



Research paper

CO₂ permeability of plastic ripening films and of natural dry ripening influences eye formation in experimental Emmentaler cheese

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ABSTRACT

The influence of CO₂ diffusion on eye formation during the ripening of an experimental Swiss-type cheese was studied. Three plastic films with defined CO₂ permeability (198, 576, or 1050 cm³ m⁻²·24 h⁻¹ bar⁻¹) were compared to natural dry-ripening (control cheese without plastic film), testing two different cultures of *Propionibacterium freudenreichii*. Gross composition and volatile carboxylic acids were measured at the end of ripening. Physical parameters, such as eye volume, eye number, and eyeless border zones, were analysed by computed tomography. With increasing permeability, the relative eye volume decreased from 13.6% to 9.0% ($p < 0.01$). Surprisingly, the total number of openings increased from 570 to 711 ($p < 0.05$), which led to generally smaller openings with a mean value of ~1000 mm³ compared to ~2000 mm³ ($p < 0.001$). The control cheese with natural dry ripening showed a distinctive eyeless border zone with completely different diffusion properties compared to the samples ripened in films. The quantitative findings on eye formation dependent on plastic film permeability and dry ripening underscore the high importance of the diffusion barrier and are highly relevant for the cheese manufacturing industry.

1. Introduction

Propionic acid fermentation by *Propionibacterium freudenreichii* (Von Freudenreich & Orla-Jensen, 1906) is a unique characteristic shared by all Swiss-type cheeses, conferring the characteristic round shiny openings (also called eyes) and a nutty and sweetish flavour. Swiss-type cheese was originally manufactured in the Emmentaler region of Switzerland. Its predecessor was mountain cheese, dating back to the twelfth century. Emmentaler PDO is probably the best-known Swiss-type cheese (Fröhlich-Wyder et al., 2017). Therefore, the size, number, shape, and distribution of the eyes in Swiss-type cheese are considered important quality traits. Eye formation in cheese is a complex process that is influenced by numerous factors. Achieving the characteristic, consistent, and reproducible eye formation throughout the year is therefore a major challenge in the production of Swiss-type cheeses:

Moulding submerged in whey to prevent air bubble inclusion, complete fusion of the cheese curd, controlled carbon dioxide (CO₂) formation, presence of eye nuclei, a soft and viscoelastic texture, solubility of CO₂ in the cheese matrix (water, fat, protein), and a diffusion barrier on the cheese surface are requirements for achieving regular and characteristic eye formation in cheese (Fröhlich-Wyder et al., 2017; Guggisberg et al., 2015).

Adequate CO₂ formation is achieved by controlling cheese environmental conditions such as pH, temperature, salt content, and a_w (water activity) during manufacture and ripening to achieve optimal CO₂

formation with a selected combination of starter and non-starter cultures (lactic acid fermentation) and *Propionibacterium freudenreichii* (propionic acid fermentation) (Bisig et al., 2019; Fröhlich-Wyder et al., 2002). The preferred pH is 5.3–5.4 after lactic acid fermentation is completed on day 1. The temperature between day 10 and around day 70 should be 20–24°C in the warm room for Emmentaler PDO (Daly, McSweeney, & Sheehan, 2010; Flückiger, 1980).

The addition of fine hay particles provides a controlled way of introducing nucleation sites into milk that has had these sites removed. For research purposes, this allows for standardised, reproducible experiments: first, the milk is microfiltered, and then defined amounts of hay particles are added (Guggisberg et al., 2015). Approximately 1–2 mg hay particles of < 100 µm per 90 L of cheese milk can sufficiently induce the optimal number of cheese eyes, as found experimentally in a dose-response experiment (Guggisberg et al., 2015) and applied in the cheese industry.

Studies show that CO₂ solubility in cheese is not a fixed value but depends on temperature and matrix composition. Pauchard et al. (1980) found that the solubility of CO₂ in Emmentaler cheese at 22°C was about 37.5 mmol kg⁻¹ or 840 mL kg⁻¹. In their experiment, eye formation started as early as at 50% CO₂ saturation. At a lower temperature of 12°C, CO₂ solubility in water increased by 29% compared to 22°C, while it increased 17% only in Emmentaler cheese. Jakobsen et al. (2009) showed that with a temperature increase in semi-hard cheese, the reduced CO₂ solubility in the aqueous phase was partially compensated

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by a solubility increase in the fat phase. Fröhlich-Wyder et al. (2023) reported that the relative eye volume in semi-hard and hard cheeses decreased with increasing fat content. These findings are relevant for eye formation because only CO₂ that exceeds the solubility capacity of the cheese matrix can contribute to eye formation and growth.

Lamichhane et al. (2021) found a non-linear relationship between CO₂ solubility and the moisture-to-protein ratio in renneted model casein matrices: At about 1.5:1 moisture-to-protein and 5°C, CO₂ solubility was lowest, around 33 mmol kg⁻¹·atm⁻¹, but the influence of protein content in the range relevant for cheeses was small. Acerbi et al. (2016) studied the influencing parameters on CO₂ diffusion, reporting that at 13°C, CO₂ diffusion was about 4 times higher than at 4°C and 60% higher than at 25°C. Salt clearly reduced diffusion, while moisture non-linearly influenced diffusion, with the highest value at 42%.

The basics of gas transfer in ideal solutions and cheeses, such as Henry's law and Fick's second law, are well described by Acerbi et al. (2020). To control the loss of CO₂ by diffusion at the cheese surface, either a rind with varying permeability is formed during dry ripening or, as alternatives, waxing or plastic films with constant CO₂ permeability can be applied during ripening (Acerbi et al., 2016; Flückiger, 1980; Kraggerud et al., 2009).

One of the first CO₂ diffusion trials on the surface of cheese was performed by Flückiger (1980). He and his colleagues compared the CO₂ diffusion of two Emmentaler cheeses—one ripened at 80% relative humidity and the other at 40%. The cheese ripened at 80% relative humidity had a greater CO₂ loss than the cheese ripened at 40%. At 80%, the surface remained more porous. Kraggerud et al. (2009) showed that the diffusion of CO₂ out of cheese can be influenced by four different plastic films as a diffusion barrier during ripening of rindless cheese by comparing the relative eye volume development during warm room ripening with a low-resolution X-ray instrument and image processing software. However, the segmentation results were not 100% accurate, according to the authors, and the relative eye volume development was not significantly different for the four different plastic films in their study. To our knowledge, no further work has been carried out to demonstrate the importance of surface permeability in relation to CO₂ diffusion and eye formation in cheese, although this is highly relevant from an industrial and practical perspective.

Analyses of the accurate volume, number, and distribution of eyes within the cheese are of paramount importance. Computed tomography (CT) is a non-invasive imaging technology that has been employed to define the precise relationship between artificial eyes (hollow balls) in the cheese matrix and the determined eye volume (Guggisberg et al., 2013). Several imaging software tools were compared quantitatively by Schuetz et al. (2016).

The objective of the present study was to investigate the influence of three different CO₂-permeable plastic films with specific CO₂ permeability and a control without film on eye formation in cheese samples from two different propionic acid bacteria (PAB) cultures under highly standardised cheese ripening conditions. The experimental Emmentaler cheese samples were analysed using CT.

2. Materials and methods

2.1. Cheesemaking and ripening

Eight experimental Emmentaler cheeses, 30 cm in diameter, were manufactured from 90 L of milk each in the Agroscope pilot plant (Bern, Switzerland) in eight stainless steel vats. The summer milk was adjusted after skimming to a fat content of 35 g kg⁻¹. The number of eye nuclei was standardised by adding powdered hay particles of microbiological food quality. A suspension containing 20 mg of powdered hay (<100 µm; Agroscope, Bern, Switzerland) in 100 mL tap water was prepared in a flask with a screw cap. The powdered hay was kept in suspension by continuous shaking, and 10 mL of the suspension were added to the individual vat milks at the beginning of cheesemaking.

Eight litres of water containing 9 mL aqueous copper sulphate solution (39.3 g·L⁻¹ of copper (II) sulphate pentahydrate) were added to the vat milk (90 L) at a level to ensure concentrations of copper (Cu) in the final cheese were close to that of traditional Emmentaler Switzerland PDO. Cu is known to regulate microbial activity (Mato Rodriguez et al., 2011). After adding 200 mL of a bulk starter containing various strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *lactis* (RMK 101, Liebefeld Kulturen, Bern, Switzerland), the milk was pre-ripened at 31–32°C for 30 min. Thereafter, the milk was inoculated with a culture of PAB (*Propionibacterium freudenreichii*)—either Prop 96 (2 strains, low aspartase activity and lower CO₂ formation; Liebefeld Kulturen) or Prop 01 (4 strains, higher aspartase activity and CO₂ formation; Liebefeld Kulturen)—at a level of approximately 10³ cfu mL⁻¹ (Turgay et al., 2022). The subsequent manufacturing and ripening processes were previously described in detail by Guggisberg et al. (2015). Briefly, the control was dry ripened without film and turned and washed weekly at a low relative humidity of 75–85%. The remaining variants were wrapped in plastic films (Südpack, Baar, Switzerland) and sealed under vacuum of –995 mbar (VC999 Verpackungssysteme AG, Herisau, Switzerland), after salting for 16 h at 12°C in a 21°Bé brine, following Table 1.

A full factorial experimental design was applied, with two factors at two and four levels. One factor was PAB culture applied at two levels: Prop 96 or Prop 01 (N = 8 for each level). The second factor was the ripening condition, with four levels: natural dry ripening and three different plastic films (N = 4 each). The experiment was conducted in two replicate trials, with the second trial taking place 48 h after the first.

2.2. Cheese sampling

At 4 h and 24 h, the pH of the strained whey was measured to control the starting conditions. No further sampling was carried out at these time points to keep the cheese loaves intact for the CT images. Cheeses were sampled after 120 days of ripening. After the CT measurements, the 4-month-old cheeses were cut in half. One half was used for the chemical and biochemical analyses. The other half served as a retention sample and was deep frozen at –20°C. Prior to taking samples for the analysis, the rind of the hoop side and the two cheese faces were discarded by cutting off a thickness of 0.5 cm.

2.3. Chemical and biochemical analyses

For the chemical and biochemical analyses, the cheese was grated and mixed. L- and D-lactate and succinate were measured enzymatically

Table 1

Experimental design of a Swiss-type model cheese manufactured using two different propionic acid bacteria cultures. The cheese samples were ripened with three different plastic films (water vapour permeability: 4 g m⁻² · d⁻¹; thickness: 110 µm ± 10%) or by natural dry-ripening without film (cheeses no. 1–8). Replicates of all eight cheeses were produced two days later (day 2; cheeses no. 9–16).

Cheese number	Production day	Type of ripening	PAB culture
1, 9	1, 2	Natural dry ripening	Prop 96
2, 10	1, 2	Plastic film with permeability of 198 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 96
3, 11	1, 2	Plastic film with permeability of 576 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 96
4, 12	1, 2	Plastic film with permeability of 1050 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 96
5, 13	1, 2	Natural dry ripening	Prop 01
6, 14	1, 2	Plastic film with permeability of 198 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 01
7, 15	1, 2	Plastic film with permeability of 576 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 01
8, 16	1, 2	Plastic film with permeability of 1050 [cm ³ · m ⁻² · d ⁻¹ · bar ⁻¹]	Prop 01

using commercial kits. Briefly, for the determination of D-/L-lactate and succinate, 1.25 g or 5 g of grated cheese, respectively, were first homogenised in 10 mL of water using an OmniPrep Multi-Sample Homogenizer (Omni International, Kennesaw, USA) at 13,000 rpm for 40 s, followed by the addition of a further 10 mL of water. The samples were then clarified using Carrez solutions with rigorous mixing between each addition (Carrez I: 1.25 mL of potassium hexacyanoferrate(II) trihydrate (85 mmol/L); Carrez II: 1.25 mL of zinc sulphate heptahydrate (250 mmol/L); Neutralisation: 1.25 mL of 0.2 M NaOH). The homogenates were then incubated at 2°C for 20 min. The concentrations of D- and L-lactate were determined using an automated UV-Vis spectrophotometer (Gallery, Thermo, Switzerland) and a kit from R-Biopharm (Murten, Switzerland), while succinate was determined using a manual UV-Vis spectrophotometer (Lambda Bio 25, PerkinElmer, Switzerland) and a kit from Roche (Basel, Switzerland). The assays are based on the specific enzymatic conversion of the target analyte coupled to the reduction of NAD⁺ to NADH. Water content was determined with the dry matter method (IDF Standard, 2004a), and fat content with the Van Gulik method (IDF Standard, 2008a, b). Volatile carboxylic acids (C1–C6) were analysed by gas chromatography with a flame ionisation detector and headspace technology after esterification with ethanol, as described in detail by Badertscher et al. (2023). Sodium chloride content was calculated from the Cl concentration using the potentiometric titration method (IDF Standard, 2006). Total nitrogen (TN) was analysed using the Kjeldahl method (IDF Standard, 2004b), and water-soluble nitrogen (SN) at pH 4.6 and 12% trichloroacetic acid soluble nitrogen (TCA-SN) were measured using the Kjeldahl method (Collomb et al., 1990).

2.4. Physicochemical analysis and computed tomography of cheese

The pH value of the cheeses was determined using a pH meter (Metrohm 605, Metrohm AG, Herisau, Switzerland). Dissolved CO₂ in the cheese matrix was analysed using a method published by Bosset et al. (1980). The samples were emulsified under reduced pressure in an alkaline citrate medium using a mixer. CO₂ was quantitatively displaced into the headspace by adding excess sulphuric acid. Measurements were carried out using a specific non-dispersive infrared detector.

X-ray CT measurements of all cheeses were carried out after a ripening period of 120 days using a CT scanner (Somatom Volume Zoom, Siemens, Zürich, Switzerland). The following scan parameters were applied: 120 kV, 60 mA s, and 0.5 mm slice thickness. The resolution for the CT cross-section was set constant by choosing a constant field of view for all cheeses. Before the scan procedure, the dry ripened cheeses were packed in plastic bags (but not vacuumed) to protect the CT scanner from grease stains and placed on a Styrofoam-Plexiglas support for optimal positioning. The procedure and image analysis with VG Studio Max, Version 3.4 (Volume Graphics, Heidelberg, Germany) were carried out as previously described by Bisig et al. (2019) and Guggisberg et al. (2015). This software determines the volume for each eye and defines its centre using a three-dimensional coordinate system.

The eyeless border zone was analysed in 3-D using VGStudio Max (Hexagon, Heidelberg, Germany). The 10 outermost cheese eyes were taken and the distances from their centres to the border (x_{1-10}) were measured and summed up. The average value $\bar{x}$ and standard deviation were calculated as measures for the eyeless border zone. As a second value for the eyeless border zone, 10% of the outermost eyes were taken into consideration in the calculation.

2.5. Calculation of CO₂ formation

The formation of CO₂ during cheese ripening was estimated based on the molar concentrations of the metabolites propionic acid, butyric acid, and succinate, according to Bisig et al. (2019). Citrate was not taken into account because the cultures used did not metabolise it. Butyric acid content was negligible. The ratio to the CO₂ formed was 2 for propionic

acid, 2 for succinate, and ½ for butyric acid, resulting in the following equation:

$$c(\text{CO}_2) = \frac{c(\text{propionic acid})}{2} + \frac{c(\text{succinic acid})}{2} + 2 \times c(\text{butyric acid}) \quad (2)$$

The calculated amounts of CO₂ (mmol kg⁻¹ cheese) were converted into mL kg⁻¹ cheese (normal conditions: T = 273.15 K and P = 101.3 kPa). Under these conditions, the molar volume of the ideal gas was 22.4 L. The CO₂ diffused out of the cheese was estimated by subtracting the CO₂ dissolved in the cheese matrix and the CO₂ in the cheese eyes from the calculated total CO₂ formation.

2.6. Statistical analysis

A full factorial design with two factors (plastic film and propionic acid bacteria, treated as categorical variables) at four levels (natural dry ripening and plastic film for CO₂ permeability of 1050, 576, and 196 cm³ m⁻²·24 h⁻¹ bar⁻¹) and two levels (Prop 96 and Prop 01), respectively, was applied (Table 1). The production day (day 1 and day 2 as independent biological replicates) was also treated as a factor and included in the model to take its effect into account. The interactive effect between “plastic film” and “propionic acid bacteria” was also included in the present three-way ANOVA. The data were summarised as the mean and standard deviation. The instrumental data were analysed using ANOVA with R software (www.r-project.org, version 4.6.0). Significant differences between various levels within a factor were accepted at $p \leq 0.05$. Pairwise comparisons (post hoc tests) were assessed using the Tukey Honest Significant Difference (Tukey-HSD) method, and the p-value was adjusted according to Tukey.

3. Results and discussion

3.1. Cheeses after fabrication

We analysed the pH of the cheese samples to control the optimal conditions for the CO₂ producing PAB. The pH of the 16 cheeses after 4 h ranged from 5.59 to 5.67, and the pH after 24 h reached 5.22–5.27, which were both within the target range of 5.60–6.00 and 5.20–5.40, respectively (Daly et al., 2010; Fröhlich-Wyder et al., 2017). To avoid disturbing the CT images, the pH was measured in the whey expelled under the press at both time points, and no other samples were taken at 24 h.

On the second day, the 24-h pH of the corresponding eight cheeses was significantly lower, indicating delayed acidification on this day of production. The ripened cheeses on the second production day had, on average, a lower moisture and fat content and, therefore, a higher TN content. This influenced the eyeless border zone, which was double the size on the second production day. Due to the full factorial design, these significant differences between the two production days did not influence the variance of the factor levels under investigation (results of production days not shown).

3.2. Cheese composition at 120 days

No significant differences between the factor levels were detected for NaCl, pH, fat, SN (pH = 4.6), and TCA-SN at 120 days (Table 2). Lactate was completely metabolised by PAB cultures Prop 96 and Prop 01, expecting maximal CO₂ formation in the cheeses (Table 4). Natural dry ripening showed significantly increased moisture loss, as expected, and therefore increased TN. In contrast, moisture content and TN were not significantly different among the three plastic film-ripened cheeses.

Additionally, the PAB cultures had a significant influence on dry loss, as the Prop 96 samples showed slightly more moisture than the Prop 01 samples (Table 2). This difference in the dry loss content was minor (around 5%). An explanation for this phenomenon might be the larger gas flow through the gas barrier for Prop 01, which could also entail a

Table 2

Mean values and standard deviation of moisture content, sodium chloride, pH, fat, total nitrogen, water soluble nitrogen at pH 4.6 and 12% trichloroacetic acid soluble nitrogen (TCA-SN) in the experimental Swiss-type cheeses (120 days) manufactured with two different propionic acid bacteria cultures (Prop 96 or Prop 01). The cheese samples were ripened with three different plastic films or by natural dry ripening without plastic film (control). The cheeses were manufactured over two days. The results of the production day are not shown.

Factors	Level	N	Moisture [g kg ⁻¹]	Sodium chloride [g kg ⁻¹]	pH [–]	Fat [g kg ⁻¹]	TN [g kg ⁻¹]	SN (pH = 4.6) [g kg ⁻¹]	TCA-SN [g kg ⁻¹]
Permeability	Natural	4	349 ± 5.32 ^a	7.80 ± 0.510	5.69 ± 0.022	313 ± 7.93	46.4 ± 0.866 ^a	9.51 ± 0.530	6.54 ± 0.538
	198	4	364 ± 7.63 ^b	7.68 ± 0.299	5.66 ± 0.020	310 ± 7.44	45.5 ± 0.974 ^b	9.17 ± 0.551	6.50 ± 0.594
	576	4	366 ± 7.42 ^b	7.02 ± 0.624	5.66 ± 0.034	310 ± 3.86	45.4 ± 1.33 ^b	9.35 ± 0.514	6.48 ± 0.478
	1050	4	362 ± 2.22 ^b	7.00 ± 0.497	5.66 ± 0.022	314 ± 5.92	45.1 ± 0.984 ^b	9.25 ± 0.285	6.57 ± 0.332
Culture	Prop 96	8	363 ± 8.08	7.31 ± 0.45	5.66 ± 0.03	309 ± 4.51	45.5 ± 0.95	9.60 ± 0.37	0.48 ± 0.02
	Prop 01	8	357 ± 7.40	7.44 ± 0.65	5.67 ± 0.01	315 ± 6.00	45.7 ± 1.12	9.04 ± 0.30	0.45 ± 0.03
ANOVA (P- value)	Permeability		** (*)	n.s.	n.s.	n.s.	** (*)	n.s.	n.s.
	Prop		* (n.s.)	n.s.	n.s.	n.s.	n.s.	*** (**)	n.s.
	Permeability x Prop		n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. not significant; values within a column with a different superscript were significantly different (Tukey HSD, $p_{adj} < 0.05$). In brackets: FDR-adjusted p-values.

Table 3

Mean values and standard deviation of formic acid, acetic acid, propionic acid, butyric acid, caproic acid, and total volatile carboxylic acids in the experimental Swiss-type cheeses (120 days), manufactured with two different propionic acid bacteria cultures and ripened within three different plastic films and a control with natural dry-ripening without plastic film. The cheeses were manufactured over two days. The results of the production day are not shown.

Factors	Level	N	Formic acid [mmol kg ⁻¹]	Acetic acid [mmol kg ⁻¹]	Propionic acid [mmol kg ⁻¹]	Butyric acid [mmol kg ⁻¹]	Caproic acid [mmol kg ⁻¹]	Total volatile carboxylic acids [mmol kg ⁻¹]
Permeability	Natural	4	0.162 ± 0.037	56.2 ± 7.59	104 ± 10.7	0.675 ± 0.178	0.252 ± 0.091	161 ± 18.1
	198	4	0.210 ± 0.139	54.1 ± 2.09	100 ± 5.67	0.728 ± 0.132	0.282 ± 0.068	156 ± 5.32
	576	4	0.178 ± 0.066	55.4 ± 3.59	102 ± 2.91	0.775 ± 0.055	0.295 ± 0.035	159 ± 3.67
	1050	4	0.215 ± 0.118	55.2 ± 3.72	101 ± 1.32	0.902 ± 0.083	0.325 ± 0.031	158 ± 3.47
Culture	Prop 96	8	0.21 ± 0.11	52.08 ± 3.11	102.22 ± 6.75	0.75 ± 0.14	0.28 ± 0.07	156 ± 9.9
	Prop 01	8	0.17 ± 0.05	58.36 ± 2.26	101.36 ± 3.95	0.79 ± 0.13	0.30 ± 0.05	161 ± 6.1
ANOVA (P- value)	Permeability		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Prop		n.s.	** (*)	n.s.	n.s.	n.s.	n.s.
	Permeability x Prop		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. not significant. In brackets: FDR-adjusted p-values.

Table 4

Mean values and standard deviation of succinate, dissolved CO₂, calculated CO₂ based on propionic acid (C3) and succinate, CO₂ diffused out of the cheese, relative eye volume, eye number, and thickness of the eyeless border zone in the experimental Swiss-type cheeses (120 days), manufactured with two different propionic acid bacteria cultures and ripened within three different plastic films and a control without film. The cheeses were manufactured over two days. The results of the production day are not shown.

Factors	Level	N	Succinate [mmol kg ⁻¹]	CO ₂ dissolved [mmol kg ⁻¹]	CO ₂ calculated from C3 and succinate [mL kg ⁻¹]	Estimated CO ₂ diffused out of the cheese [mL kg ⁻¹]	Rel. eye volume [%]	Eye number [–]	Eyeless border zone (10 eyes from outside) [mm]	Eyeless border zone (10% eyes from outside) [mm]
Permeability	Natural	4	8.48 ± 5.64	32.6 ± 1.66 ^a	1254 ± 160	429 ± 102 ^a	9.22 ± 3.84 ^a	426 ± 191 ^a	6.7 ± 1.08 ^b	9.36 ± 0.79 ^b
	198	4	8.10 ± 4.84	29.0 ± 2.39 ^a	1214 ± 45	416 ± 65 ^a	13.6 ± 2.26 ^b	570 ± 56.2 ^a	0.74 ± 0.53 ^a	2.20 ± 0.58 ^a
	576	4	8.62 ± 5.52	29.0 ± 1.84 ^a	1236 ± 56	485 ± 17 ^a	9.91 ± 2.23 ^a	683 ± 135 ^b	1.60 ± 1.50 ^a	3.58 ± 2.20 ^a
	1050	4	9.05 ± 5.96	24.6 ± 2.74 ^b	1238 ± 65	596 ± 106 ^b	8.97 ± 1.41 ^a	711 ± 74.8 ^b	1.88 ± 2.02 ^a	4.34 ± 2.98 ^a
Culture	Prop 96	8	3.91 ± 0.49	28.71 ± 2.89	1189 ± 79	459 ± 70	8.47 ± 2.16	534 ± 139	2.887 ± 2.80	5.004 ± 3.45
	Prop 01	8	13.19 ± 1.63	28.89 ± 3.92	1283 ± 54	504 ± 118	12.39 ± 2.18	661 ± 147	2.586 ± 2.46	4.734 ± 2.85
ANOVA (P- value)	Permeability		n.s.	**	n.s.	**	**	* (n.s.)	** (*)	**
	Prop		***	n.s.	0.0585 (0.151)	n.s.	***	0.060 (0.151)	n.s.	n.s.
	Permeability x Prop		n.s.	0.0518 (0.220)	n.s.	** (*)	n.s.	n.s.	n.s.	n.s.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. not significant; values within a column with a different superscript were significantly different (Tukey HSD, $p_{adj} < 0.05$). In brackets: FDR-adjusted p-values.

larger moisture flow and therefore moisture loss. As will be explained later, the culture Prop 01 produces higher amounts of CO₂. The type of PAB culture also had an effect on proteolysis; as already observed in other cheese trials and in practice, Prop 96 often had a greater impact on the proteolysis rate than Prop 01 (Fröhlich-Wyder et al., 2002). Taken together, these preliminary results suggest that plastic film ripening has

a significant influence on water loss by reducing it during cheese ripening.

3.3. Propionic acid fermentation

As shown in Fig. 1, eye formation varied between some of the

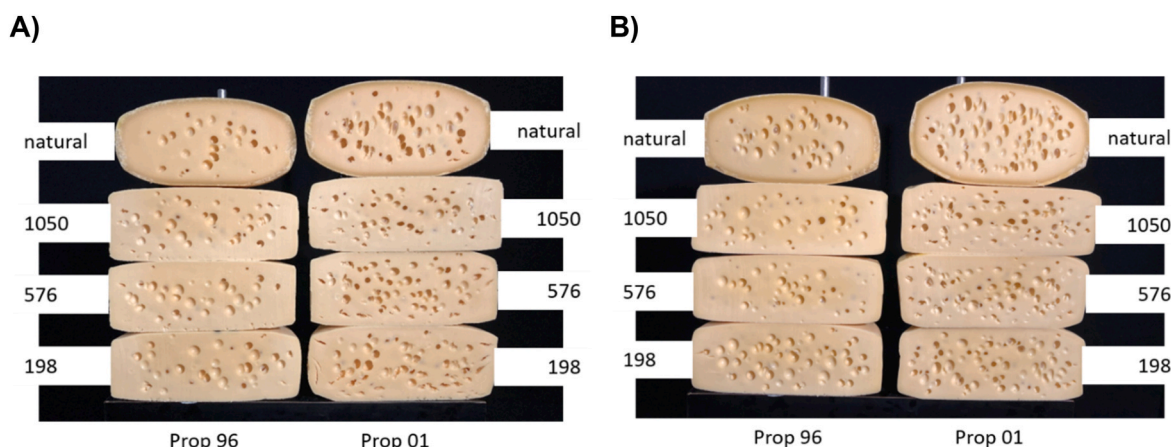


Fig. 1. Cross sectional view of the ripened (120 days) experimental Emmentaler cheeses manufactured with one of two different propionic acid bacteria cultures—Prop 96 (left in both views) or Prop 01 (right). The samples were ripened within a plastic film of defined CO₂-permeability (from bottom to top 198, 576, and 1050 cm³ m⁻²·d⁻¹·bar⁻¹ or with natural dry-ripening without film, respectively). A: Cheeses 1–8 manufactured on day 1; B: Cheeses 9–16 manufactured on day 2.

samples, which prompted the question of whether similar variations could be observed for propionic acid fermentation. The volatile carboxylic acids are summarised in Table 3. As expected, acetic and propionic acids were the main volatile acids in this experiment, but their values were not significantly affected by the ripening conditions of the natural versus plastic film. Only acetic acid was significantly higher in the Prop 01 samples, which could be attributed to its high aspartase activity. The simultaneous fermentation of lactate and aspartate leads not only to propionic acid and acetic acid but also to succinate, as shown in Table 3, ammonia, and to additional acetic acid and CO₂ (Turgay et al., 2020). The low levels of butyric acid (≤ 1 mmol kg⁻¹) proved that no interference fermentation by butyric acid bacteria was involved during the ripening of the cheese samples.

Notably, we observed an interesting trend: ripening without a film induced slightly higher levels of acetic, propionic, and total carboxylic acid. The water loss may have been responsible for a concentration of these carboxylic acids. However, the production of volatile carboxylic acids (representing fermentation) was not significantly influenced by the treatments. To summarise, plastic film ripening had no effect on propionic acid fermentation and, hence, on total CO₂ formation.

3.4. Estimated CO₂ diffusion

At the end of ripening, the production rate of CO₂ was probably almost zero, as the precursors (D-/L-lactate) were completely consumed, and an equilibrium state between CO₂ enrichment and diffusion was expected. During ripening, the cheese matrix was enriched with CO₂ before eye formation, but some CO₂ was lost by diffusion through the plastic film or the dry surface. CO₂ is therefore allocated in three ways and is found in the cheese eyes, dissolved in the cheese matrix, and diffused out of the cheese. The CO₂ dissolved in the cheese matrix was analytically measured, the CO₂ in the cheese eyes was represented by the relative eye volume, and the CO₂ diffused out of the cheese was estimated by the metabolic products from propionic acid fermentation, as described in Section 2.5.

Our results show that the quantity of dissolved CO₂ in the cheese matrix depended on the type of surface permeability: the higher the permeability, the lower the amount of dissolved CO₂ in the cheese matrix, and vice versa (Fig. 2a). Even though the trend, as shown in Fig. 2a, looks straightforward, only the cheeses ripened in plastic film with the highest permeability showed significantly less dissolved CO₂ compared to the other variants (Table 4).

The range we found in our experiment for dissolved CO₂ of 22–32 mmol kg⁻¹ in the matrix of cheeses with propionic acid

fermentation corresponds with those reported by Martley and Crow (1996) and Fröhlich-Wyder et al. (2017). Pauchard et al. (1980) observed that the saturation limit was 37 mmol kg⁻¹ but that eye formation started already at only 13–18 mmol kg⁻¹. They supposed that local saturation was sufficient to start eye formation. Martley and Crow (1996) reported that saturation of the cheese matrix with CO₂ was >18–36 mmol kg⁻¹ and a prerequisite for eye formation. This wide range is probably due to the fact that eye formation does not begin only once the cheese body has reached absolute saturation.

The dissolved CO₂ mean value of ~29 mmol kg⁻¹ for the lowest permeability was rather close to the dry-ripened cheese, with a mean value of ~32 mmol kg⁻¹, showing that the dry-ripened cheese produced a relatively impermeable rind during ripening as a consequence of moisture loss (Table 4). This observation was also supported by visual inspection of the cross-sectional views of the different cheeses after 120 days of production (Fig. 1). The developed rind was quite hard, thick, and clearly distinguishable by a darker colour. Bisig et al. (2019) compared the border and core zones of dry ripened experimental cheeses and found that the border zone had lower moisture and higher salt content than the core zone.

The cheeses with the highest permeability (1050 cm³ m⁻²·24 h⁻¹ bar⁻¹) showed a significantly reduced amount of dissolved CO₂ in the cheese matrix of barely 25 mmol kg⁻¹ (Fig. 2a and Table 4). An interesting interaction was observed, as Fig. 2 clearly shows ($p = 0.0518$): The cheeses produced with PAB culture Prop 01 and ripened in the plastic film with the highest permeability had the lowest CO₂ dissolved in the cheese matrix. The two cultures are very distinct cultures, especially in their aspartase activity, with Prop 01 being the more active culture producing more CO₂ faster. The lower amount of CO₂ dissolved in the cheese matrix therefore indicates a higher estimated amount of CO₂ diffused out of the cheese, as Fig. 2b suggests. Prop 01, with its more intense propionic acid fermentation, was responsible for the higher estimated diffusion of CO₂ out of the cheeses ripened with the most permeable plastic film.

Fig. 3 shows an overview of the estimated CO₂ percentage balance between diffusion out of the cheese, dissolution in the cheese matrix, and entrapment within the cheese eyes. It is arguable that each ripening condition—natural dry ripening or ripening with the three plastic films—has a unique pattern. Natural dry ripening stood out for the highest amount of dissolved CO₂, nearly 60% remained in the cheese matrix. The plastic film with the lowest permeability (“198”) had the highest amount of CO₂ entrapped in the eyes: on average 12% compared to 7%–8% for the remaining variants. The plastic film with an intermediate permeability (“576”) showed corresponding values that fell

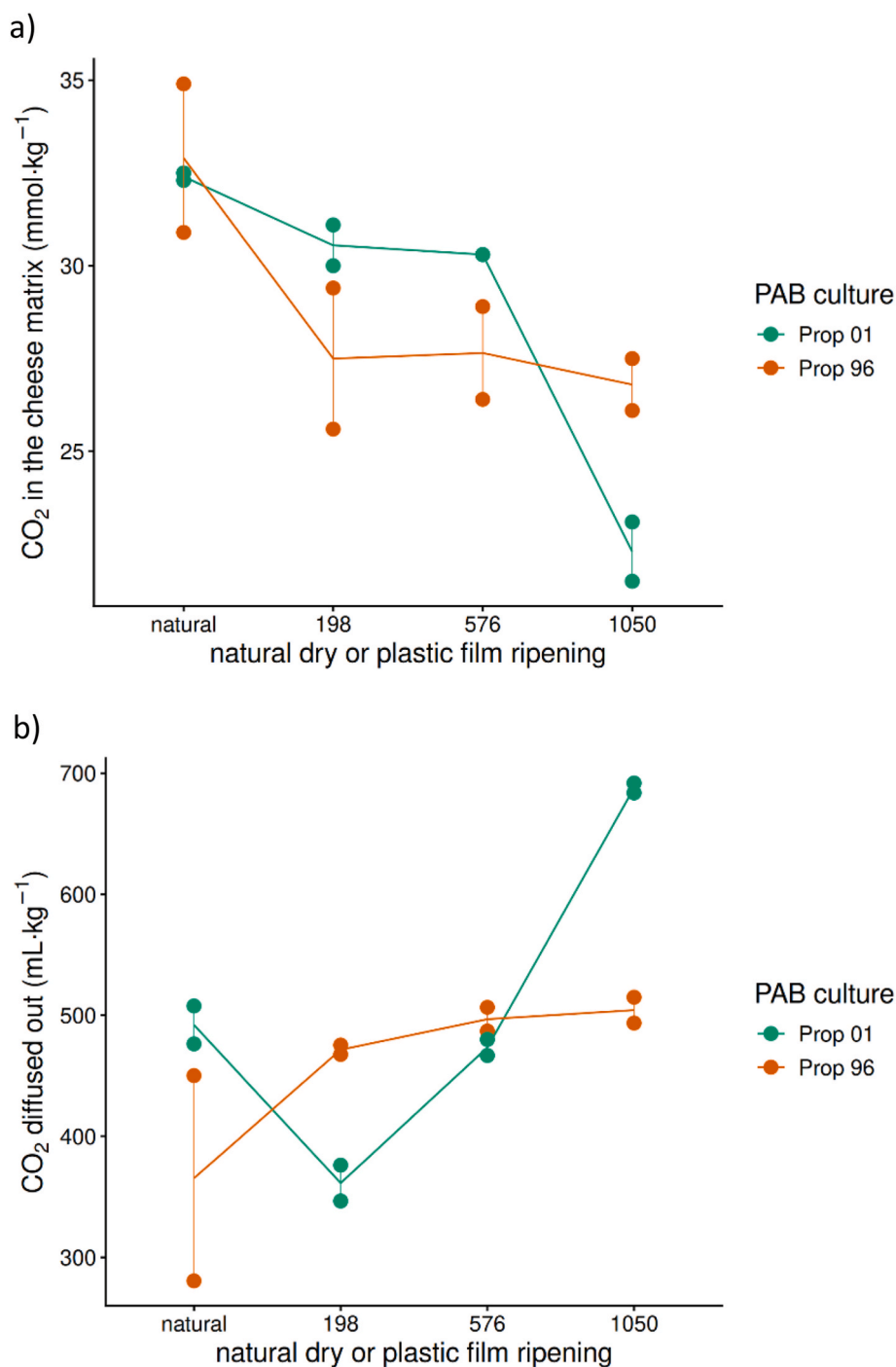


Fig. 2. Carbon dioxide dissolved in the cheese matrix (a) and diffused out of the cheeses (b) for experimental Emmentaler cheeses manufactured with either propionic acid bacteria culture Prop 96 or Prop 01 and ripened for 120 days with natural dry-ripening (without plastic film) or within a plastic film of three different CO₂-permeabilities (198, 576, or 1050 cm³ m⁻²·d⁻¹·bar⁻¹, respectively). CO₂ dissolved in the cheese was measured by an infrared-based method, and CO₂ diffused out of the cheeses was calculated from the metabolites propionate and succinate. The interaction in panel (a) had a p-value of $p = 0.0518$, and the interaction in panel (b) was significant, with $p < 0.01$.

between the two extremes; these cheeses had less CO₂ dissolved in the cheese matrix and slightly higher estimated diffusion values. Finally, the cheeses ripened in the plastic film with the highest permeability ("1050") stood out for nearly equal diffused (estimated ~48%) and dissolved (~45%) CO₂ quantity.

Obviously, it is possible to influence the degree of saturation with CO₂ not only by the cheese matrix, as discussed in the introduction (fat, water, protein content, NaCl), but also by a diffusion barrier (plastic films). Importantly, different PAB cultures may react differently to

diffusion barriers, although the reason remains unclear.

3.5. Eye formation

The findings concerning eye formation are described by the values of the relative eye volume and the total eye number. Significantly greater eye volume was found (Table 4, Fig. 4a) for the lowest plastic film permeability (198 cm³ m⁻²·24 h⁻¹ bar⁻¹). An average value of 13.6% of relative eye volume is quite a high value and is not often encountered.

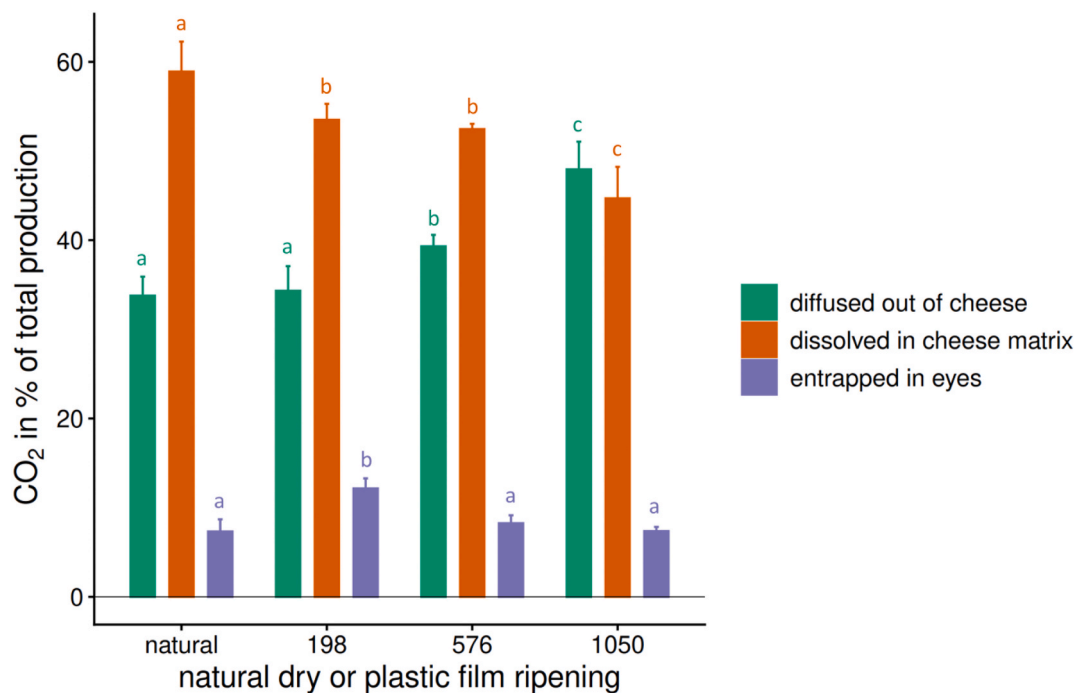


Fig. 3. Carbon dioxide distribution for experimental Emmentaler cheeses manufactured with either propionic acid bacteria culture Prop 96 or Prop 01 and ripened for 120 days with natural dry-ripening (without plastic film) or within a plastic film of three different CO₂-permeabilities (198, 576, or 1050 cm³ m⁻² d⁻¹ bar⁻¹, respectively). The three volume parts CO₂ dissolved in the cheese, CO₂ in the openings, and CO₂ diffused out of the cheese were measured by an infrared-based method, assessed by CT, or calculated from the metabolites propionate and succinate, respectively. Different letters within the same parameter indicate significant differences (n = 4).

Emmentaler-type medium-fat cheeses may have high eye volumes; Fröhlich-Wyder and co-workers observed eye volumes of >16% (Fröhlich-Wyder et al., 2023). However, in a normal dry-ripened experimental Emmentaler cheese, values of 5–7% relative eye can be expected when about 1/4 of the lactate was still available and not completely fermented after 130 days, using Prop 96 (Fröhlich-Wyder et al., 2023; Guggisberg et al., 2015).

Reports on total eye volume in the literature are scarce. Two studies report eye volumes, albeit in cheeses without propionic acid fermentation. Lee et al. (2012) found values from 1 to 10% for Gouda-type cheeses. The sample with 10% relative eye volume was presumably an outlier, showed abnormal and unwanted butyric acid fermentation, and could not be counted as a good reference. Therefore, 0.5–1.5% can be used as a reference for Gouda cheese. An Appenzeller-type cheese was found to have 0.44–1.07% eye volume, depending on the fat content (Fröhlich-Wyder et al., 2023).

A range of 9–10% relative eye volume was found for the remaining plastic films with a higher permeability of 575–1050 cm³ m⁻² d⁻¹ bar⁻¹, which confirms the trends observed by Kraggerud et al. (2009): the lower the permeability of the plastic film, the higher the eye volume (Fig. 4a). However, the results obtained by Kraggerud et al. (2009) were not significant but showed a clear trend. The sample without a plastic film (control) reached values comparable to those of the cheeses with a plastic film of high and middle permeability, but the values fluctuated. Even though the naturally ripened cheese was found to have the surface with the lowest permeability (see Section 3.4), it was not the variant with the highest eye volume (but it had the highest loaves, Fig. 1): most of the produced CO₂ remained dissolved in the cheese matrix (Fig. 3). By contrast, the number of eyes was lowest in the naturally ripened cheeses and increased with the increasing permeability of the surface (Fig. 4b). This is an interesting and unexpected observation because hay particles were added to the cheese milk to standardise the number of eye nuclei. High number and low total volume of eyes indicate smaller openings, which can be seen in

the visualised spatial distribution of the cheese eyes by two-dimensional CT images in Fig. 5: the variant plastic film “198” seemed to have the largest eyes, several because of cracking or coalescent openings. In fact, the openings in the cheeses ripened with the ‘198’ plastic film were significantly larger than those in naturally dry-ripened cheeses and the remaining two types of plastic film (see Fig. 6). The smallest eyes were found in the cheeses with highest surface permeability. Unfortunately, the software tool VGStudio Max that was used could not count all individual cheese eyes, as some of these eyes were connected to other eyes, so that some connected eyes were counted as only one opening. Under these conditions, the values for the number of cheese eyes were less trustworthy but still allowed some relationship to the permeability of the cheese surface to be observed.

Together, these observations suggest that the plastic film with the lowest permeability was responsible for the highest total and individual eye volume; the highest eye numbers and smallest eyes were observed in the cheeses ripened in the two plastic films with high permeability.

3.6. Eyeless border zone

Variation in the eyeless border zone (the zone where no eyes were formed) was studied in relation to permeability (Table 4). The eyeless border zone from the 10 outermost eyes in the plastic film ripened experimental cheeses increased significantly by increasing permeability (0.74 mm–1.88 mm). Thus, when the plastic film had only a small permeability, the cheese eyes could also form close to the surface as a result of the reduced diffusion of CO₂ out of the cheese (Table 4). For the cheese without a plastic film (control), the cheese eyes could not grow in a larger border zone of around 7 mm. It can be hypothesised that at the beginning of the ripening, the diffusion of CO₂ out of the cheese was bigger in that zone than the chance to start eye growth. The large eyeless zone is clearly visible in Fig. 4. A similar relationship was observed by calculating the eyeless border zone with 10% of the outermost eyes (Table 4).

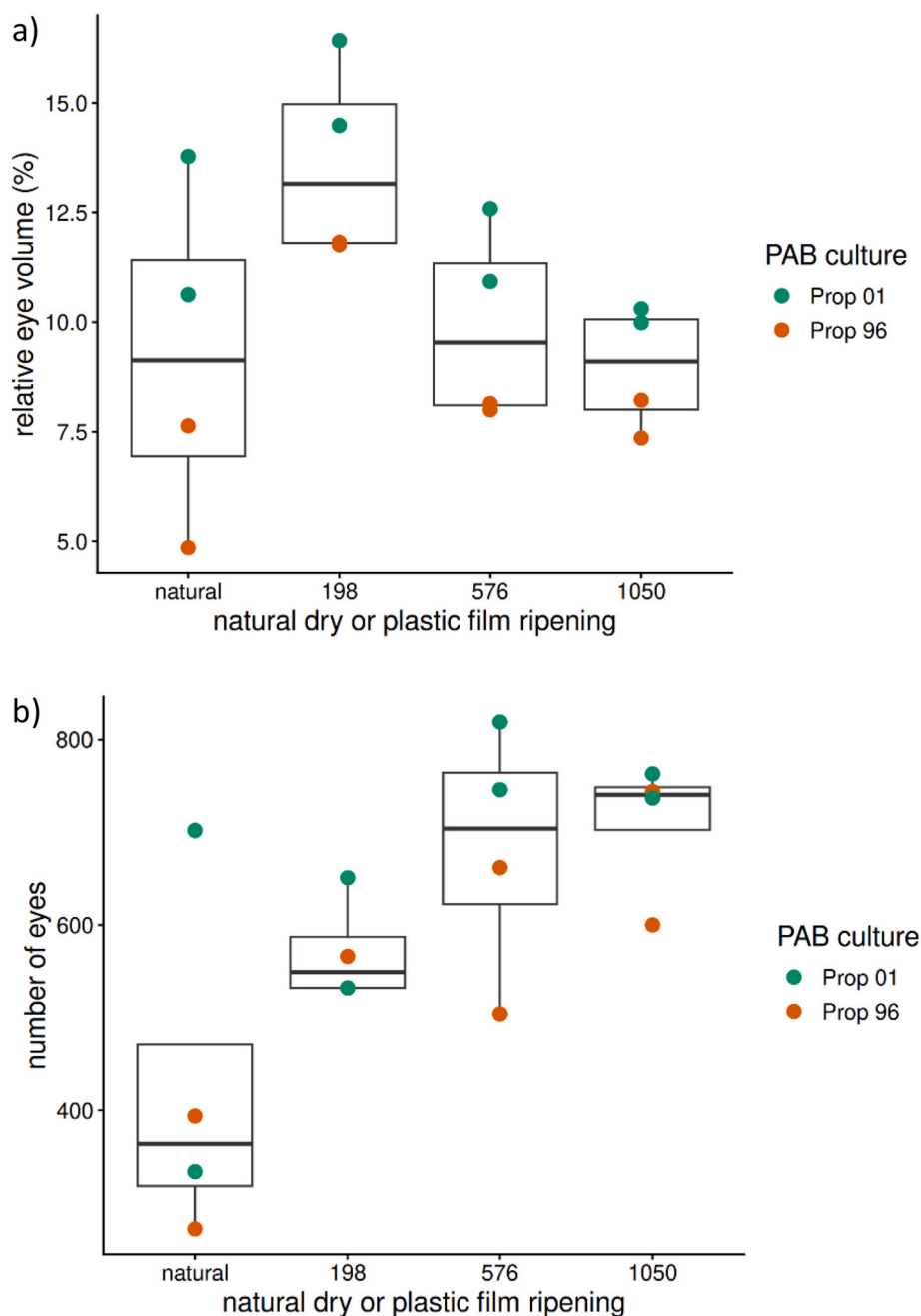


Fig. 4. Relative eye volume (a) and number of eyes (b) for experimental Emmentaler cheeses manufactured with either propionic acid bacteria culture Prop 96 or Prop 01 and ripened for 120 days with natural dry-ripening (without plastic film) or within a plastic film of three different CO₂-permeabilities (198, 576, or 1050 cm³ m⁻²·d⁻¹·bar⁻¹, respectively). Both relative eye volume and eye number were measured by CT. The p-values can be found in [Table 4](#).

Apart from CO₂, water also diffused out of the cheese during ripening, especially in the naturally ripened cheeses. The water loss in the outer zone of these cheeses led to a dense matrix of protein and fat, as shown in [Fig. 1](#). This explains why eyes in the centre of a cheese are usually larger and more numerous than those in peripheral zones ([Huc, Challoy, et al., 2014](#)). Furthermore, this zone usually has higher NaCl contents, which inhibits the growth of PAB and, thus, leads to less CO₂-production ([Bisig et al., 2019; Huc et al., 2014a, 2014b](#)). [Pauchard et al. \(1980\)](#) observed that the CO₂ concentration near the surface was lower than in the centre of the cheese. Thus, the eyeless border zone was especially large in the natural dry ripened cheeses but not in the plastic film ripened cheeses, where eyes could form near the cheese surface. This zone can be regarded as one main reason why the dry ripened control cheeses showed the fewest eyes, despite the addition of hay

particles to standardise eye formation.

4. Conclusion

Considerable progress has been made in recent years in understanding eye formation in cheeses, including improved standardisation of the conditions for eye formation by adding eye nuclei, adjusting the protein-to-fat ratio and combining X-ray computed tomography with advanced image analysis to quantify the individual factors that influence eye formation. These advances make it possible to develop cheese varieties with tailor-made eye formations. In the present study, we investigated the effects of surface permeability on eye formation in cheese made with two PAB cultures forming different amounts of CO₂ by applying three different plastic films and natural dry ripening. The

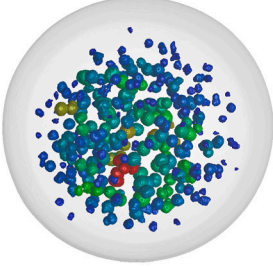
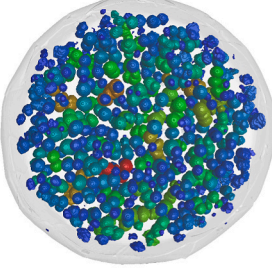
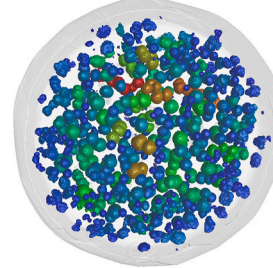
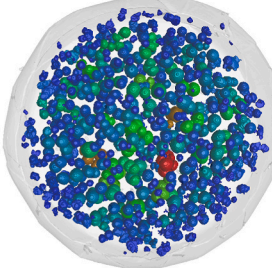
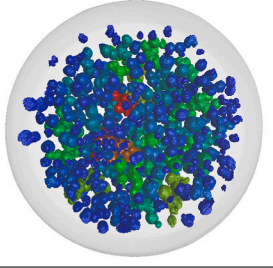
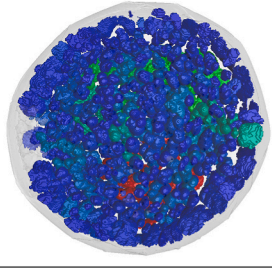
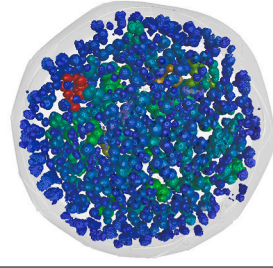
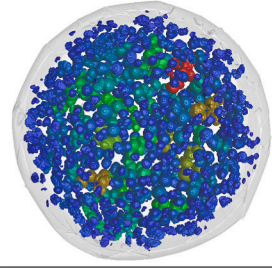
	Natural dry ripening	Plastic film CO ₂ permeability [cm ³ ·m ⁻² ·d ⁻¹ ·bar ⁻¹]		
	Natural	198	576	1050
Prop 96				
eye-number / rel. volume	272 / 4.9%	532 / 11.8%	504 / 8.0%	744 / 8.2%
Prop 01				
eye-number / rel. volume	334 / 10.6%	532 / 14.5%	819 / 10.9%	763 / 10.0%

Fig. 5. CT images of experimental Emmentaler cheeses manufactured on day 1, either with propionic acid bacteria culture Prop 96 or Prop 01, and ripened with natural dry-ripening without plastic film or within a plastic film of the indicated CO₂-permeability during 120 days. The number of openings and the relative opening volume (total opening volume expressed as a percentage of the total cheese volume) are the averages of the two replicates produced on day 1 and day 2.

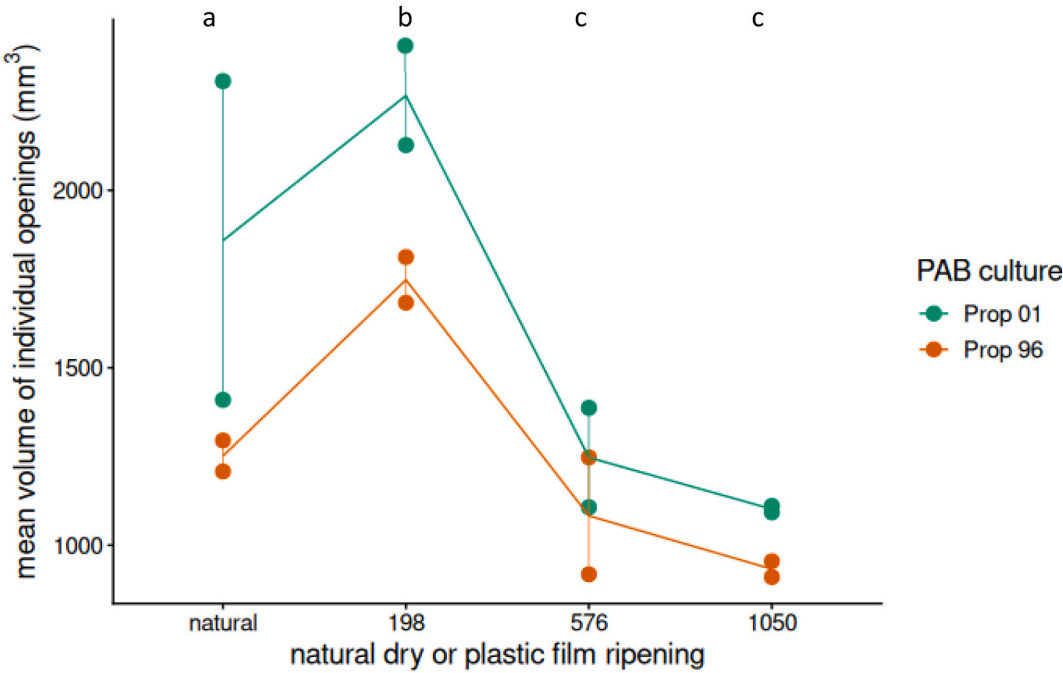


Fig. 6. Mean volume of individual eyes in the experimental Emmentaler cheeses manufactured with either propionic acid bacteria culture Prop 96 or Prop 01 and ripened for 120 days with natural dry-ripening (without plastic film) or within a plastic film of three different CO₂-permeabilities (198, 576, or 1050 cm³ m⁻² d⁻¹ bar⁻¹, respectively). The individual eye volumes were measured by CT. Different letters indicate significant differences (n = 4) at p < 0.001.

results clearly demonstrate that varying permeability of the plastic films influences CO₂ diffusion, with impact on the amount of eye formation and the size of the eyeless border zone. Increased permeability resulted in increased estimated CO₂ diffusion out of the cheese, lower CO₂

dissolution in the cheese matrix, decreased eye volume, and decreased eyeless border zone thickness. Interestingly, the number of openings increased significantly, which led to generally smaller openings.

The naturally dry-ripened cheeses behaved differently from the film-

ripened cheeses. Based on our experience, we assume that during the first phase of ripening, the permeability of the natural rind is higher. As this first phase is crucial for eye formation, fewer cheese eyes are formed, and total eye volumes are lower. As ripening advances with time, the rind becomes drier, harder, and more compact, resulting in low CO₂ permeability and an increased risk of defective eye formation as CO₂ production goes on. Such defects can be oval eyes, splits, or cracks, as observed in the experimental Emmentaler cheeses (Fig. 1). Our findings suggest that during this phase, the rind was not only more compact, preventing CO₂ from diffusing out of the cheese, but it also became more rigid, inhibiting the cheese from growing even higher. Both factors acted as a counterforce for the surplus CO₂, which subsequently remained in the cheese but did not find its way to an opening. The CO₂ dissolved in the cheese matrix was highest in the naturally ripened cheeses. It could be recommended that measures to promote rind formation be taken in the early stages, followed by more humid conditions in the later stages to allow CO₂ to diffuse out of the cheese.

The present study confirms that eye formation in cheese is a complex dynamic process that is strongly influenced by the properties of the applied ripening conditions, such as dry-ripening or the use of plastic films with different permeabilities. The permeability of the applied plastic film or coating must be considered, especially during the ripening of cheeses with openings. Thus, CO₂ permeability of the ripening film could be an option for controlling eye formation in Swiss-type cheese. However, when choosing a highly permeable plastic film, caution should be taken, as the employed PAB culture might have a significant impact on CO₂ diffusion but not necessarily on eye formation.

CRediT authorship contribution statement

Walter Bisig: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization. **Dominik Guggisberg:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Lotti Egger:** Methodology, Writing – review & editing. **Marie-Therese Fröhlich-Wyder:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that there is no financial interest that could have influenced the work in this paper.

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Data availability

Data will be made available on request.

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