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Mechanism and control of the eye formation in cheese

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ABSTRACT

The production of Swiss-type cheeses with a typical number, size, and distribution of eyes is a difficult task, especially when bactofuged or microfiltrated milk is utilised. In this study, the potential of microparticles (plant origin) to influence eye formation in cheese, was assessed. Eight experimental Emmental cheeses were produced with one replicate from microfiltrated milk with addition of 0.0625–4.000 mg of powdered hay to the milk (90 L) and ripened for 130 days. Eye formation was quantified by means of X-ray computed tomography (between 30 and 130 days). The contents of fat, water, citric acid, lactic acid, and volatile carboxylic acids were determined at 130 days. The results demonstrate that microparticles of plant origin act as eye nuclei that control the number ($P < 0.001$) and size of the eyes in cheese in a dose-dependent manner. The findings also provide new insights into the formation of eye defects.

1. Introduction

The size, number, shape, and distribution of eyes in Swiss-type cheese, such as Emmental, are crucial quality parameters. The eye formation in Swiss-type cheese is mainly the result of propionic acid fermentation during warm room storage, involving the conversion of lactate into propionate, acetate, and CO_2 (Fröhlich-Wyder & Bachmann, 2004; Thierry et al., 2010). The formation of eyes is also a characteristic feature of various other cheese varieties without propionic acid fermentation, such as Gouda, Appenzeller, or Tilsit cheese, although the eyes are smaller.

Quality assessment of eye formation is traditionally carried out by listening to the type of sound made while tapping with a special hammer on the surface of a cheese loaf, by visual inspection of a small cylinder of cheese using a cheese trier, or by viewing a section of a cheese loaf cut into halves. These methods, however, are highly dependent on professional skills and are not quantitative. Newer methods of non-invasive imaging, using ultrasound (Albrecht, Ulmann, & Bosset, 1998; Conde, Mulet, Clemente, & Benedito, 2007; Eskelinen, Alavuotunki, Haeggström, & Alatossava, 2007), magnetic resonance imaging (MRI: Huc et al., 2014a; Musse, Challois, Huc, Quellec, & Mariette, 2014; Rosenberg, McCarthy, & Kauten, 1992), X-ray (Akkerman, Walstra, & Van Dijk, 1989; Blanc & Hättenschwiler, 1973; Kraggerud, Wold, Hoy, & Abrahamsen, 2009) and X-ray computed tomography (X-ray CT) (Guggisberg et al., 2013; Lee et al., 2012; Schuetz et al., 2013; Strand, 1985) are applied for the investigation of eye formation in cheese, in particular for growth kinetics, size, number, spatial distribution, and defects of cheese eyes.

The phenomenon of eye formation in cheese has attracted the attention of scientists since the early beginnings of cheese research. A review of Clark (1917) on the formation of eyes in Emmental cheese shows that very precise ideas about the formation of eyes in cheese already existed at the beginning of the last century. Although important sources of gas production in cheese, such as propionic acid fermentation or butyric acid fermentation, had not been discovered yet, bacterial action was assumed to be the cause of gas production in cheese. Former cheese scientists differentiated already between normal holes,

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blow holes, thousand eyes, and pin holes and tried to identify the factors that influence the number, size, and distribution of the eyes in cheese. At that time, the unequal number and size of eyes was mainly associated with differences in the distribution and growth of bacteria in cheese. However, this assumption was questioned when experiments showed that the whole cheese body contained considerable amounts of CO_2. Clark postulated that the formation of eyes in cheese would occur at "favoured localities" that have no necessary relation to bacterial growth. He hypothesised that eye formation in cheese could be a phenomenon similar to crystallisation from a supersaturated solution, where the start of the crystal growth is imperatively triggered by small "seeds" or "irregularities" and the size of the final crystals is dependent on the number of seeds added. As a second example, Clark mentioned the formation of raindrops in a vapour-saturated atmosphere by the presence of "dust particles". However, the identification of the nature of the eye nuclei remained an unsolved question (Thierry et al., 2010).

An increase in the production of CO_2 in the warm room generates an overpressure (2–4 kPa) in the cheese body, which causes the gas to begin to diffuse into the eye nuclei and form small eyes (Flückiger, 1980). Martley and Crow (1996) found that saturation of the cheese matrix with CO_2 gas (>18–36 mmol kg^{-1}) is a prerequisite for eye formation and suggested that microscopic air bubbles trapped in the curd serve as "sites" or "nuclei" into which CO_2 dissociates from the soluble state in the cheese body. Polychroniadou (2001) mentioned other factors such as nitrogen from milk, CO_2 from starters, solid microparticles, and small mechanical openings that could act as eye nuclei.

A saturation of the cheese body with CO_2 can only be realised by a high rate of CO_2 production and a relatively low rate of diffusion out of the Emmentaler-type cheese. The intense production of CO_2 by propionibacteria starts with a delay of about 30 days during the warm room storage (21–23 °C) and is slowed down after 60 days by cooling the cheese to a temperature of about 11 °C (Fröhlich-Wyder & Bachmann, 2004; Thierry et al., 2010). Fröhlich-Wyder, Bachmann, and Casey (2002) showed that the strain-dependent aspartase activity of propionic acid bacteria (PAB) and their interactions with proteolytic lactic acid bacteria and facultatively heterofermentative lactobacilli strongly influence the formation of CO_2. They recommended the use of adapted culture systems to control the average size of the eyes and improve the storage quality of Emmentaler PDO (protected designation of origin) cheese.

Certain treatments of cheese milk (cleaning filtration in milking systems or cheese dairies prior to cheese making; centrifugation; bactofugation; and microfiltration) are known to affect the openness of cheese and usually lead to a drastic reduction of the number of eyes, which can entail a downgrading of the quality of cheeses (Fragnière & Schafroth, 2004; Thierry et al., 2010). Water, fat, and the high calcium content in Emmental curd contribute to its soft, elastic structure, which is an important prerequisite for the formation of eyes. However, elevated water content accelerates proteolysis and thereby makes the cheese body brittle in the later ripening stage, which provokes the formation of cracks and reduces the storage quality of Swiss-type cheese (Fröhlich-Wyder & Bachmann, 2007). Apart from processing and composition, the season also seems to influence the openness of cheese. Kurmann, Gehriger, and Kaufmann (1975) reported that the transformation of winter milk increased the risk of an excessive number of small eyes in Emmentaler PDO cheese. In contrast, cheeses produced during the summer season tend to have a lower number of eyes. We reasoned that hay and grass feeding could affect the entry of microparticles into raw milk and hypothesised that dust particles originating from hay could act as highly effective eye nuclei and induce the formation of eyes in cheese. The objective of the present study was therefore to assess the ability of powdered hay to "seed" eyes in cheese and to understand the influence of the hay powder concentration on the number and volume of the formed cheese eyes.

2. Material and methods

2.1. Production of experimental Emmental cheeses

Raw milk was filtered in the milking equipment on the farms with milk filter socks made of mixed cellulose-synthetic fabric having a pore size of 100 μm. After milk collection, skimming and further cleaning was carried out in a commercial dairy with a centrifuge Westfalia MSD 50 (GEA Westfalia, Oelde, Germany) at 40 °C and 6250 rev min^{-1} (6650 × *g*). Skimmed milk was transported to the experimental cheese plant and microfiltrated at 50 °C through a 1.4 μm membrane (Membralox, Pall Corporation, Saint-Germain-en-Laye, France) at 240 kPa. Heat-treated cream (70 °C, 10 s) was added to the microfiltrated skim milk to a fat content of 35 g kg^{-1}. Eight variants of experimental Emmental cheese were produced in parallel at the Agroscope pilot plant (Bern, Switzerland) from the same batch of microfiltrated and standardised milk as follows: after the addition of water (8 L), the milk (90 L) was warmed to 31 °C. The milk was inoculated with 2‰ (v/v) of a bulk starter containing strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *lactis* (RMK 101, Agroscope). After pre-ripening (32 °C, 30 min), the milk was inoculated with a commercial culture of *Propionibacterium freudenreichii* (PAB, Prop 96, Agroscope) at a level of approximately 10^3 cfu mL^{-1}. Milk was coagulated in approximately 35 min and the coagulum was cut into grains of about 0.3–0.5 cm in 15 min. After the addition of water (1 L), the mixture of curd grains and whey was warmed to 53 °C in 30 min, scalded at 53 °C for 35 min, poured into the mould, pressed (50–25 °C, 1 day), and brine-salted (12 °C, 1 day) to achieve a salt content of 5 g kg^{-1}. The cheeses (30 cm in diameter, between 6 and 7 kg) were initially stored at a cool temperature (12 °C) for 10 days, then stored in a warm room (22 °C, 80% relative humidity (RH)) for 60 days, and finally ripened in the cool room (12 °C, 70% RH) to the age of 130 days. To study the effect of microparticles of a size of <100 μm originating from powdered hay on eye formation, a suspension containing 10 mg of powdered hay (Agroscope) in 100 mL tap water was prepared in a flask with a screw cap. The microparticles were kept in suspension by continuous shaking, and different volumes of the suspension were transferred into the individual milks at the beginning of cheese making as listed in Table 1. A second run (randomly distributed) of the eight variants of

Table 1
Dosage of the added hay powder to cheese milk before the production of the experimental Emmental cheeses.[a]

Cheese variants	Volume (mL per 90 L milk)	Dose (mg per 90 L milk)
Control	0.000	0.000
Variant 1	0.625	0.0625
Variant 2	1.25	0.125
Variant 3	2.5	0.25
Variant 4	5.0	0.50
Variant 5	10.0	1.0
Variant 6	20.0	2.0
Variant 7	40.0	4.0

[a] Volume is the volume of hay powder suspension added to cheese milk; dose is the estimated dose of hay powder in cheese milk.

experimental Emmental cheeses was produced in the same week, using the same procedure as described above.

2.2. Analysis of cheese composition

The fat content of the cheese was determined by the Gerber–van Gulik method (ISO, 2008). Water content was determined with the dry matter method (IDF, 2003) by calculation of the weight difference of the cheese sample after drying at 102 °C for 4 h. Volatile carboxylic acids (C1–C6) were analysed by gas-chromatography and a flame ionisation detector with headspace technology after esterification with ethanol, as described by Fröhlich-Wyder et al. (2013). All measurements were carried out in duplicate. Total lactic acid and citrate were analysed enzymatically according to the instruction protocol of the kit manufacturer (R-Biopharm, Darmstadt, Germany).

2.3. X-ray computed tomography measurement

X-ray CT measurements of all the cheeses were carried out during their ripening (at four different ripening stages: day 30, day 45, day 60, and day 130), using a CT scanner (Somatom Volume Zoom, Siemens, Zürich, Switzerland) with the following scan parameters: 120 kV, 60 mA s and 0.5 mm slice thickness. The cheese was placed on a Styrofoam–Plexiglas support to be properly and reproducibly positioned. The pixel size in each slice was adjusted to each cheese individually, ranging from 0.66 mm to 0.62 mm. The individual pixel spacing was automatically calculated, as determined by the field of view. The cheeses were directly transported from the cool/warm room (12 °C/22 °C) to the CT scanner and packed in a plastic bag, but not vacuum-packed. The CT scan time for each cheese was about 95 s. After scanning, the cheeses were returned to the cool/warm room and unpacked.

2.4. Image analysis of CT data

The tomographic slices were transformed from DICOM (Digital Imaging and Communication in Medicine) format to TIFF (Tagged Image File Format), employing the Dicom2Tiff version 0.9.9 software (Disect Systems, Ipswich, England). In the tomographic images of the 30-day-old cheeses, the gantry had to be removed with image processing techniques: first, the binarised image (grey level larger than 10^4) was eroded with a disk of three pixels. The largest component in the resulting binary image was dilated with a disk of five pixels and employed as a mask on the tomographic images. For the other tomographic images, this procedure was not necessary because a thicker plate of Styrofoam was employed so that an additional separation of gantry and cheese was not necessary. The position, diameter, and volume of the cheese eyes was determined with VG Studio Max 2.2 (Volume Graphics, Heidelberg, Germany) using the pore identification module with the default parameters and exported to a text file. The grey level threshold separating cheese matrix and air was chosen separately for each measurement by employing the automatic threshold tool from VG Studio Max. The small differences in the pixel size were eliminated in the analysis by working only with physical dimensions (mm). Erroneously detected pores in the gantry system were removed by hand, based on their position outside the cheese matrix. Total eye volume was obtained by adding up the volumes of the identified eyes. Finally, the relative eye volume was calculated (total eye volume expressed as percentage of the total cheese volume) to facilitate the comparison of the results.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) of hay powder microparticles was carried out on a Hitachi S-3000N with a magnification of 2000. The picture was taken in the normal mode, after sample preparation with an argon plasma sputtering device (gold) and a Baltzers metal evaporator (gold), see e.g. Juan, Fernandez, and Pastor (1997).

2.6. Statistical analysis

Statistical analysis of the data was carried out using analysis of variance (ANOVA) with the general linear model (GLM) using SYSTAT 13 (SPSS Inc., Chicago, IL, USA). Differences between the various factors are considered statistically significant at $P \leq 0.05$. The addition of hay powder and the replicate were treated as categorical variables.

3. Results and discussion

3.1. Chemical composition of the experimental Emmental cheeses

The composition and biochemical parameters of the experimental cheeses at the end of cheese ripening (130 days) are presented in Table 2. As expected, the statistical analysis of the results showed no significant differences for the eight variants of experimental Emmental cheeses manufactured with different additions of powdered hay into the cheese milk. In contrast, significant differences for several analysed parameters were found between the two series of experimental cheeses originating from production days 1 and 2. This presumably was caused by varying conditions such as small differences in the standardisation of the cheese milk (fat in dry matter), differences in the manual cutting of the coagulum and preparation of the curd, and slight differences in the acidification activity of the bulk starters.

The slightly lower amounts of propionic and acetic acid and the higher amount of residual lactic acid in the cheeses of production day 2 indicate that propionic acid fermentation was slightly retarded. However, in total, the differences (e.g., propionic acid ~ 5 mmol kg^{-1}) between the two series of cheeses were negligible. It is also important to note that the content of butyric acid was generally low in all of the cheeses and mainly originated from lipolysis, confirming that no butyric acid fermentation had occurred. Caproic acid was present in concentrations of 0.4 ± 0.2 mmol kg^{-1}, whereas formic, iso-butyric, iso-valeric, and iso-caproic acid were below detection limits (data not shown). Citrate was not degraded during ripening as there was no addition of facultatively heterofermentative adjunct cultures of *Lactobacillus casei* or *Lactobacillus rhamnosus* to the cheese milk and because no relevant contamination of facultatively heterofermentative bacteria in the raw milk occurred. Therefore, this potent source of CO_2 had no influence on the eye formation in the investigated cheeses.

In summary, it can be concluded that all of the experimental cheeses had undergone a very similar and intense propionic acid fermentation and showed no signs of faulty fermentation. Therefore, it was concluded that comparable and sufficient quantities of CO_2 had been produced in all of the investigated cheeses over ripening time, which would allow for a characteristic and typical formation of eyes, as is to be expected in Swiss-type cheeses. The arithmetic mean of the calculated CO_2-production over all 16 experimental cheeses was 1027 ± 41 mL kg^{-1} under standard conditions. The classical propionic acid fermentation – predominant in Prop 96 – was taken as a basis for calculation.

Table 2
Composition, biochemical parameters and CO_2-formation of the experimental Emmental cheeses at the end of ripening (130 days).[a]

Cheeses	Number of cheeses	Dose of hay powder (mg 90 L^{-1})	Water (g kg^{-1})	Fat (g kg^{-1})	Acetic acid (mmol kg^{-1})	Propionic acid (mmol kg^{-1})	Butyric acid (mmol kg^{-1})	Total volatile carboxylic acids (mmol kg^{-1})	Total lactic acid (mmol kg^{-1})	Citric acid (mmol kg^{-1})	Produced CO_2 (mL kg^{-1})
Control	2	0.000	355 ± 5	308 ± 10	36.5 ± 1.7	89.7 ± 1.1	1.2 ± 0.2	127.6 ± 0.1	34 ± 1	8.2 ± 0.2	1004 ± 18
Variant 1	2	0.0625	355 ± 4	310 ± 7	35.8 ± 2.4	91.6 ± 2.1	1.4 ± 0.1	129.1 ± 4.2	20 ± 2	7.4 ± 0.5	1026 ± 33
Variant 2	2	0.125	354 ± 4	311 ± 8	34.7 ± 2.4	90.6 ± 5.3	1.5 ± 0.0	127.3 ± 7.7	26 ± 8	7.5 ± 0.3	1014 ± 84
Variant 3	2	0.25	353 ± 2	309 ± 7	34.8 ± 2.0	92.3 ± 4.2	1.5 ± 0.1	129.1 ± 6.1	24 ± 11	7.8 ± 0.1	1034 ± 67
Variant 4	2	0.5	352 ± 1	311 ± 10	35.9 ± 2.0	91.0 ± 3.9	1.4 ± 0.1	128.8 ± 5.8	26 ± 12	7.9 ± 0.1	1019 ± 63
Variant 5	2	1.0	351 ± 5	313 ± 10	36.3 ± 1.9	94.3 ± 3.3	1.5 ± 0.0	132.7 ± 5.1	18 ± 4	8.0 ± 0.1	1057 ± 53
Variant 6	2	2.0	351 ± 3	313 ± 9	34.9 ± 1.3	91.6 ± 2.4	1.5 ± 0.1	128.5 ± 3.6	28 ± 1	8.1 ± 0.2	1026 ± 38
Variant 7	2	4.0	352 ± 5	308 ± 12	36.0 ± 1.9	92.7 ± 1.1	1.4 ± 0.1	130.4 ± 2.7	25 ± 7	8.1 ± 0.1	1038 ± 17
Production day 1	8		356 ± 2	301 ± 3	37.5 ± 0.7	94.4 ± 2.6	1.3 ± 0.2	133.6 ± 2.7	20 ± 7	8.0 ± 0.2	1057 ± 29
Production day 2	8		349 ± 2	319 ± 2	33.6 ± 0.8	89.1 ± 2.0	1.5 ± 0.0	124.8 ± 2.7	31 ± 6	7.8 ± 0.4	997 ± 23
Both productions	16		353 ± 4	310 ± 9	35.6 ± 2.1	91.7 ± 3.5	1.4 ± 0.1	129.2 ± 5.2	25 ± 8	7.9 ± 0.3	1027 ± 41
GLM-ANOVA											
Variant			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Production day			***	***	***	**	**	**	*	n.s.	**

[a] CO_2-formation calculated from propionic acid content [classical (Fitz) pathway] and compared with 22.414 L from 1 mol ideal gas under norm-conditions (universal gas law): 0.1 MPa and 273.17° K. GLM-ANOVA significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.5$; n.s., not significant.

3.2. Monitoring of eye formation and growth by CT

To minimise the influence of intrinsic and extrinsic factors of raw milk on eye formation and to carry out the present eye formation study at the most standardised conditions, naturally-occurring eye nuclei such as microscopic air bubbles and solid microparticles were eliminated prior to cheese making by cleaning-filtration and centrifugation of the raw milk and subsequent microfiltration (1.4 μm) of the skim milk.

The formation of eyes in the experimental Emmental cheeses was monitored by X-ray computed tomography after ripening periods of 30, 45, 60, and 130 days. Fig. 1 shows an example of this non-destructive 3D visualisation of the eye formation (cheese variant 3 with 0.25 mg hay powder per 90 L milk, see Table 1). At day 30 of the ripening (20 days after the start of the warm room storage) numerous eyes were already detectable, but their size was still very small (Fig. 1A). At day 45, eyes were formed in almost all of the cheeses, but most of them were still rather small (Fig. 1B). However, during the last 15 days of the warm room storage, the size and volume of the eyes increased markedly (Fig. 1C). In contrast, the number of cheese eyes remained almost constant during the final 60 days of cheese ripening in the cool room, and the growth of the eyes slowed down (Fig. 1D).

These findings are in good agreement with the results of several other studies. Thierry, Salvat-Brunaud, and Maubois (1999) reported that eye formation started in traditional Emmental cheese between day 20 and 30 of cheese ripening. In an eye formation study by Kurmann and Wüthrich (1975), the formation of new eyes in Emmentaler PDO cheese occurred mainly between days 40 and 66, with a maximum at day 54. Similarly, Steffen, Eberhard, Bosset, and Rüegg (1993) reported for Emmentaler PDO cheese a strong expansion of eyes and an increase in the number of eyes after about 50 days. The formation of new eyes in the cool room was weak when the production of CO_2 decreased.

3.3. Dose–response relationship of hay powder addition and eye number

One hundred years ago, Clark (1917) had already postulated that cheese eyes are “seeded” at the moment of cheese making. However, the nature of these suggested “favourable points” leading to the formation of eyes during cheese ripening remained an unsolved mystery. Fig. 2 clearly illustrates the dose-dependent effect of hay powder on the number of eyes in the experimental Emmental cheeses. The influence of the hay powder on the openness of the cheeses is not only apparent with regard to the number of cheese

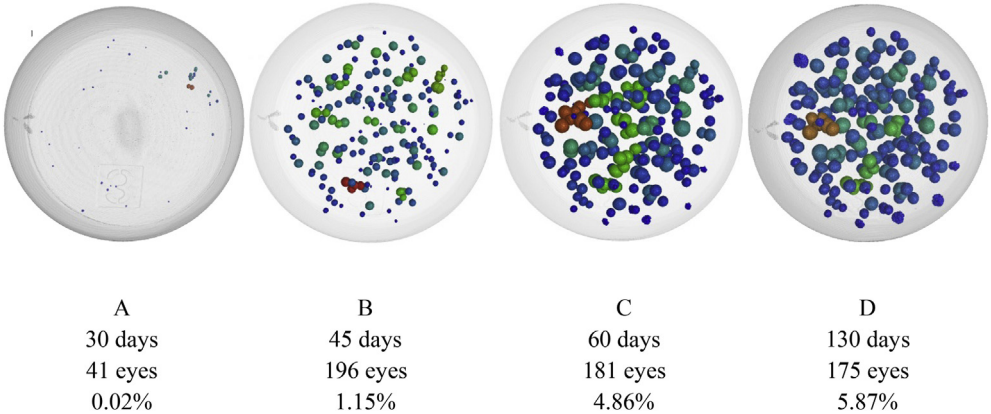

Fig. 1. Example of the visualisation by X-ray computed tomography of eye formation and growth in cheese 3 after ripening periods of: A, 30 days; B, 45 days; C, 60 days; D, 130 days. The cheese was made with addition of 0.25 mg powdered hay to 90 L milk. The number of eyes and the relative eye volume (total eye volume expressed as percentage of the total cheese volume) are indicated in the figure. The two defect eyes on the left side of the cheese (erased by the software tool) are artefacts originating from the pH measurements after one day.

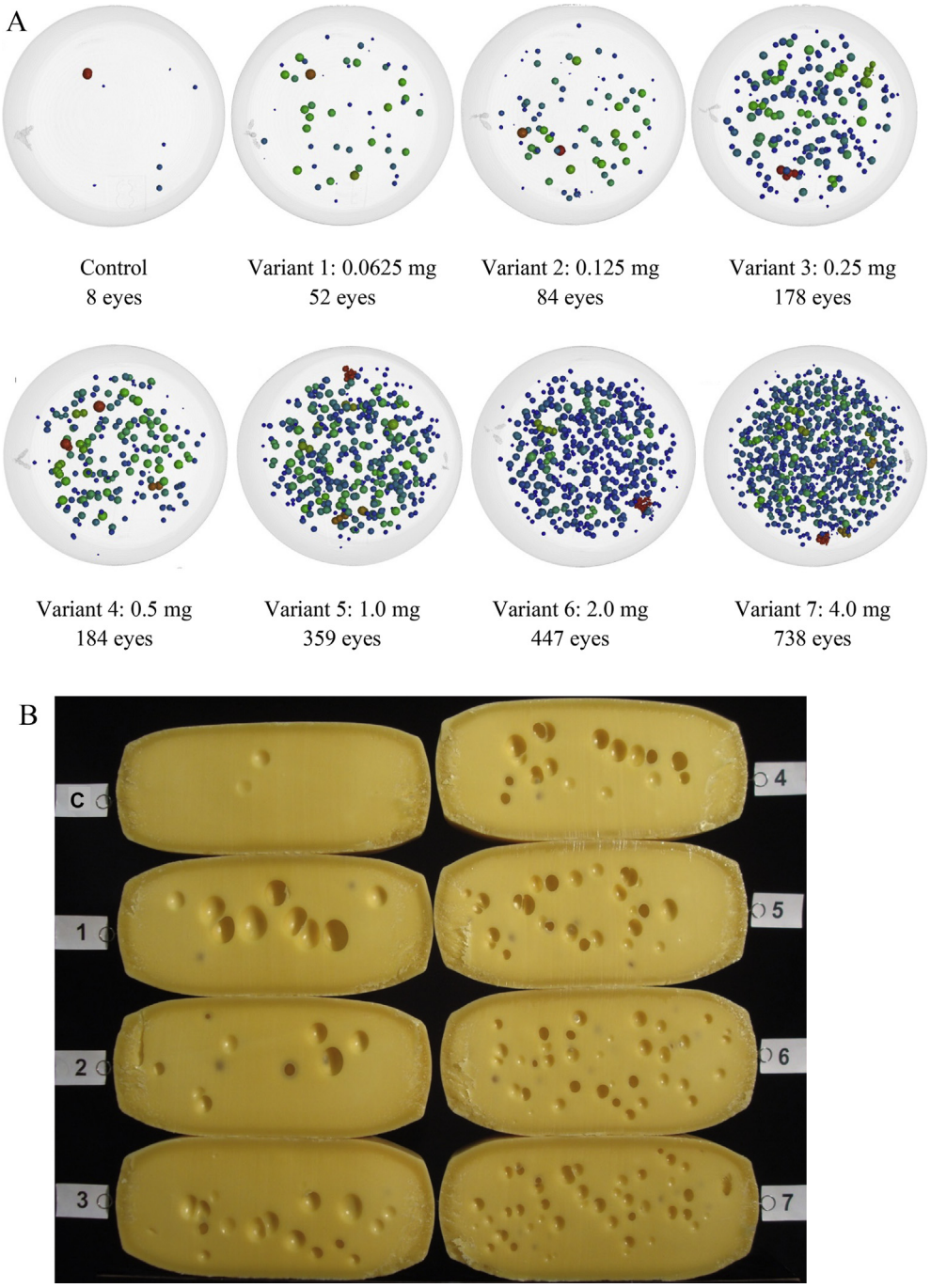

Fig. 2. Visualisation of the eye formation by X-ray computed tomography data in 45-day aged experimental Emmental cheeses from the first production day (A) and sectional view of the cheeses at the end of ripening (130 days: B). The eight cheeses (control, C, and variants 1–7) were produced with additions of powdered hay as indicated in Table 1.

eyes but is also evident in the stronger increase in the height of the cheeses, as seen by comparison of the two stacks of cheeses in Fig. 2b.

Martley and Crow (1996) assumed that microscopic bubbles trapped in the curd structure serve as nuclei into which CO_2 dissociates from solution to accumulate as gas, initiating the development of visible eyes. In our study, a causal relationship between microparticles obtained from hay powder and the number of eyes could be evidenced. The relationship between the increasing amounts of hay powder and the growing number of eyes was highly significant ($P < 0.001$). Fig. 3 shows the dose–response relationship of the hay powder and the eye numbers obtained in the experimental Emmental cheeses aged 45 days. The lowest dose of hay powder was 0.0625 mg per 90 L milk, and this amount was doubled six times, up to 4 mg per 90 L milk. Doses of hay powder in the range of 0–1 mg to 90 L caused a nearly-linear increase in the number of cheese eyes ($R^2 = 0.900$). In contrast, a saturation effect was observed with doses in the range of 2–4 mg, indicating that overpressure of CO_2 became a limiting factor when very high numbers of eyes were formed.

According to Willows and Hatschek (1914), the surface tension of a spherical bubble of gas suspended in a liquid is characterised by the equation $p = 2\sigma/r$, in which p is the pressure, σ is the surface tension and r is the radius of the bubble. The enclosed gas in an air

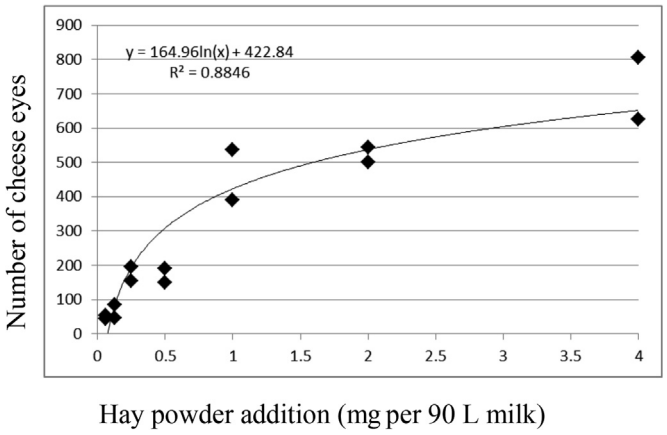

Fig. 3. Dose–response relationship of hay powder addition and eye numbers in 45-day aged experimental Emmental cheeses (n = 14, graph presented without control cheeses due to logarithmic function used for the fitting of the data).

bubble must overcome the surface tension if the bubble is to expand. The smaller the radius of an air bubble, the higher the pressure required to overcome the surface tension. If high numbers of eyes and eye nuclei are present in a cheese body, diffusion of CO_2 into bigger eyes is favoured over diffusion of CO_2 into micro-bubbles. In such a situation, the level of gas overpressure in the cheese body is sufficient to continue growing the visible eyes, but insufficient to expand micro-bubbles and, respectively, eye nuclei. As a result, the formation of "new eyes" from microscopic bubbles stops, while the growth of visible eyes continues.

3.4. Relationship between eye number and eye volume

Fig. 4 illustrates the relationship between the number of cheese eyes (means of two runs) and the resulting relative eye volume (eye volume expressed as percentage of the total cheese volume, means of two runs) at the different ripening stages (day 45, 60, and 130). At day 45, an almost-linear relationship between the number of eyes and the relative eye volume was found ($R^2 = 0.9729$). The cheese eyes were still quite small at this ripening stage, and each eye was clearly separated from the next eye. Between day 45 and day 60, a marked increase of the relative eye volume took place (logarithmic fit of the date, $R^2 = 0.9748$ and $R^2 = 0.9258$). However,

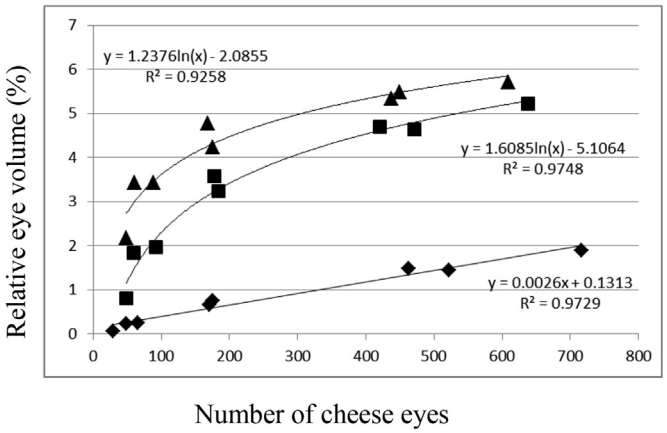

Fig. 4. Relationship between eye number and relative eye volume (total eye volume expressed as percentage of the total cheese volume) obtained at different stages of ripening in experimental Emmental cheese: ◆, 45 days; ■, 60 days; ▲, 130 days. The curves in the graph represent mean values (n = 2).

in cheeses with high eye numbers, the increase of the relative eye volume was no longer linear. It seems that the rate of production of CO_2 in these cheeses was insufficient to maintain a linear growth of relative eye volume. As a result, the average size of the cheese eyes was distinctly smaller than in cheeses with fewer eyes.

Regarding the number of computed cheese eyes, a slight decrease was observed at advanced stages of ripening in cheeses with high eye numbers. This decrease can be explained by the fact that some adjacent cheese eyes started to coalesce and, as a result of this phenomenon, the software tool underestimated the total number of eyes in cheeses aged for 60 and 130 days. The analysis software only counted eyes correctly if the eyes were separated by a layer of cheese matrix with sufficient thickness (one voxel). If two or more eyes were touching each other or even coalesced, VG Studio did not recognise the septa and merged multiple adjacent eyes into a single coherent cavity. Similarly, Musse et al. (2014) obtained a decrease in eye numbers during ripening.

3.5. Dynamics of the increase in eye volume during cheese ripening

In the production of Swiss-type cheese, *P. freudenreichii* is inoculated into cheese milk, typically at a level of 10^3–10^4 cfu mL^{-1}. During storage in the warm room, the counts of PAB in the curd increase to a level of 10^8 cfu g^{-1}, leading to intense gas and eye formation (Turgay et al., 2011). After a storage period of 30 days (20 of those days in the warm room), only a few eyes were visible, and the relative eye volume was still very small (<0.1%).

A drastic increase of the relative eye volume occurred during the second part of the warm room ripening between day 45 and day 60 (Fig. 5). However, the increase of the relative eye volume was strongly dependent on the amount of added hay powder and the resulting eye number. At day 60, the relative eye volume was 0.8% in the control cheeses (without hay powder addition), whereas in the variant, with a hay powder addition of 4 mg to 90 L of cheese milk, the relative eye volume was 5.2%, despite the fact that PAB fermentation and production of CO_2 were in all variants comparable (Table 2). It seems that the different additions of hay powder and, accordingly, the widely varying numbers of cheese eyes influenced the share of CO_2 escaping out of the eight variants of

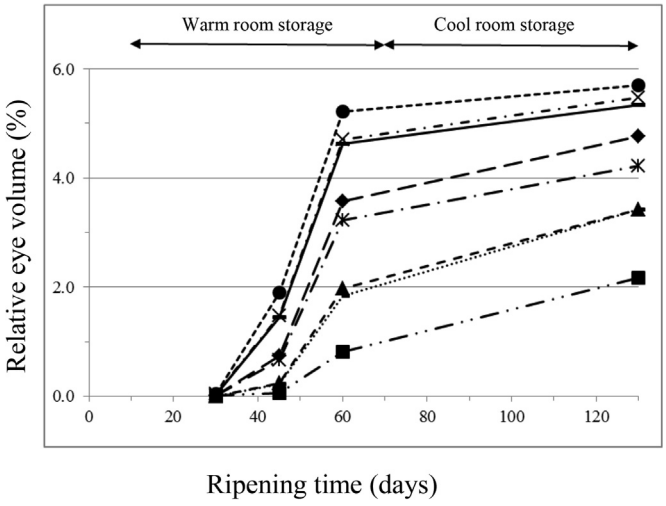

Fig. 5. Increase of the relative eye volume (total eye volume expressed as percentage of the total cheese volume) in experimental Emmental cheeses during storage in the warm room (days 11–70) and the cool room (days 71–130). Cheeses were produced with addition of different amounts of powdered hay to the cheese milk (90 L): ■, 0 mg; ····, 0.0625 mg; ▲, 0.125 mg; *, 0.25 mg; ◆, 0.5 mg; ×, 1.0 mg; —, 2.0 mg; ●, 4.0 mg. The curves in the graph represent mean values (n = 2).

experimental Emmental cheese. Although the formation of CO_2 during the last 60 days of ripening in the cool room was slowed down, there was still some increase in the relative eye volume, again depending upon the number of already-existing eyes. Stronger increases were observed in cheeses with lower numbers of eyes (Fig. 5), suggesting that CO_2 overpressure was higher in such cheeses at advanced stages of cheese ripening.

According to the list of requirements of the Association of Emmental PDO Cheese, the size of the eyes of first-quality cheese should be between the size of a cherry (approximately 4 cm^3) and a walnut (>4 cm^3). Table 3 shows the percentage of eyes in the cheese variants that were above 4 cm^3. This example illustrates that not only the number but also the size of eyes in cheese can be designed by a controlled seeding of nuclei in the cheese. The ability to design eye size and number has marketing ramifications for the industry. Italian consumers prefer Emmental cheese with walnut-sized eyes, whereas commercial manufacturers of sliced cheeses ask for cheese with smaller eyes and higher eye numbers to obtain slices with an optimal openness.

Furthermore, it must be noted that the growth dynamic of the observed eye is not representative of the behaviour of other eyes in the same cheese, since this is determined by a number of factors, such as the location of an eye in the cheese body and its distance from the cheese rind, as well as the amount and size of competing eyes nearby.

3.7. Radial distribution of cheese eyes

In Figs. 1 and 2a, clear differences in the radial distribution of the cheese eyes can be observed. Although it can be assumed that the added eye nuclei were homogeneously distributed in the experimental cheeses, almost no cheese eyes could be detected in the outermost 30 mm. The diffusion of CO_2 out of the cheese prevented the build-up of a sufficiently high CO_2 overpressure in this zone and thus disabled the formation of eyes. Furthermore, it must be noted that apart from CO_2 diffusion, other technological factors such as the initially increased salt content (brine salting) and the on-going decrease of the water content (dry ripening) reduced the potential for eye formation in the border zone (Blanc et al., 1973; Flückiger, 1980; Huc et al., 2014b).

Table 3
Percentage of cheese eyes larger than "cherry-sized" (4 cm^3) at the end of ripening.

Cheeses	Dose of hay powder (mg per 90 L milk)	Index of eyes bigger than 4 cm^3 (%)	Total number of eyes
Control	0	31 ± 22	49
Variant 1	0.0625	34 ± 8	61
Variant 2	0.125	22 ± 1	89
Variant 3	0.25	7 ± 4	169
Variant 4	0.5	4 ± 4	175
Variant 5	1.0	1 ± 1	449
Variant 6	2.0	0 ± 0	438
Variant 7	4.0	0 ± 0	609

3.6. In-depth study of the growth dynamic of an individual eye

Fig. 6 illustrates the growth of an individual eye over the whole period of ripening in the control cheese from production day 1. The growth rate of this eye was calculated using the voxels obtained from CT data. The calculated volume and growth rates of the observed eye are listed in Table 4. At day 30, the observed eye had a very small volume of only 0.091 cm^3, but at day 45 it had already reached a volume of 1.25 cm^3. By day 60, the eye volume increased to 6.8 cm^3, and at the end of ripening (130 days) an eye volume of 17.6 cm^3 was reached. The largest increase in eye volume occurred between day 45 and day 60 of the ripening period (warm room), with an average increase in eye volume of 0.37 cm^3 day^{-1}. During the subsequent storage in the cool room, the average growth of the volume of the observed eye decreased to 0.16 cm^3 day^{-1}.

3.8. Formation of eye defects

Eye defects such as cracks, slits, or splits are usually developed in the advanced stages of cheese ripening. The formation of eye defects becomes visible, either as a new crack (Fig. 6D) or as tearing off of an existing eye (Fig. 6E). In our experience, blind cheeses or cheeses with insufficient eye numbers show an elevated risk for the formation of such eye defects. The high susceptibility of such cheeses for eye defects presumably results from the higher CO_2 overpressure in the cheese body. In such cases, the addition of eye nuclei to cheese milk may help to prevent eye defects. However, several other factors influence the formation of

Table 4
Growth rate of an individual eye in an experimental Emmental cheese (control production day 1).

Ripening period (days)	Ripening conditions	Eye volume		
		Volume at end of period (cm^3)	Increase (cm^3)	Average daily increase (cm^3 per day)
10–30	Warm room (22 °C)	0.091	0.091	0.005
30–45	Warm room (22 °C)	1.249	1.158	0.077
45–60	Warm room (22 °C)	6.798	5.549	0.370
60–130	Cool room (12 °C)	17.636	10.838	0.155

Similar studies were previously carried out with Gouda-type cheese by Lee et al. (2012) using CT, and with foil-ripened semi-hard cheese blocks by Musse et al. (2014) using MRI. However, it is difficult to compare these results with our findings, since the type of fermentation (up to 8.8 mg butyric acid per 100 mg cheese) and the ripening conditions were very different in these studies. eye defects, such as acidification (solubilisation of calcium), moulding and pressing (technical openness), proteolysis (reducing the elasticity of the cheese body), low storage temperature (reducing the plasticity of the cheese body), sudden and significant increases in storage temperature (affecting the solubility and partial pressure of CO_2), as well as seasonal variations in the

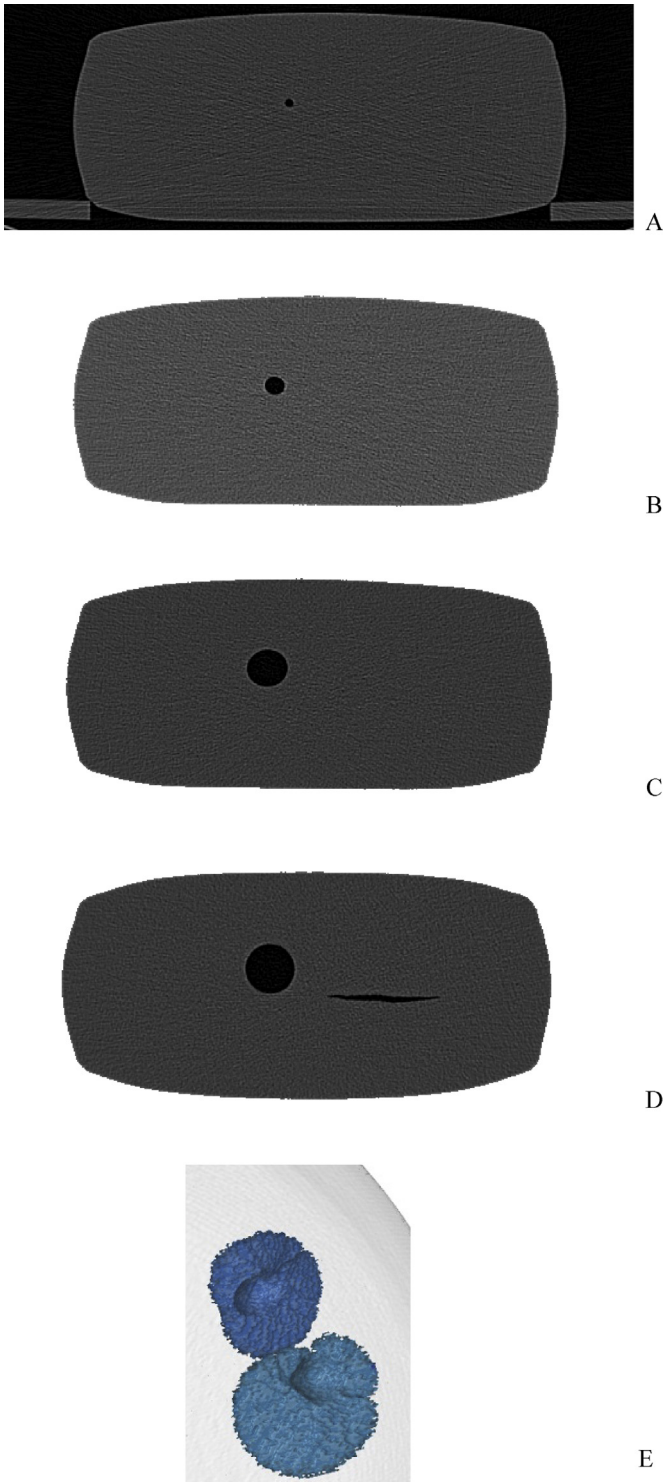

Fig. 6. In-depth study on the dynamic growth of a large-sized eye in a control cheese without addition of hay powder and on the development of a crack in the adjacent zone. The volume of the observed eye increased as follows: A, 0.09 cm^3 at day 30; B, 1.25 cm^3 at day 45; C, 6.78 cm^3 at day 60; D, 17.64 cm^3 at day 130, with crack formation in the final stage of ripening between day 60 and 130; E, three-dimensional image of two smaller cracks in the same cheese with small-sized eyes as starting points for crack formation.

hardness of the milk fat (Fröhlich-Wyder & Bachmann, 2007; Jakobsen, Jensen, & Risbo, 2009). Daly, McSweeney, and Sheehan (2010) recommend considering milk and cheese microflora as a solution to the problem of eye defects.

3.9. Microstructure of eye nuclei present in powdered hay

Clark's hypothesis (1917) that eyes are "seeded" in a cheese has been substantiated by a number of practical observations, such as the seasonal variation in eye numbers and the negative influence of centrifugation, bactofugation, and microfiltration on eye formation in cheese. The potential for eye nuclei has been attributed to a number of factors, such as nitrogen from milk, CO_2 from starters, solid microparticles, and small mechanical openings (Polychroniadou, 2001). However, with the exception of solid microparticles, none of these factors explain the season-dependent variation of eye formation in cheese, and none of these factors have been shown to allow a reproducible "seeding" of eyes in cheese. In our studies, we investigated the potential of various solid microparticles such as powdered cellulose, powdered wheat fibre, potato starch, and several spices such as powdered ginger, rosemary, thyme, and pepper to serve as eye nuclei and observed huge differences (data not shown), indicating that the botanical origin and the type of preparation of these solid microparticles determine their potential to serve as eye nuclei. Highly processed products such as powdered cellulose and wheat fibre, as well as powders originating from tubers or roots, such as potato starch and ginger powder, proved to be ineffective, whereas powders obtained from dried botanical tissues such as leaves and stems showed promising results. Therefore, we assumed that such microparticles would contain specific structural properties.

We started to investigate the microstructure of microparticles of the highly effective hay powder, using SEM. The SEM micrograph in Fig. 7 shows fragments of intact capillaries (xylem strands) that are likely to be the highly effective structural elements that act as eye nuclei in cheese milk and enable eye formation during cheese ripening. Possible entrapment of air in such capillaries allows diffusion of CO_2 from the cheese body into the microparticles, as proposed by Martley and Crow (1996). The subsequent growth of such cavities leads finally to the formation of visible eyes in the cheese.

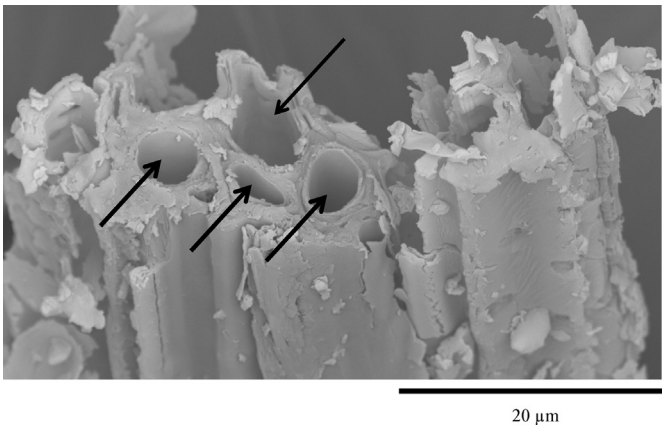

Fig. 7. Scanning electron micrograph of a microparticle from hay powder. The visible capillary structures (see arrows) are likely to be the highly effective structural elements that act as eye nuclei in cheese milk and enable eye formation during cheese ripening. Entrapment of air in such capillaries allows the diffusion of CO_2 from the cheese body into the microparticles. The subsequent growth of the cavities leads finally to the formation of visible eyes in the cheese (scale bar 20 μm).

4. Conclusions

The results of the present study clearly show that trace amounts of hay powder in microfiltrated milk induce eye formation during cheese ripening. To our knowledge, this is the first scientific report demonstrating an active and successful seeding of eyes in cheese. It

is likely that trace amounts of hay dust entering in raw milk have always been the natural source of eye nuclei, triggering the start of eye formation in cheese. Therefore, the applied addition of traces of hay powder represents a controlled use of a naturally occurring phenomenon, as opposed to an artificial "seeding" of eyes.

The microscopic investigation of hay powder microparticles revealed that capillary structures present in plant tissues from leaves or stems that act as eye nuclei in cheese milk and enable eye formation during cheese ripening. Possible entrapment of air in such capillaries allows the diffusion of CO_2 from the cheese body into the microparticles. The subsequent growth of the cavities leads finally to the formation of visible eyes in cheese. A linear relationship between the amount of added hay powder and the number of eyes in Emmental cheese was found up to 1 mg per 90 L cheese milk ($R^2 = 0.900$). Higher traces of hay powder (up to 4 mg per 90 L cheese milk) led to a saturation effect regarding eye number and eye volume.

The nearly complete elimination of eye nuclei from the cheese milk by microfiltration disabled eye formation almost completely, although sufficient CO_2 must have been produced by propionic acid fermentation in the control cheeses without the addition of eye nuclei. Therefore, it can be concluded that the absence of eye nuclei in the cheese body leads to a higher CO_2 overpressure, which increases the diffusion rate of CO_2 out of the cheese. Similarly, the results obtained for the radial distribution of the eyes revealed that, despite the presence of sufficient eye nuclei, no eyes could be induced in the border zone of the cheeses, most likely due to the higher diffusion rate of CO_2 out of the cheese.

The findings of our study are of high practical importance for the understanding and control of eye formation in cheese. They also provide new insights into the formation of eye defects such as cracks and slits. Years of practical experience show that latter are often coupled with a lack of eye nuclei and therefore a low number of eyes. In the control cheeses without the addition of eye nuclei, it is thought that cracks and slits were formed during cool-room ripening. This indicates that the lack of eye nuclei and, accordingly, insufficient numbers of eyes increase the risk for the formation of cracks and slits in the less elastic cheese body, weakened by proteolysis, at advanced stages of ripening. Under such conditions, a high overpressure of CO_2 in the cheese body leads to an uncontrolled tearing off of eyes or the spontaneous formation of cracks in weakened zones of the cheese body.

These findings highlight the competing interests of hygienic milk production and the need to contaminate milk with solid microparticles to induce eye formation. During the last few decades, important technological improvements have been achieved in milk production. Traditional milking in the stable with milk buckets has been replaced by closed and automated milking systems in separated areas. This, along with the application of fine-pored filters in milking systems, has resulted in a drastic reduction of solid microparticles in raw milk. Therefore, it is not surprising that even in the traditional production of cheeses made from raw milk, such as Emmentaler PDO cheese, cheese makers are increasingly facing the problem of missing cheese eyes in their production and have difficulty meeting the high quality standards. The findings of this study may help to overcome the problem of a too-spare openness. Moreover, the dilemma of requiring hygienic milk production that not only reduces microbiological contaminations but also lowers the entry of solid microparticles (as eye nuclei) into the cheese milk are addressed.

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References

Akkerman, J. C., Walstra, P., & Van Dijk, H. J. M. (1989). Holes in Dutch-type cheese. 1. Conditions allowing eye formation. *Netherlands Milk and Dairy Journal, 43*, 453.
Albrecht, B., Ulmann, H. R., & Bosset, J. O. (1998). Nachweis von Rissen in Käselaiben mit Ultraschall-Echografie. *Deutsche Milchwirtschaft, 49*, 1162–1163.
Blanc, B., & Hättenschwiler, J. (1973). Neue Möglichkeiten für die Anwendung der Radiologie in der Käsereitechnologie. *Schweizerische Milchwirtschaftliche Forschung, 2*, 1–16.
Clark, W. M. (1917). On the formation of "eyes" in Emmental cheese. *Journal of Dairy Science, 1*, 91–113.
Conde, T., Mulet, A., Clemente, G., & Benedito, J. (2007). Detection of internal cracks in Manchego cheese using the acoustic impulse-response technique and ultrasounds. *Journal of Dairy Science, 91*, 918–927.
Daly, D. F. M., McSweeney, P. L. H., & Sheehan, J. J. (2010). Split defects and secondary fermentation in Swiss-type cheeses – a review. *Dairy Science and Technology, 90*, 3–26.
Eskelinen, J. J., Alavuotunki, A. P., Haeggström, E., & Alatossava, T. (2007). Preliminary study of ultrasonic structural quality control of Swiss-type cheese. *Journal of Dairy Science, 90*, 4071–4077.
Flückiger, E. (1980). CO_2- und Lochbildung im Emmentalerkäse. *Schweizerische Milchzeitung, 106*, 473–474, 479–480.
Fragnière, C., & Schafroth, K. (2004). Neue Erkenntnisse zur Lochbildung in Käse aus mikrofiltrierter Milch. *Schweizerische Milchzeitung, 7*, 27.
Fröhlich-Wyder, M.-T., & Bachmann, H. P. (2004). Cheeses with propionic acid fermentation. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese: Chemistry, physics and microbiology* (3rd ed., Vol. 2, pp. 141–156). London, UK: Elsevier Academic.
Fröhlich-Wyder, M.-T., & Bachmann, H. P. (2007). Swiss cheese. In P. L. H. McSweeney (Ed.), *Cheese problems solved* (pp. 246–267). Cambridge, UK: Woodhead Publishing Ltd.
Fröhlich-Wyder, M.-T., Bachmann, H. P., & Casey, M. G. (2002). Interaction between propionibacteria and starter/non-starter lactic acid bacteria in Swiss-type cheese. *Lait, 82*, 1–15.
Fröhlich-Wyder, M.-T., Guggisberg, D., Badertscher, R., Wechsler, D., Wittwer, A., & Irmler, S. (2013). The effect of *Lactobacillus buchneri* and *Lactobacillus parabuchneri* on the eye formation of semi-hard cheese. *International Dairy Journal, 33*, 120–128.
Guggisberg, D., Fröhlich-Wyder, M.-T., Irmler, S., Greco, M., Wechsler, D., & Schuetz, P. (2013). Eye formation in semi-hard cheese: X-ray computed tomography as a non-invasive tool for assessing the influence of adjunct lactic acid bacteria. *Dairy Science and Technology, 93*, 135–149.
Huc, D., Mariette, F., Challois, S., Barreau, J., Moulin, G., & Michon, C. (2014a). Multiscale investigation of eyes in semi-hard cheese. *Innovative Food Science and Emerging Technologies, 24*, 106–112.
Huc, D., Roland, N., Grenier, D., Challois, S., Michon, C., & Mariette, F. (2014b). Influence of salt content on eye growth in semi-hard cheeses studies using magnetic resonance imaging and CO_2 production measurements. *International Dairy Journal, 35*, 157–165.
IDF. (2003). *Dry matter (total solids)*. IDF Standard 4A. Brussels, Belgium: International Dairy Federation.
ISO. (2008). *Cheese – Determination of fat content – Van Gulik method*. ISO3433:2008 (IDF 222). Geneva, Switzerland: International Organisation for Standardisation.
Jakobsen, M., Jensen, P. N., & Risbo, J. (2009). Assessment of carbon dioxide solubility coefficients for semihard cheeses: the effect of temperature and fat content. *European Food Research and Technology, 229*, 287–294.
Juan, R., Fernandez, I., & Pastor, J. (1997). Morphological and anatomical studies on fruits of *Veronica* from south-west Spain. *Botanical Journal of the Linnean Society, 123*, 157–171.
Kraggerud, H., Wold, J. P., Hoy, M., & Abrahamsen, R. K. (2009). X-ray images for the control of eye formation in cheese. *International Journal of Dairy Technology, 62*, 147–153.
Kurmann, J. L., Gehriger, G., & Kaufmann, H. (1975). Ueber die Bedeutung des Pressdruckes für den Lochansatz beim Emmentaler. *Schweizerische Milchzeitung, 101*, 365–366.
Kurmann, J. L., & Wüthrich, A. (1975). Radiologische Untersuchungen während der Käsereifung. *Schweizerische Milchzeitung, 101*, 1–2.
Lee, K., Uegaki, K., Nishii, C., Nakamura, T., Kubota, A., Hirai, T., et al. (2012). Computed tomographic evaluation of gas hole formation and structural quality in Gouda-type cheese. *International Journal of Dairy Technology, 65*, 1–5.
Martley, F. G., & Crow, V. L. (1996). Open texture in cheese: the contribution of gas production by microorganisms and cheese manufacturing practices. *Journal of Dairy Research, 63*, 489–507.
Musse, M., Challois, S., Huc, D., Quellec, S., & Mariette, F. (2014). MRI method for investigation of eye growth in semi-hard cheese. *Journal of Food Engineering, 121*, 152–158.

Polychroniadou, A. (2001). Eyes in cheese: a concise review. *Milchwissenschaft, 56*, 74–77.
Rosenberg, M., McCarthy, M., & Kauten, R. (1992). Evaluation of eye formation and structural quality of Swiss-type cheese by magnetic resonance imaging. *Journal of Dairy Science, 75*, 2083–2091.
Schuetz, P., Guggisberg, D., Jerjen, I., Fröhlich-Wyder, M.-T., Hofmann, J., Wechsler, D., et al. (2013). Quantitative comparison of the eye formation in cheese using radiography and computed tomography data. *International Dairy Journal, 31*, 150–155.
Steffen, C., Eberhard, P., Bosset, J. O., & Rüegg, M. (1993). Swiss type varieties. In P. F. Fox (Ed.) *Cheese: Chemistry, physics and microbiology: Vol. 2. Major cheese groups* (2nd ed., pp. 83–110). London, UK: Chapman & Hall.
Strand, A. H. (1985). Scanning of Jarlsberg using data-tomograph. *Meieriteknikk, 39*, 2–7.
Thierry, A., Berthier, F., Gagnaire, V., Kerjean, J. R., Lopez, C., & Noël, Y. (2010). Eye formation and Swiss-type cheeses. In B. A. Law, & A. Y. Tamime (Eds.), *Technology of cheesemaking* (2nd ed., pp. 360–383). West Sussex, UK: Wiley-Blackwell.
Thierry, A., Salvat-Brunaud, D., & Maubois, J. L. (1999). Influence of thermophilic lactic acid bacteria strains on propionibacteria growth and lactate consumption in an Emmental juice-like medium. *Journal of Dairy Research, 66*, 105–113.
Turgay, M., Irmler, S., Isolini, D., Amrein, R., Fröhlich-Wyder, M.-T., Berthoud, H., et al. (2011). Biodiversity, dynamics, and characteristics of *Propionibacterium freudenreichii* in Swiss Emmentaler PDO cheese. *Dairy Science and Technology, 91*, 471–489.
Willows, R. S., & Hatschek, E. (1914). Surface tension and surface energy and their influence on chemical phenomena. In *The chemical world* (Vol. 3, no. 5, p. 146). London, UK: J. & A. Churchill.