International Dairy Journal 39 (2014) 259–269

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

# Spatial characterisation of eye-growing kinetics in semi-hard cheeses with propionic acid fermentation

D. Huc [a, b, c, d, e], S. Challois [a, b], M. Monziols [f], C. Michon [c, d, e], F. Mariette [a, b, *]

[a] Irstea, UR TERE, 17 Av. de Cucillé, CS 64427, F-35044 Rennes, France
[b] Université Européenne de Bretagne, France
[c] AgroParisTech, UMR1145 Ingénierie Procédés Aliments, F-91300 Massy, France
[d] INRA, UMR1145 Ingénierie Procédés Aliments, F-91300 Massy, France
[e] CNAM, UMR1145 Ingénierie Procédés Aliments, F-75003 Paris, France
[f] IFIP Inst. Porc, F-35651 Le Rheu, France

ARTICLE INFO

*Article history:*
Received 2 September 2013
Received in revised form
23 June 2014
Accepted 23 June 2014
Available online 18 July 2014

ABSTRACT

An important characteristic of semi-hard cheeses with propionic acid fermentation is the eyes. However, growth mechanisms of the eyes are only qualitatively understood. In this study, X-ray computed tomography was used to monitor eye growth inside cheeses during ripening without disrupting any mechanism. Chemical and rheological analyses carried out in two different zones of the cheeses revealed a spatial-dependency, with the position the eyes significantly correlated to their final volume, and a different $CO_2$ production by $CO_2$-producing propionic acid bacteria with 27.7 mmol $kg^{-1}$ in the centre and 18.7 mmol $kg^{-1}$ in the outer zone. Furthermore, the present study allowed quantification using image processing: the overall cheese porosity grew from 0.03% at the beginning of ripening up to 4.60% at the end. Moreover, the porosity was 10 times lower, and the volume of eyes 13 times lower, in the outer zone than in the centre of the cheese.



## 1. Introduction

A characteristic feature of semi-hard cheeses with propionic acid fermentation is the presence of holes, usually referred to as eyes. They represent an important quality parameter as a result of their number, size, shape and spatial distribution. Indeed, eyes need to display specific characteristics for each type of cheese. For example, in Swiss-type cheeses, they are required to be "round and regular, from medium to large size" (Bachmann, Butikofer, & Isolini, 2002; Fröhlich-Wyder & Bachmann, 2004; Steffen, Eberhard, Bosset, & Rüegg, 1993). Their appearance is also an important criterion for the consumer, since it has an impact on product choice.

However, eye growth mechanisms are still largely unknown or generally explained only qualitatively. Product innovation is thus difficult to achieve since the consequences of a new process or recipe on eye development cannot be anticipated. Several fields of interest must be taken into account to obtain a better understanding of theses mechanisms. Steffen et al. (1993) summarised the four requirements for good eye formation and development as: (i) $CO_2$ production, (ii) number and size of nuclei, (iii) $CO_2$ pressure and diffusion rates; and (iv) cheese body texture. However, the investigation of composition, rheology and eye growth in cheese is complex, due to its intrinsic heterogeneities. Indeed, several elements such as bacteria counts, organic acids concentrations and rheological properties appeared to be spatially-dependent within cheeses.

Higher water content and lower salt/moisture (S/M) ratios were found in the centre of Gouda and Swiss-type cheeses (Guinee, 2004; Hollywood & Doelle, 1984; Reinbold, Hussong, & Stine, 1958; Saurel, Pajonk, & Andrieu, 2004). $CO_2$-producing propionic acid bacteria (PAB) were also found to be 5 to 1000 times more numerous in the centre of Swiss-type cheeses than at the periphery (Reinbold et al., 1958). Hollywood and Doelle (1984) obtained PAB counts that were 12 times higher in the centre of Swiss-type cheeses at the end of ripening than at the periphery, associated with quantities of acetate and propionate that were twice as high. Moreover, Accolas, Veaux, Vassal, and Mocquot (1978) showed that lactic acid bacteria (LAB; specifically *Streptococcus thermophilus* and *Lactobacillus helveticus*) were also 12 to 15 times more numerous in the centre of Gruyère cheese after 20 h of pressing than at the periphery. Finally, Culioli and Sherman (1976) and Prentice (1987)

* Corresponding author. Tel.: +33 2 23 48 21 78.
*E-mail address:* francois.mariette@irstea.fr (F. Mariette).

http://dx.doi.org/10.1016/j.idairyj.2014.06.010
0958-6946/

found a fracture force and a level of firmness twice as high at the periphery of Gouda and Cheddar cheeses at the end of ripening compared with the core zone. The amount of $CO_2$ inside Emmental wheels was also higher in the core zone in both the vertical and horizontal directions, compared with the outer zones (Flückiger, 1980). Moreover, the magnitude of the spatial-dependency of cheese components evolves during ripening, increasing or equilibrating inside the cheese. For example, the salt content difference is maximal after brining (12 times higher in the outer zone) and continues to decrease thereafter (2.54% in the centre compared with 2.81% in the outer slice), as was found in Cheddar cheese between 1 and 48 h after salting (Morris, Guinee, & Fox, 1985).

However, despite the empirical knowledge stating that there are fewer eyes under the rind, no study quantifying the spatial-dependency of eyes has been done for cheese. Moreover, whereas the interest of X-ray imaging techniques to study eye growth has already been demonstrated (Blanc & Hättenschwiler, 1973; Guggisberg et al., 2013; Hättenschwiller, 1976; Kurmann & Würthrich, 1975; Schuetz et al., 2013), a kinetic approach has been carried out during the ripening process in the recent literature only by Lee et al. (2012), who used X-ray computed tomography (CT) to monitor increase of porosity in Gouda during ripening, even though Kurmann and Würthrich (1975) studied the kinetics of the eye development in Swiss Emmentaler cheese based on conventional X-ray technology and manual measurements in the X-ray images. However, they observed the formation and growth of individual eyes from 20 days to 150 days of ripening. However, no quantitative differentiation was ever made between eyes in the centre and in the outer zone. Therefore, there is a link missing between, on the one hand, the characterisation of cheese chemical and rheological properties, and on the other hand, eye characteristics. Finally, the identification of key factors that influence eye development would be necessary to further increase our knowledge and to attempt not only to outline mechanisms, but to prioritise them as well.

The aim of the current study was to investigate and quantify the intensity and impact of the spatial-dependency of eye growth, chemical and rheological properties in semi-hard cheeses with propionic acid fermentation, and to investigate the correlations with eye development. Several techniques were used to fulfil this objective: chemical analysis for the investigation of cheese composition, rheological tests for the evaluation of cheese matrix resistance, and CT to monitor eye growth kinetics and location in cheese during ripening. Two zones of interest were identified – the outer layer and the centre – and all data were inter-linked statistically to better understand, prioritise and define dependences of eye growth mechanisms.

## 2. Materials and methods

### 2.1. Sample preparation

The cheeses studied were industrial pressed and non-cooked cheeses (fat/dry matter content 47%, w/w, water content 42%, w/w) shaped into blocks of 11 kg (24 cm × 10 cm × 48 cm) that can be described as semi-hard cheeses with propionic acid fermentation. They were produced in nine main steps: preparation of milk with the addition of lactic and propionic strains, renneting, cutting the gel in curd grains separated from the serum during the draining step, moulding, pressing, brining by immersion of the products in a NaCl saturated solution. After brining, they were packaged under a plastic film before entering ripening. The packaging film possessed a 300 mL $m^{-2}$ $d^{-1}$ permeability to $CO_2$ (23 °C, 0% RH), a 12 g $m^{-2}$ $d^{-1}$ permeability to $H_2O$ (38 °C, 100% RH) and an 87 mL $m^{-2}$ $d^{-1}$ permeability to $O_2$ (23 °C, 50% RH). The ripening was divided in two periods: 10 d at 12 °C (production room) and 15 d at 20 °C (warm room). Ten cheeses were monitored throughout the whole ripening cycle using CT, whereas 10 other cheeses were sampled at five ripening times to perform destructive measurements. To study the potential spatial-dependency of eyes, two different locations were chosen: one slice was taken in the centre of the cheeses (C slice) and the other one was taken at 1 cm underneath the rind (UR slice). The location of these two 16 cm × 16 cm × 1.5 cm slices can be seen in Fig. 1. Both UR and C slices were divided into two parts; one part was frozen at −20 °C and used for chemical analysis, the other was stored at +4 °C and used for rheological tests.

### 2.2. Chemical tests

Half of the UR and C slices were ground before performing chemical analysis: fat, dry matter, and salt contents were determined using the ISO (2002), ISO (2004a), and ISO (2007) methods, respectively, and pH was determined using the ISO (2009) method. The total and soluble nitrogen (TN and SN, respectively) were determined using the ISO (2004b) method, based on a Kjeldahl protocol at pH 4.6 for SN. The organic acids were determined using HPLC (with a 1 mM HCl eluent gradient with a 3–5 mL $min^{-1}$ flow, separation on an ionic exclusion column ICE-AS6 (ThermoFisher Scientific, Waltham, MA, USA), detection by conductimetry (20 µs cell) coupled to an anionic suppressor, and quantification using chemical standards injected using the same conditions as the tested solutions). Chemical tests were carried out at five different times during ripening, two in the production room (Day 3 and Day 7 at 12 °C) and three in the warm room (Day 12, Day 21 and Day 25 at 20 °C). $CO_2$ production was estimated using the acetic acid tracker, according to the hypothesis that both components are equimolar, which has been recently validated by Huc et al. (2014b). Each chemical analysis was carried out on two samples taken from the centre slice and two samples taken from the outer slice at each ripening time previously mentioned.

### 2.3. Rheological tests

Cylinders of cheese were cut using a cork-borer with a diameter of 7 mm in the second half of UR and C slices at 4 °C. These cylinders were then cut into discs with a height of 3.5 mm using a cutting tool with two razor blades separated by a 3.5 mm spacer, allowing

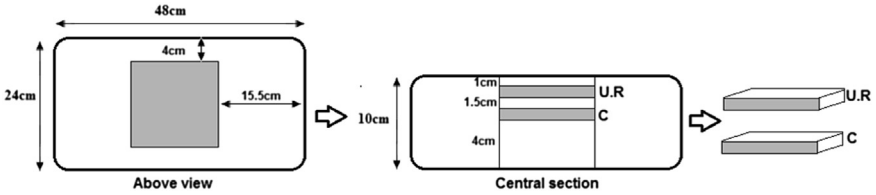


**Fig. 1.** Schematic outline of the cheese block sampling (Under-Rind slice: UR; Core slice: C).