarenga et al. 2008). In fact, the observed increase on hardness is related with loss of moisture, but also related with the increase of pH, especially until 15d. The storage modulus $G'_{1\,Hz}$ presented a similar evolution as hardness during ripening time, starting with an initial value around 32.11 kPa, at 0d, increasing to values around 920.46-1168.42 kPa at 25d (Table 2). The results did not present an influence of the location on the results of G'_{1Hz} ($p > 0.05$). The adhesiveness (- N.s) of cheese presented initial values of 1.64, increasing progressively until 25d (Table 2). The larger values of adhesiveness were observed in samples ripened in locations FL and TL ($p < 0.05$), thus presenting higher

Table 2 Mean values of physical–chemical and microbiological results during 25d of ripening time

	0 days	10 days				25 days			
		FL	TL	TR	FR	FL	TL	TR	FR
Moisture (%)	63.9^{a}	36.3^{cd}	39.1^{bc}	33.4^{de}	42.3^{b}	31.5^{e}	24.7^{f}	19.4^{g}	26.9^{f}
	(0.6)	(1.7)	(1.5)	(0.5)	(4.1)	(1.4)	(1.0)	(0.4)	(0.6)
pH (−)	6.63^{a}	5.09^{e}	5.14^{de}	5.17^{de}	5.24^{cd}	5.13^{de}	5.31^{c}	5.74^{b}	5.75^{b}
	(0.07)	(0.05)	(0.08)	(0.10)	(0.07)	(0.09)	(0.22)	(0.05)	(0.10)
Titrable acidity (mL NaOH N/100 g)	1.53^{c}	9.27^{a}	9.93^{a}	7.27^{ab}	8.20^{ab}	9.00^{ab}	8.07^{ab}	6.00^{b}	7.47^{ab}
	(0.30)	(2.08)	(1.28)	(1.03)	(1.65)	(1.31)	(1.80)	(0.25)	(0.90)
Hardness (N)	2.6^{g}	12.4^{d}	14.1^{d}	15.9^{cd}	13.6^{d}	49.2^{a}	58.6^{a}	45.7^{ab}	31.7^{bc}
	(0.4)	(5.1)	(5.4)	(7.6)	(7.4)	(16.8)	(22.6)	(29.0)	(13.6)
Adhesiveness (− N.s)	1.64^{d}	11.16^{b}	7.47^{bc}	4.65^{cd}	5.15^{cd}	22.04^{a}	21.33^{a}	8.47^{bc}	5.49^{cd}
	(1.00)	(4.58)	(4.81)	(2.33)	(2.51)	(8.29)	(6.94)	(4.89)	(2.40)
$G'_{1\,Hz}$ (kPa)	32.11^{b}	648.95^{ab}	678.47^{ab}	587.41^{ab}	334.17^{ab}	1168.42^{a}	920.46^{ab}	1020.25^{a}	931.50^{ab}
	(5.03)	(424.33)	(345.75)	(331.94)	(224.43)	(836.78)	(884.29)	(793.88)	(798.47)
Area of holes (%)	1.24^{c}	3.47^{bc}	8.84^{ab}	13.30^{a}	10.79^{a}	1.72^{c}	2.36^{bc}	11.51^{a}	14.56^{a}
	(1.02)	(3.11)	(4.08)	(4.09)	(1.81)	(1.23)	(1.49)	(1.88)	(4.11)
WI ext (−)	91.20^{a}	69.84^{bc}	69.85^{bc}	69.15^{c}	71.78^{b}	61.73^{e}	62.57^{de}	64.31^{d}	61.44^{e}
	(0.79)	(1.36)	(1.30)	(2.03)	(2.12)	(2.74)	(2.80)	(1.38)	(1.16)
WI int (−)	92.11^{a}	79.96^{bc}	81.04^{bc}	79.33^{c}	82.27^{b}	72.14^{d}	71.14^{d}	69.66^{d}	70.24^{d}
	(0.78)	(1.89)	(1.25)	(2.44)	(1.37)	(2.25)	(3.32)	(3.28)	(2.99)
Total mesophilic bacteria ($\log_{10}$ cfu/g)	7.62^{cd}	7.49^{d}	7.60^{cd}	7.77^{bc}	7.64^{cd}	7.57^{cd}	7.57^{cd}	7.91^{b}	8.34^{a}
	(0.12)	(0.11)	(0.03)	(0.01)	(0.04)	(0.08)	(0.09)	(0.05)	(0.01)
Lactic acid bacteria ($\log_{10}$ cfu/g)	6.49^{e}	7.41^{cd}	7.49^{c}	7.51^{c}	7.46^{cd}	7.20^{d}	6.74^{e}	7.79^{b}	8.10^{a}
	(0.15)	(0.01)	(0.02)	(0.01)	(0.01)	(0.08)	(0.02)	(0.13)	(0.01)
Enterococci ($\log_{10}$ cfu/g)	5.22^{e}	5.51^{d}	5.71^{bc}	5.66^{c}	6.04^{a}	5.64^{c}	5.48^{d}	5.79^{b}	5.73^{bc}
	(0.05)	(0.03)	(0.01)	(0.02)	(0.01)	(0.04)	(0.04)	(0.01)	(0.02)
Enterobacterias ($\log_{10}$ cfu/g)	4.97^{e}	3.82^{g}	5.99^{c}	6.60^{b}	7.23^{a}	4.30^{f}	5.00^{e}	6.17^{c}	5.54^{d}
	(0.23)	(0.02)	(0.13)	(0.07)	(0.01)	(0.01)	(0.04)	(0.02)	(0.01)
Yeasts ($\log_{10}$ cfu/g)	1.89^{g}	5.70^{c}	5.87^{bc}	5.94^{b}	5.79^{bc}	6.28^{a}	4.74^{d}	4.01^{f}	4.47^{e}
	(0.14)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)

$^{a,b,c,...}$Means in the same row marked with different letters are significantly different ($p < 0.05$, n = 3, Scheffé test). Standard deviation is presented between parentheses

humidity (Table 1). It is important to notice that all rheological parameters presented high values of standard deviation (Table 2), which is a common situation in cheeses produced with raw milk (Alvarenga et al. 2008), in part due to the indigenous microflora, affecting proteolysis and lipolysis (Reis and Malcata 2011).

The appearance of cheese during ripening was evaluated through the estimation of whiteness index (WI), on a scale from 0 to 100. Both interior (WI_{int}) and exterior (WI_{ext}) of cheese presented initial values around 91-92, as expected, due to the natural white appearance of fresh cheese (Table 2). During ripening, both exterior and interior of cheese presented a progressive decrease until 25d, obtaining WI_{int} around 70-72 and WI_{ext} around 61-64. The lower results on the exterior are consequence of the drying process during ripening time, presenting a shift to a yellowish appearance as stated in previous studies (Alvarenga et al. 2008). No influence was observed due to the location ($p > 0.05$).

The production of gas in cheeses has been identified with several microbiota groups such as coliforms, yeasts, citrate-positive lactococci, *Leuconostoc* spp., heat-resistant streptococci, propionibacteria (Guggisberg et al. 2015) or enterobacterias (Tabla et al. 2016). The observed results of digital image analysis in the cross section of cheese conclude that the percentage occupied by gas holes at 0d was around 1.2% (Table 2). During the first 10d of ripening time, all cheese samples presented a significant growth to percentages ranged from 3.5 to 13.3%, higher values observed in locations TR and FR. At 25d, a decrease was observed in locations FL and TL, to values around 1.7-2.5%, but locations TR and FR increased the percentage of holes to 11.5 and 14.6%, respectively (Table 2).

Microbiological results

The evolution of the microbiological counts during ripening included total mesophilic bacteria, total mesophilic lactic acid bacteria (LAB), enterococci, enterobacterias and yeasts (Table 2). All analysed groups presented a significant growth during ripening, regardless of the location of cheese inside the ripening room. According to previous studies on Queijo de Évora PDO, there is an apparent relationship between microbial viable numbers and degree of environmental contamination (Freitas and Malcata 2000), depending in the airflow pattern inside the ripening room. The total mesophilic bacteria presented an initial count of 7.62 log units (Table 2), similar to cheese La Serena (Del Pozo et al. 1988). No significant changes were observed during the first 15d ripening time, nevertheless, locations TR and FR presented the highest counts at 25d, where values 7.91 and 8.34 log units were observed ($p > 0.05$). The presence of swirls near locations TR an FR (Fig. 3) may contribute to such higher mesophilic counts.

LAB was the dominant microbiological group during ripening time, as in other studies (Freitas and Malcata 2000), presenting values at 0d around 6.5 log units (Table 2). Similar values were observed in previous studies in Queijo de Évora (Freitas and Malcata 2000). LAB, mainly from milk, constitute the main microbial group of cheese, forming part of its natural microflora, together with other bacteria, yeasts and/or molds (Beresford et al. 2001). Starter LAB (SLAB) participate in the lactose fermentation process, whereas some others are implicated in the maturation of cheese, indicated as non-starter LAB (NSLAB) (Settanni and Moschetti 2010).

During the early 15d, a significant growth of LAB was observed in all cheese samples, around ten folds when compared with 0d, being responsible for the conversion of lactose into organic acids (Beresford et al. 2001), mainly lactic acid (Delgado et al. 2009), thus decreasing pH value, as observed in Table 2. Locations FL and TL presented a reduction in the LAB group from 10 to 25d ripening time, but locations TR and FR presented a considerable growth to 7.79 and 8.10 log values, respectively (Table 2). Cheeses in these positions also have the highest odor scores, which seems to reflect the importance of NSLAB in these attributes. The observed results are similar to cheese Queijo Serra da Estrela PDO (Dahl et al. 2000).

Enterococci are a relevant part of native microflora in a wide range of traditional products from the Mediterranean area (Freitas and Malcata 2000) not inoculated with starter cultures and is widely accepted as an indicator for sanitary conditions. On the other hand, this group plays an important role in the ripening of traditional cheeses, through proteolysis, lipolysis, and citrate breakdown, which contribute to the typical taste and flavor (Moreno et al. 2006). In some Portuguese cheeses, enterococci is one the most predominant groups inside LAB (Dahl et al. 2000). In the present study, at 0d enterococci presented results around 5.2 log count (Table 2), higher than in previous studies on cheese Queijo de Évora (Freitas and Malcata 2000) but lower than La Serena (Del Pozo et al. 1988). During the early 15d, a growth was observed in all locations, followed by a decrease until 25d, to values from 5.5 to 5.8 log counts. Higher values were observed at locations TR and FR (Table 2).

The enterobacterias are an excellent indicator of the hygienic quality of a product and high values on milk or cheese can be an indicator of poor hygiene practices during milking and cheese production (Kornacki et al. 2013). The initial counts of enterobacteria were already high, around 5 log count (Table 2), still this value increased in some locations until the end of ripening time. The highest values were observed in locations TR and FR, at 10d, where results over 6.5 log count were observed. Although these values may seem high, are similar to previous studies on raw ewe's milk cheeses from Spain (Tabla et al. 2016) and Portugal (Tavaria and Malcata 1998) where the maximum counts of enterobacteria were observed in the midterm, around 10-18 days ripening time. After, excepting location FL, all cheese samples presented a decrease until 25d. According to Macedo et al. (1996), this fact can be attributed to physicalchemical conditions prevailing in the cheese, unfavorable to this group (Macedo et al. 1996), like increase in NaCl and decrease in pH. The evolution of percentages of gas holes in cheese is coherent with the evolution of enterobacterias (Table 2), which may indicate that this group is the responsible by the production of gas holes.

The yeasts can be found commonly in traditional cheeses produced with raw ewe's milk. Previous studies on similar Portuguese cheese, using raw's ewe milk, identified the main species as *Debaryomyces*, *Candida*, *Rhodotorulla* (Freitas and Malcata 2000; Pereira-Dias et al. 2000) and *Yarrowia* (Santos et al. 2017). In the present study, values ranged from 1.9 log count, at 0d, to a maximum value 6.3 log count at 25d ripening time, at location FL. The obtained values are comparable to previous studies on Queijo de Evora (Pereira-Dias et al. 2000), La Serena (Del Pozo et al. 1988) and Queijo Serra da Estrela (Dahl et al. 2000). Although, yeast flora has been related with the increase of pH at the final stages of cheese ripening, they are also responsible by lactose fermentation, lipolysis, proteolysis, production of aromatic compounds, important for the typical characteristics of such cheese (Santos et al. 2017; Pereira-Dias et al. 2000). On the other hand, yeasts can also be responsible for cheese spoilage, like off flavours flavours, texture losses, excessive gas formation, acidity increase or surface discolourations (Pereira-Dias et al. 2000; Santos et al. 2017).

Sensorial results

The scores of descriptors at 25d for appearance, texture and aroma/taste are reported in Table 3. Considering appearance, the sensory results clearly identified two major groups: a first group including cheeses ripened at locations FL and TL, and a second group including locations TR and FR. Indubitably, this difference is result of the heterogeneity of the environmental conditions inside ripening room, affecting directly and undirectly the properties of cheese. Considering the environmental measurements (Table 1), it is possible to observe that environmental conditions at locations TR and FR presented lower humidity values, increasing the diffusion of moisture from the cheese, affecting both colour outside and inside (Table 2), as reported in previous studies on cheeses using raw ewe's milk (Alvarenga et al. 2008). The presence of holes is considered normal in this kind of cheese using raw ewe's milk, due to the activity of enterobacterias or NSLAB, nevertheless, according to the regulation of Queijo de Évora PDO (Despacho No. 29/94), is mandatory that size and number of such holes to be strict to “few small holes", which requires a special attention to the quality of raw milk and to the environmental conditions. As observed in Table 3, cheeses from location FL and TL presented a low number of holes, while cheeses from locations TR and FR present a very high number of holes ($p < 0.05$). These results are according to the digital image analysis (Table 2), where the percentage of holes in cheeses from locations FL and TL was clearly lower than in locations TR and FR ($p < 0.05$).

The firmness parameter can be related with the resistance of the sample to a very slight opening and closing of the jaws (Bárcenas et al. 2007). This parameter presented the highest score at location TR (Table 3), while the lowest were observed at locations TL, probably consequence of environmental humidity. However, the sensorial perception for firmness does not fully agree with the instrumental evaluation (Table 2), where the lowest hardness value was observed at location FR. The unctuosity is related with the perception of greasiness, therefore with spreadability. As expected, the lowest value was observed at location TR, with a score 2.3, due to the highest value on firmness (Table 3).

According to studies in cheeses made from raw ewe's milk, the aroma/taste in this kind of cheese is consequence of volatiles produced from the catabolism of lactate, free amino acids (especially valine and leucine) and lipids (free fatty acids), as a result of activity of the natural flora like *Lactobacillus*, *Lactococcus*, enterococci, enterobacterias and yeasts (Dahl et al. 2000). This effect has been stated in previous studies in Portuguese

traditional cheeses (Reis and Malcata 2011). In the present study, the development on odor and flavor after 25d ripening time was also identified by the sensorial panel (Table 3), however no correlation was observed between these parameters and location environmental conditions ($p > 0.05$).

Table 3 Mean values of sensorial scores of cheeses

	Appearance			Texture		Aroma/taste	
	Colour outside	Colour inside	Holes inside	Firmness	Unctuosity	Intensity of odor	Intensity of flavor
FL	1.7^{b}	2.6^{c}	1.3^{b}	4.5^{b}	3.3^{a}	2.8^{a}	2.8^{a}
	(0.8)	(0.9)	(0.5)	(1.1)	(1.5)	(1.5)	(1.0)
TL	1.8^{b}	3.0^{bc}	1.8^{b}	3.7^{b}	3.9^{a}	3.4^{a}	2.8^{a}
	(0.9)	(1.2)	(0.5)	(1.3)	(1.3)	(1.5)	(1.2)
TR	4.0^{a}	4.6^{a}	5.5^{a}	5.6^{a}	3.1^{a}	3.6^{a}	2.5^{a}
	(1.4)	(0.9)	(1.1)	(0.8)	(1.9)	(1.3)	(1.2)
FR	3.0^{a}	3.9^{ab}	5.3^{a}	4.2^{b}	3.6^{a}	3.1^{a}	3.1^{a}
	(1.5)	(1.4)	(1.1)	(1.1)	(1.5)	(1.2)	(1.2)

$^{a,b,c,...}$Means in the same row marked with different letters are significantly different ($p < 0.05$, n = 20, Scheffé test). Standard deviation is presented between parentheses

Conclusion

The present study confirms a reality in a considerable number of traditional cheese factories: the heterogeneity of the environmental conditions inside the ripening room. The results pointed that the evolution of cheeses during ripening was strongly affected by local environmental conditions, especially airflow pattern and humidity. In fact, cheeses ripened with lower environmental humidity presented lower moisture content, higher bacterial counts, darker appearance and higher number of holes inside. Considering sensorial evaluation, locations that favored the activity of the secondary microbial flora promoted a larger neutralization of the paste and presented a higher intensity of smell, flavor and aroma, together with lower hardness values, showing the effect of other factors rather than humidity. The CFD modelling and simulation was an additional tool for the prediction of streamlines inside the ripening room and made possible the identification of swirl areas inside the stacks of cheese which may influence the evolution of cheese during ripening. However, based only in the results of the present study it is difficult to take objective and ultimate conclusions, as the ripening of cheeses produced from raw milk are also dependent of many other variables. Nevertheless, this study is intented to be a step towards a larger homogeneity in the ripening process of Queijo de Évora PDO cheese.

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