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The total eye volume of cheese is influenced by different fat-levels

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ABSTRACT

The impact of fat content in cheese on eye formation was investigated. Observations in practice have shown that fat-reduced cheeses tend to have more eyes than equivalent full-fat counterparts. A semi-hard cheese with CO_2 production through citrate catabolism by *Lacticaseibacillus paracasei* and a hard Swiss-type cheese with CO_2 production by *Propionibacterium freudenreichii* were produced with different fat content. Four different fat-in-dry-matter levels (~100 to ~480 g kg^{-1}) were applied to the semi-hard cheeses and three (~330 to ~560 g kg^{-1}) to the hard cheeses. The direct influence of the fat content on eye formation was distinguished from the consequentially altered cheese composition on bacterial fermentation (i.e., on CO_2-production). An increasing fat content had a significant (p <0.05) inhibitory effect on relative eye volume in semi-hard and hard cheeses by increasing the capacity of the cheese matrix to solubilise more CO_2.

1. Introduction

More than 1000 different cheese varieties have been established worldwide. They differ in terms of characteristics such as composition, functionality, appearance and flavour (McSweeney, Ottogalli, & Fox, 2004). An important characteristic of many semi-hard cheeses, such as Appenzeller®, Swiss Tilsiter and Gouda, is the presence of primarily round shiny openings known as eyes. The size, number, shape and distribution of eyes in these semi-hard cheeses are considered important quality parameters. The growth mechanism of the eyes is a complex process that is dependent on multivariate parameters such as moulding and pressing technology, cheese curd fusion, gas formation by the starter and/or adjunct bacteria, presence of eye nuclei, a soft and viscoelastic texture, salt, pH and temperature, the solubility of CO_2 in the cheese matrix (water, fat, protein) and the diffusion properties (Fröhlich-Wyder, Bisig, Guggisberg, Jakob, & Wechsler, 2017; Guggisberg et al., 2015).

The formation of cheese eyes requires an amount of total CO_2 produced that is larger than the amount solubilised in the cheese matrix and which diffuses out of the cheese (Pauchard, Flückiger, Bosset, & Blanc, 1980). When CO_2 is produced in cheese by bacteria, one part is solubilised in the cheese matrix, one part diffuses to the eye nuclei and another part is lost by diffusion out of the cheese (Guggisberg et al., 2015). According to Flückiger (1980) and Flückiger and Walser (1976), 30–52% of the CO_2 produced remains in the cheese matrix of a typical Swiss Emmentaler, 15% diffuses into the eyes and the remaining 33–55% diffuses out of the cheese. Analyses of ripened cheeses have shown mean CO_2 concentrations of 29 mmol kg^{-1} in Swiss Emmentaler AOP (eyes from propionic acid fermentation), 17 mmol kg^{-1} in Appenzeller® (eyes from citric acid fermentation) and 6 mmol kg^{-1} in Le Gruyère AOP (without eyes) (Fröhlich-Wyder et al., 2017).

CO_2 production in cheese plays an important role in eye formation, but only a few studies have described the solubility and diffusivity of CO_2 in cheese. Henry's law, which states that partial pressure of a gas over a liquid is directly proportional to its concentration in the liquid, when applied to cheese, has to consider both water and fat as solvents. Cheese is therefore regarded as a two-phase system of water and fat distributed in a protein network. CO_2 solubility within a cheese matrix largely depends on the cheese composition, temperature and partial pressure. CO_2 is highly soluble in both the cheese fat and water phases due to its symmetrical structure and dipole moment of zero; however, the CO_2 solubility in each phase is temperature-dependent (Jakobsen, Jensen, & Risbo, 2009). CO_2 solubility in the aqueous phase decreases with increasing temperature, while it increases in the fat (Jakobsen et al.,

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2009). The opposite temperature effects partly compensate each other. Jakobsen et al. (2009) considered cheese as a two-phase system and proposed the following equation to estimate CO_2 solubility (S_{cheese}; mol kg^{-1} Pa^{-1}) in cheese (Eq. (1)):

$$S_{cheese} = w_w \times S_w(T) + w_f \times S_f(T) \quad (1)$$

Eq. (1) is based on the weight fraction of water (w_w) and fat (w_f) and the corresponding CO_2 solubility in water (S_w) and in fat (S_f) in a temperature (T) range of 10–20 °C. However, Jakobsen et al. (2009) did not consider the salt content in the water phase of the studied semi-hard cheeses. Acerbi, Guillard, Guillaume, and Gontard (2016) investigated the effects of temperature, partial pressure, salt and moisture content on CO_2 solubility in full-fat semi-hard cheese and observed a decrease in CO_2 solubility with an increasing temperature and salt level. Pauchard et al. (1980) studied the effect of temperature, pH, NaCl content, water and fat content on CO_2 solubility. They found that CO_2 solubility in the water phase decreases with increasing temperature and NaCl concentration and increases with increasing pH. This was also confirmed by Lamichhane et al. (2021). This temperature-dependent decrease in CO_2 solubility could also be observed in the cheese matrix, but to a much lesser extent. They explained this phenomenon in terms of the capacity of the other components, namely fat and protein, to dissolve CO_2. In the meantime, the role of the three main components in cheese has been elucidated by several researchers, who assessed CO_2 solubility coefficients for the water and fat phases at different temperature and salt combinations (Acerbi et al., 2016; Chaix, Guillaume, Gontard, & Guillard, 2015; Chaix, Guillaume, & Guillard, 2014; Jakobsen et al., 2009; Lamichhane et al., 2021; Truong, Palmer, Bansal, & Bhandari, 2017).

There has been little research on the role of the protein phase (Acerbi et al., 2016), although a recent study by Lamichhane et al. (2021) confirmed the temperature, salt, partial pressure and pH-dependent solubility of CO_2 in renneted casein matrixes with a water–protein ratio of 2:1. The behaviour of the CO_2 solubility in the renneted casein matrix resembled very closely its behaviour in water. For example, the gradient of the CO_2 solubility coefficients at varying salt contents was comparable between water and the casein matrix, with a water–protein ratio of 2:1, and only a negligible difference of 1.1×10^{-7} mol kg^{-1} Pa^{-1}. The water–protein ratio as such also had an effect on CO_2 solubility coefficients; however, in the range occurring in cheese, its influence was stable (Lamichhane et al., 2021; Pauchard et al., 1980). Therefore, it was decided that the two-phase model of water and fat (Eq. (1)) would be a convenient approach for the present study.

The current study investigated and quantified the influence of CO_2 solubility in cheese on the eye-formation of semi-hard and hard cheese as a function of the fat content. Appenzeller®, as a cheese type with CO_2 formation by citric acid fermentation by *Lacticaseibacillus paracasei*, was taken as reference semi-hard cheese, and Swiss Emmentaler AOP, as a cheese type with propionic acid fermentation by *Propionibacteriuim freudenreichii*, as reference hard Swiss-type cheese. The manufacturing processes of both cheese types were adapted according to the desired fat level. To quantify the CO_2 solubility in cheese, Eq. (1), proposed by Jakobsen et al. (2009), was applied using CO_2 solubility coefficients for water (S_w; mol kg^{-1} Pa^{-1}) that had been adjusted to their individual salt content and a ripening temperature of approximately 12 °C, according to Pauchard et al. (1980) (Eq. (2)).

$$S_w = 4.67 \times 10^{-7} - 1.78 \times 10^{-8} \times NaCl_{aq} \quad (2)$$

As described by Bisig et al. (2019) and Wenzel et al. (2018), CO_2 formation in cheese can be estimated on the basis of the metabolised substrate citrate (citrate lyase pathway; Díaz-Muñiz et al., 2006) or of the accumulated metabolites (classical Fitz pathway; Crow, 1986). The analysis of the accurate volume, number and distribution of eyes within the cheese matrix is of high importance. Computed tomography (CT) was applied as a non-invasive imaging technology, as proposed by Guggisberg et al. (2013) and Schuetz et al. (2013). The CO_2 theoretically produced and dissolved in the different semi-hard and hard Swiss-type cheeses was compared with relative eye volume to explain the impact of cheese composition and of CO_2 production on eye formation.

2. Material and methods

2.1. Production of semi-hard cheese (model Appenzeller)

As summarised in Table 1, a total of eight semi-hard cheeses (Appenzeller-type, diameter 30 cm) were produced from 70 to 100 L of micro-filtered skimmed cow milk from the same batch. The fat content was adjusted with pasteurised (72 °C, 15 s) cream according to the following desired fat levels: 37 g kg^{-1} for a full-fat (FF) cheese; 20 g kg^{-1} for a medium-fat (MF) cheese, 9 g kg^{-1} for partially skimmed (PS) cheese and 0.5 g kg^{-1} for a skim (S) cheese. The presence of eye nuclei was ensured by adding tiny hay particles to micro-filtered cheese milk during manufacture, as reported by Guggisberg et al. (2015). This is an efficient measure to standardise eye formation preconditions in cheese. A suspension containing 100 mg of powdered hay (<100 μm; BA Heublumen gemahlen BIO-K, Kennel AG, Baar, 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 7–10 mL of the suspension (0.1 mg hay L^{-1} milk) was added into the individual vat milks. Five litres of water and 7–10 mL aqueous copper sulphate solution (39.3 g L^{-1} copper (II) sulphate pentahydrate) were added to the vat milk at a level to ensure a concentration of Cu in the final cheese close to that of traditional Appenzeller® (12.5–19.8 mg kg^{-1}; Sieber, 2012). After the addition of 0.5–0.2‰ bulk starter culture MK 401 (Agroscope, Liebefeld, Switzerland, containing various strains of *Lactobacillus delbrueckii* subsp. *lactis*, *Streptococcus thermophilus* and *Lactococcus lactis* subsp. *lactis*), 0.5–0.8‰ bulk starter culture RMK 150 (Agroscope, containing various strains of *L. delbrueckii* subsp. *lactis* and *S. thermophilus*) and adjunct direct culture MK 3008 (Agroscope, containing *Lacticaseibacillus paracasei*), the milk was pre-ripened at 31–32 °C for 30 min.

For coagulation, 13–17 mL (~0.18‰) of calf rennet (Winkler GR Orange, Winkler, Konolfingen, Switzerland) was diluted in 1 L of water and added to the milk, which was then renneted at 32 °C for 35 min. According to the manufacturer's instructions, the strength of the rennet was as follows: 1 part of rennet clots 9000 parts of non-heated full-fat cow milk (pH 6.65 at 32 °C) within 30 min, equivalent to 194 IMCU mL^{-1}. The coagulum was cut into cubes of about 4–8, 8–15, 8–20 and 8–20 mm for FF, MF, PS and S cheese, respectively, using a cheese harp with vertical wires and stirred for 20 min, to obtain the necessary water content. Thereafter, in addition to the water added with the copper sulphate solution and the rennet, 10–14 L of water (~14%) was added to the curd grains/whey mixture, which was heated to 45, 40, 40 and 40 °C for the FF, MF, PS and S cheeses, respectively, for 15 min, followed by a final stirring (45, 38, 38 and 38 °C, respectively, 15 min).

For air-bubble-less moulding and whey removal, the mixture was transferred into perforated moulds (Ø 30 cm) and thereafter into pressing chambers, where they were pressed and drained at 10,000 N m^{-2} for 4 h at 34 °C, followed by 4 h at 32 °C, 8 h at 28 °C and finally at 26 °C until they reached a pH of 5.1–5.2. Immersion in brine solution 20% (w/w) for 16 h at 11–13 °C and ripening at

Table 1
Main steps of the manufacture of the full fat, medium fat, partially skimmed and skim experimental semi-hard cheeses with citric acid fermentation.[a]

Item	Full fat	Medium fat	Partially skimmed	Skim
Fat content of the vat milk	37 g kg^{-1}	20 g kg^{-1}	9 g kg^{-1}	0.5 g kg^{-1}
Quantity of vat milk	70 L	80 L	100 L	100 L
Addition of aqueous copper sulphate solution (39.3 g L^{-1}) in 5 L water	14 mL	16 mL	20 mL	20 mL
Addition of hay suspension (100 mg in 100 mL water)	7 mL	8 mL	10 mL	10 mL
Addition of starter culture MK 401	35 mL	24 mL	20 mL	20 mL
Addition of starter and adjunct culture RMK 150 and MK 3008	35 mL	56 mL	80 mL	80 mL
Pre-ripening	31–32 °C, 30 min	31–32 °C, 30 min	31–32 °C, 30 min	31–32 °C, 30 min
Addition of rennet (Winkler GR orange) in 1 L of water	13 mL	15 mL	17 mL	17 mL
Coagulation	32 °C, 35 min	32 °C, 35 min	32 °C, 35 min	32 °C, 35 min
Cutting coagulum into cubes of	4–8 mm	8–15 mm	8–20 mm	8–20 mm
Stirring	20 min	20 min	20 min	20 min
Addition of water	10 L	11 L	14 L	14 L
Scalding	45 °C, 15 min	40 °C, 15 min	40 °C, 15 min	40 °C, 15 min
Final stirring	45 °C, 15 min	38 °C, 15 min	38 °C, 15 min	38 °C, 15 min

[a] Cheeses were manufactured in parallel and in duplicate from the same batch of milk.

14–15 °C and 90–96% relative humidity for 90 d followed. During the first 10 d of ripening, the cheeses were smeared daily with brine solution (3%, w/v, NaCl) that previously had been inoculated with a mixture of *Brevibacterium linens*, *Arthrobacter* ssp. and *Debaryomyces hansenii* (OMK 702; Agroscope); afterwards, smearing with the brine solution was carried out twice a week.

2.2. Production of Swiss-type hard cheese (model Emmentaler)

The cheeses were produced according to the standard production of model Emmentaler from 90 L of milk published by Guggisberg et al. (2015). The fat content was adjusted with pasteurised (72 °C, 15 s) cream to attain the desired fat level, as shown in Table 2. Since the quantity of vat milk was different for each fat level, the ingredients and parameters had to be adjusted accordingly (Table 2).

2.3. Cheese sampling

The cheeses were sampled 24 h after moulding by cutting a cheese slice from the hoop side. Before sampling the 3-month-aged cheeses, CT measurements were performed (section 2.6). The cheeses were then cut in half; one half was used for the rheological analysis (section 2.5), and a quarter for the chemical and biochemical analyses (section 2.4). The remaining quarter served as a backup sample and was deep-frozen at −20 °C. For all the analyses, with a thickness of 0.5 cm, the rind of the hoop side and of the two cheese faces was discarded. For the chemical and biochemical analyses, the cheese was grated and mixed.

2.4. Chemical and biochemical analyses of the cheeses

The fat content of the cheeses was determined using the Gerber–Van Gulik method (ISO/IDF, 2008a,b). Water content was determined with the dry loss method (ISO/IDF, 2004a) by measuring the weight difference of the cheese samples before and after drying at 102 °C for 4 h. Moisture content in the fat-free cheese matrix (MFFB) was calculated using the following formula (Eq. (3)):

$$\text{MFFB}\left(\text{g kg}^{-1}\right) = \text{Water content} \times 1000 \Big/ (1000 - \text{Fat content}) \quad (3)$$

Total nitrogen (TN) was determined by the Kjeldahl method (ISO/IDF, 2004b). Protein content was calculated by multiplying TN by 6.38 (standard dairy nitrogen conversion factor). Nitrogen soluble at pH 4.6 (SN) was measured using the Kjeldahl method (Collomb, Spahni, & Steiger, 1990).

Total lactate (D- and L-lactate) was determined enzymatically according to the instruction protocol of the kit manufacturer (Boehringer, Mannheim, Germany) using an automated spectrophotometric analyser (Gallery, Thermo, Switzerland).

Sodium chloride content was analysed by titration of chloride and calculated according to ISO/IDF (2006), and the NaCl content in the aqueous phase ($NaCl_{aq}$) according to the following formula (Eq. (4)):

Table 2
Main steps of the manufacture of the high fat, full fat and medium fat experimental hard cheeses with propionic acid fermentation.[a]

Item	High fat	Full fat	Medium fat
Fat content of the vat milk	42 g kg^{-1}	35 g kg^{-1}	17 g kg^{-1}
Quantity of vat milk	85 L	90 L	100 L
Addition of aqueous copper sulphate solution (39.3 g L^{-1}) in 5 L water	8.5 mL	9 mL	10 mL
Addition of water	8 L	8 L	9 L
Addition of hay suspension (100 mg in 100 mL water)	8.5 mL	9 mL	10 mL
Addition of starter culture MK 101 (young)	95 mL	100 mL	110 mL
Addition of starter culture MK 101 (old)	95 mL	100 mL	110 mL
Pre-ripening	31–32 °C, 30 min	31–32 °C, 30 min	31–32 °C, 30 min
Addition of rennet (Winkler GR orange) in 1 L of water	15 mL	16 mL	18 mL
Coagulation	35 °C, 32 min	35 °C, 32 min	35 °C, 38 min
Cutting coagulum into cubes of	4–8 mm	4–8 mm	4–8 mm
Stirring	25 min	25 min	25 min
Addition of water	5 L	1 L	–
Scalding	53 °C, 30 min	53 °C, 30 min	50 °C, 30 min
Final stirring	53 °C, 35 min	53 °C, 35 min	50 °C, 10 min

[a] Cheeses were manufactured in parallel and in duplicate from the same batch of milk.

$$NaCl_{aq}\left(g\ kg^{-1}\right) = NaCl \times 1000 \Big/ \text{Water content} \quad (4)$$

Volatile carboxylic acids (C1–C6: formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid, caproic acid and isocaproic acid) were measured using gas chromatography and flame ionisation detection (GC-FID) with headspace technology after esterification with ethanol, as described by Badertscher, Blaser, and Noth (2023).

2.5. Physicochemical analysis of the cheese samples

A uniaxial compression test (ISO 17996:2006/IDF 4:2004) was carried out at 15 ± 1 °C using a Zwick universal machine (Zwick GmbH & Co., Ulm, Germany). Cheese cylinders without openings (height 15 mm, diameter 12.5 mm) were cut vertically out of the core cheese sample. The method was described in Guggisberg et al. (2017).

The pH was measured by a Metrohm system (Metrohm 605, Metrohm, Zofingen, Switzerland) on a slurry created from the cheese sample. Rheological parameters and pH values were analysed after 90 days of ripening in all 14 cheese samples in duplicate.

2.6. Computed tomography

CT measurements of all cheeses were carried out after the ripening period of 90 days using a CT scanner (Somatom Volume Zoom, Siemens, Zürich, Switzerland). The scan parameters were published in detail by Bisig et al. (2019). The image analysis was carried out with VG Studio Max, Version 3.4.X. (Volume Graphics, Ulm, Germany).

The cheese volume, eye volume and rel. eye volume were analysed by the software.

2.7. Sensory analysis

Sensory analysis of the texture firmness of the experimental Emmental-type cheeses was performed by a panel of nine experts. Cheese samples were coded with a randomly generated number. Texture firmness was judged on a 10 cm line scale from zero (very soft) to 10 (very hard).

2.8. Statistical analysis

An experimental design with four fat levels in a semi-hard cheese and three fat levels in a Swiss-type hard cheese was applied (Tables 1 and 2). Each of the experiments were replicated on the same day (N = 2, treated as categorical variable). Statistical data analysis was carried out with the method of analysis of variance ANOVA using R (R Core Team, 2022) to determine if any of the differences between the level means were statistically significant ($p \leq 0.05$). To explain the dependent variable (eye formation), linear regression analysis was performed with one or more independent variables, which were also selected stepwise on the basis of expert knowledge using R (R Core Team, 2022). Figures in this work were plotted using the ggplot2 package by Wickham (2016).

3. Results and discussion

3.1. Composition of the experimental semi-hard and hard cheeses at 24 h

As expected, the different recipes, process parameters and fat content of the vat milk had an influence on the composition and acidification of the cheeses (Tables 3 and 4). Lower fat content was related to higher water content (with the exception of one outlier among the S cheeses), which resulted in more intensive lactic acid fermentation within the first 24 h of ripening, as shown by the elevated lactic acid content. However, this relationship was not significant. Contrary to expectations, the pH-values were higher in the reduced-fat cheeses, which was a result of elevated buffering capacity due to a shift in the fat–water–protein ratio in favour of the protein fraction (Upreti, Buhlmann, & Metzger, 2006). This was seen in the negative correlation between pH-values in the semi-hard cheeses and the different fat levels in the milk ($r = -0.902$; $p < 0.01$). Neither the fermentation process nor the buffering capacity was therefore comparable between the fat levels.

Table 3
Mean values (N = 2) of the analysis of experimental full fat, medium fat, partially skimmed and skim semi-hard cheeses with citric acid fermentation.[a]

Parameter	Full fat	Medium fat	Partially skimmed	Skim	*p*-value
Cheese 1 d					
Fat in milk (g 100 g^{-1})	3.64	1.91	0.84	0.40	n.a.
Water (g kg^{-1})	420.3	474.3	505.5	442.3†	n.s.
pH	5.22	5.26	5.41	5.41	**
Total lactic acid (mmol kg^{-1})	139.7	144.6	143.2	161.3	n.s.
Citrate (mmol kg^{-1})	6.80	7.63	7.55	7.53	n.s.
Cheese 90 d					
Fat (g kg^{-1})	296.0	179.3	94.5	52.8	***
FIDM (g kg^{-1})	477.8	322.6	182.9	101.5	***
Water (g kg^{-1})	380.3	443.3	481.0	477.8	**
MFFB (g kg^{-1})	540.5	540.5	531.4	504.8	n.s.
Protein (g kg^{-1})	280.3	323.0	361.6	406.6	**
SN pH4.6 of TN (%)	20.9	22.8	22.9	24.0	*
Total lactic acid (mmol kg^{-1})	97.2	101.4	115.2	121.7	*
Acetic acid (mmol kg^{-1})	19.0	24.9	27.9	30.0	*
Free fatty acids (C1–C6; mmol kg^{-1})	24.3	30.6	33.6	37.3	*
NaCl in aqueous phase (%)	4.87	5.08	4.99	3.85	n.s.
S_{cheese} (mmol kg^{-1}) [calculated]	27.7	24.8	22.6	21.7	***
CO_2 (mmol kg^{-1}) [basis acetic acid]	19.0	24.9	27.9	30	*
Remaining S_{cheese} (mmol kg^{-1}) [calculated]	8.7	0.0	−5.2	−8.3	**

[a] Abbreviations are: MFFB, moisture content on a fat free basis; FIDM, fat in dry matter; n.a., not analysed. The cheeses were made from the same batch of micro-filtered milk and were smear-ripened for 90 days. Each fat level was replicated once. The solubility S_{cheese} of CO_2 in cheese was calculated according to equation (5). Protein was calculated from TN with the standard dairy nitrogen conversion factor (6.38). SN pH4.6 of TN (%): Percentage of soluble nitrogen at pH 4.6 of total nitrogen. Statistical differences indicated as: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; n.s., not significant. A dagger (†) indicates one outlier not deleted.

Table 4
Analysis of experimental high fat, full fat and medium fat hard cheeses with propionic acid fermentation.[a]

Parameter	High fat	Full fat	Medium fat	*p*-value
Cheese 1 d				
Water (g kg^{-1})	380.5	383.0	411.3	n.s.
pH	5.15	5.19	5.32	**
Total lactic acid (mmol kg^{-1})	141.9	150.9	156.6	n.s.
Citrate (mmol kg^{-1})	7.00	7.75	7.43	n.s.
Cheese 90 d				
Fat (g kg^{-1})	370.8	327.8	211.0	***
FIDM (g kg^{-1})	560.0	501.5	334.1	***
Water (g kg^{-1})	337.3	345.5	368.3	**
MFFB (g kg^{-1})	536.6	514.9	467.0	***
Protein (g kg^{-1})	252.3	282.1	366.5	***
SN pH4.6 of TN (%)	19.9	21.4	23.7	**
Total lactic acid (mmol kg^{-1})	67.8	44.5	0.0	n.s.
Acetic acid (mmol kg^{-1})	30.3	49.9	64.8	***
Propionic acid (mmol kg^{-1})	60.1	95.5	127.0	***
Total free fatty acids (C1–C6; mmol kg^{-1})	91.2	146.3	192.3	***
NaCl in aqueous phase (%)	2.68	2.21	2.07	n.s.
S_{cheese} (mmol kg^{-1}) [calculated]	30.6	29.4	25.4	**
CO_2 (mmol kg^{-1}) [basis acetic acid]	30.3	49.9	64.8	***
Remaining S_{cheese} (mmol kg^{-1}) [calculated]	0.3	−20.5	−39.4	***

[a] Abbreviations are: MFFB, moisture content on a fat free basis; FIDM, fat in dry matter. The cheeses were made from the same batch of micro-filtered milk and were ripened for 90 days. Each fat level was replicated once. The solubility S_{cheese} of CO_2 in cheese was calculated according to equation (5). Protein was calculated from TN with the standard dairy nitrogen conversion factor (6.38). SN pH4.6 of TN (%): Percentage of soluble nitrogen at pH 4.6 of total nitrogen. Statistical differences indicated as: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$; n.s., not significant.

3.2. Composition and texture of the 90 d ripened experimental semi-hard and hard cheeses

Not surprisingly, adapting the fat content to reach the desired fat level resulted in a shift of the main components of water and protein in the ripened cheeses (Tables 3 and 4). As the fat content in both cheese types decreased, both the water content ($r = -0.935$) and the protein content (TN × 6.38; $r = -0.921$) increased, and the moisture in the fat-free basis decreased (significant in hard cheese only). This is in accordance with the findings of Jakobson et al. (2009) in a mild Nordic semi-hard cheese variety, Fenelon, O'Connor, and Guinee (2000) in Cheddar and Rudan, Barbano, Yun, and Kindstedt (1999) in low-moisture mozzarella as well as the general findings of Johnson and Ibáñez (2022) in reduced-fat cheese.

Lower fat content and higher water content, higher pH-values/buffering capacities and slightly lower NaCl content not only contributed to significantly stronger fermentation processes due to higher residual fermentable carbohydrates in cheese milk but also to a significantly more intensive proteolysis. This was confirmed by an increase in the content of lactic acid (semi-hard cheese), free short-chain fatty acids and the proportion of SN pH 4.6 (Tables 3 and 4). This is in accordance with the results in Cheddar cheese by Fenelon et al. (2000), who found significantly lower secondary proteolysis in full-fat cheddar than in either the half-fat or low-fat cheeses at ripening times longer than 60 d. Three independent linear regression analyses for the semi-hard cheeses in the present study showed that the variance of lactate, the variance of short chain fatty acids as well as the variance of the proportion of SN pH 4.6 could be explained each to approximately 94% with the water content, NaCl content and pH in the 1d old cheeses ($p < 0.001$, each). Similarly, in the Swiss Emmentaler, the variance of short-chain fatty acids as well as of the proportion of SN pH 4.6 could be explained each to nearly 100% with the water content in the dry matter and with pH ($p < 0.001$ for both).

To describe the textural properties of the cheeses, the strain at fracture of the semi-hard cheeses was measured (Fig. 1a). Because of the abundant eyes, it was not possible to perform the same analysis in the hard Swiss-type cheeses, which is why it was decided to describe the texture by sensory analysis (Fig. 1b). The

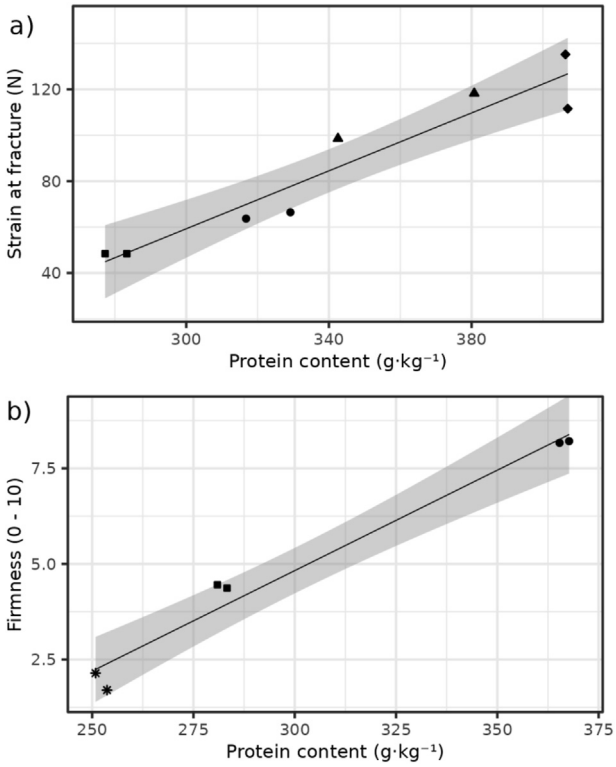

Fig. 1. Strain at fracture for semi-hard cheese (a) and "firmness" for Swiss-type cheese (b), as a function of protein content and fat level (✱, high fat; ■, full fat; ●, medium fat; ▲, partially skimmed; ◆, skim), and the predicted line from a linear regression model with the corresponding 95% confidence interval.

texture of both cheese varieties became harder with increasing protein and decreasing fat content (Fig. 1a,b). This was the result of the increasing protein content (the structure-forming part of the three main components) and of the decreasing water content in the fat-free cheese (Rogers et al., 2009). The water was not able to replace the fat on an equal basis (Rudan et al., 1999). Furthermore, the higher water-binding capacity of the accumulating proteolysis

products probably also contributed to an increasing hardness (SN pH 4.6 of TN). According to a linear regression analysis, the protein content explained 90% of the force needed at fracture in the semi-hard cheeses and 97% of the firmness of the Swiss-type hard cheeses (p <0.001). All cheeses were elastic enough to allow proper eye formation without cracks (Fig. 2a,b).

3.3. CO_2 and eye formation in the 90 d ripened experimental semi-hard and hard cheeses

The images obtained by CT show the eye formation in relation to the fat level (Fig. 2a,b), and the higher the fat level, the fewer the openings. Eye formation in cheese is the result of several

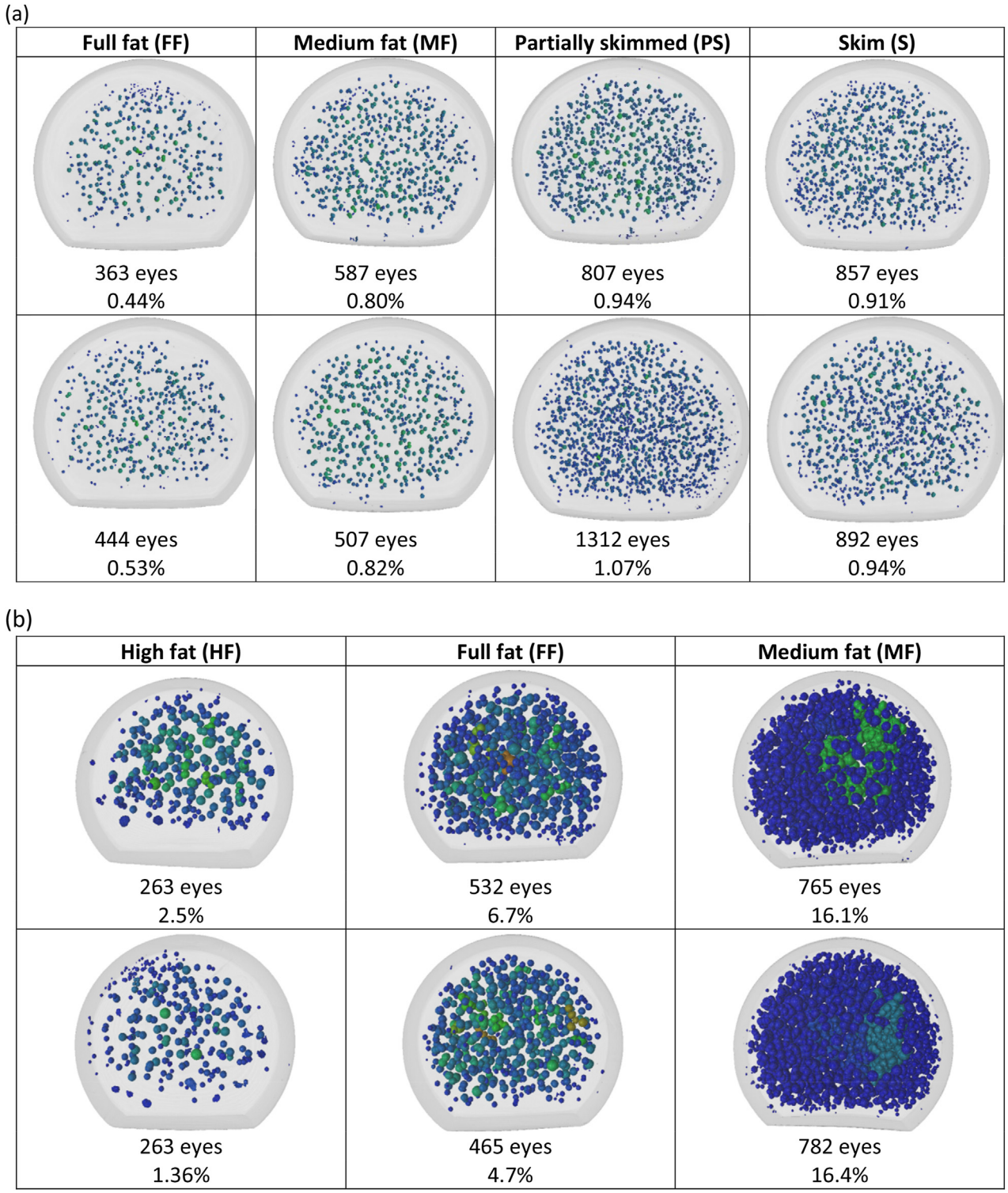

Fig. 2. Computer tomography images of semi-hard cheeses, number of eyes and relative eye volume as a function of the fat content in (a) semi-hard cheese and (b) Swiss-type cheese. The colour indicates the relative eye volume (blue, smaller; green, mid; red, larger).

interacting factors, such as the moulding and pressing technology, cheese curd fusion, sufficient gas formation, the presence of eye nuclei, viscoelastic properties, salt, pH and temperature, the solubility of CO_2 in the cheese matrix (water, fat, protein) and the diffusion barrier at the cheese surface (Fröhlich-Wyder et al., 2022).

CO_2 formation can be estimated by considering the known fermentation processes in cheese, such as the fermentation of citrate and lactose and propionic acid and butyric acid fermentation. This is only possible when all known fermentations are characterised, such as by analysing the level of the consumed carbon sources or the fermentation products, as shown by Bisig et al. (2019) and Wenzel et al. (2018). Acetic acid reflects different CO_2 sources; in the semi-hard cheeses, about 7–8 mmol kg^{-1} citrate was catabolised by the facultatively heterofermentative *lactobacilli*, and in the hard Swiss-type cheeses, the more abundant lactate was catabolised by the *propionibacteria* to equimolar amounts of acetic acid and CO_2, among other metabolic products. To distinguish between the appropriate CO_2 sources, the utilisation of citrate could be used as a basis for estimating the CO_2 production in Appenzeller® and the formation of propionic acid in the Swiss-type cheeses (Bisig et al., 2019; Wenzel et al., 2018). However, the catabolism in particular of citrate can lead to different end-products, as shown by Díaz-Muñiz et al. (2006), and therefore to different amounts of CO_2 (1–3 mmol CO_2 per mmol of citrate). As the estimation of theoretical CO_2 production will remain an approximation, the equimolar amount of acetic acid produced was taken as the basis for the estimation, which is an approach already used by other researchers (Huc et al., 2014). As can be seen in Tables 3 and 4 in both cheese types, the content of acetic acid, and therefore the CO_2 production, increased with the decreasing fat level; in the Swiss-type cheeses, as expected, this was at a much higher level. This was the result of higher initial substrate concentrations for CO_2 production in the respective cheeses, as discussed in the previous paragraph.

Similar to CO_2 production, the relative eye volume increased with each decreasing fat level and reached its maximum at the (partially) skimmed level in the semi-hard cheeses and at the medium-fat level in the hard Swiss-type cheeses (Figs. 2 and 3). In fact, the CO_2 production in the hard Swiss-type cheeses explained 82% (adjusted R-squared, p <0.01) of the relative eye volume, compared with only 52% in the semi-hard cheeses (adjusted R-squared, $p = 0.05$). Other factors may have played a role in the eye formation of the experimental cheeses. Texture properties play an important role; for example, an elastic, deformable texture is a prerequisite for a good eye formation (Daly, McSweeney, & Sheehan, 2010; Fröhlich-Wyder et al., 2022). Contrary to this knowledge, in the present study, strain at fracture correlated highly positively with relative eye volume in the semi-hard cheeses ($r = 0.847$) and firmness in the hard Swiss-type cheeses ($r = 0.980$). Furthermore, the quality of the eyes was good; hardly any cracks were recognisable (Fig. 2a,b), which would be expected in cheeses with firm texture properties (Daly et al., 2010). However, the viscoelastic properties were mainly the result of the interaction of the three main compounds (water, fat and protein), which were correlated with each other (see section 3.2). The impact of the texture independently of the cheese composition could therefore not be demonstrated.

3.4. Solubility coefficients and dissolved CO_2 and their impact on eye formation in the experimental semi-hard and hard Swiss-type cheeses

The question now arises of whether the varying composition of the cheeses, especially the fat level, had an impact on the quantity of dissolved CO_2, and therefore on eye formation. The high

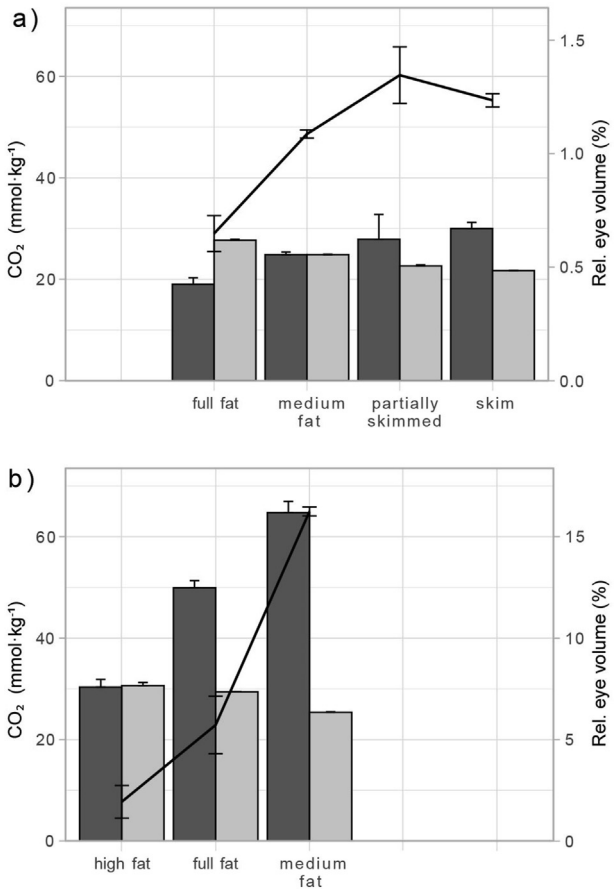

Fig. 3. Calculated amounts of (■) produced CO_2 and (■) solubility of CO_2 (primary y-axis) and the (—) relative eye volume (secondary y-axis) as a function of fat content in (a) semi-hard cheese and (b) hard Swiss-type cheese (N = 2).

correlation between fat content and relative eye volume ($r = -0.911$) at a significance level of $p = 0.002$ suggests such an influence. This is not surprising, since CO_2 is soluble in water as well as in nonpolar materials such as fat (Jakobsen et al., 2009). Recent research by Lamichhane et al. (2021) quantitatively described CO_2 solubility in a hydrated protein matrix, which was not considered here, since under the conditions found in cheese, the influence is rather stable. Knowing that CO_2 solubility is different in both phases of water and fat, the solubility capacity of the cheese is influenced by the cheese composition and has a relevant impact on eye formation. We therefore decided to compare the calculated CO_2 formation (using acetic acid as an indicator) with its solubility in the cheese and subsequently with eye formation. In the following discussion, the solubility in the cheese is displayed for conditions under a standard pressure of 101,325 Pa, and the solubility coefficient (S_{cheese}) is therefore expressed in mmol kg^{-1}.

The semi-hard cheeses produced in this study were comparable, although not identical, with those produced in the work of Jakobsen et al. (2009), who also applied different fat levels. The experimental full-fat hard Swiss-type cheeses were comparable with the Emmentaler investigated by Pauchard et al. (1980). As discussed in the introduction, Jakobsen et al. (2009) proposed an equation (Eq. (1)) to calculate the solubility coefficients and Pauchard et al. (1980) proposed considering the NaCl content (Eq. (2)). Both proposals were combined into one equation and applied in the present study (Eq. (5)). This equation applies for temperature conditions of 10–12 °C and for standard pressure:

$$S_{cheese} = w_w \times (47.32 - 1.80 \times NaCl_{aq}) + w_f \times 43.77 \left[\text{mmol kg}^{-1}\right] \quad (5)$$

The solubility coefficients (S_{cheese}) were calculated according to Eq. (5) and are shown in Tables 3 and 4 and Fig. 3a,b. As expected, the solubility of CO_2 in cheese was strongly dependent on the composition of the cheese, with higher fat-level associated with a higher solubility coefficient. Furthermore, the calculated coefficients for the semi-hard cheeses agreed well with those reviewed by Chaix et al. (2014) for cheese. In contrast, the solubility coefficients of the hard Swiss-type cheeses were lower than those found by Pauchard et al. (1980).

Comparing the amount of CO_2 produced with S_{cheese}, the remaining capacity of the cheese to dissolve CO_2, or else the excess CO_2 to diffuse towards openings or the outside of the cheese, can be estimated. A positive value of the remaining S_{cheese} describes a non-saturated cheese still able to absorb CO_2, whereas a negative value describes a saturated cheese; the excess CO_2 diffuses either towards the eye nuclei to form eyes or diffuses out of the cheese matrix. The saturation concentrations of Gouda and of Emmentaler were calculated to be 36 and 34 mmol kg^{-1}, respectively, but the eye formation began long before saturation concentration had been reached (Fröhlich-Wyder et al., 2017; Martley & Crow, 1996; Pauchard et al., 1980).

In both cheese varieties, this seemed to be the case at a CO_2 concentration of approximately 18 mmol kg^{-1}. In our case, assuming that 1 mmol of CO_2 is produced in parallel with 1 mmol of acetate, the catabolism of citrate would result in an estimated 19–30 mmol kg^{-1} CO_2 in the semi-hard cheeses and the fermentation of lactate during propionic acid fermentation would result in approximately 30–65 mmol kg^{-1} CO_2 in the hard Swiss-type cheeses (Bisig et al., 2019; Díaz-Muñiz et al., 2006; Martley & Crow, 1996). For the full-fat levels, CO_2 saturation concentration of 27.7 mmol kg^{-1} in the semi-hard cheeses and of 29.4 mmol kg^{-1} in the hard Swiss-type cheeses were calculated (Tables 3 and 4; Fig. 3a,b). In the cases of Gouda and Emmentaler, reported eye formation would start at ~17–18 mmol kg^{-1}, which corresponds with the theoretical amount of CO_2 produced in the full-fat semi-hard cheese (19 mmol kg^{-1} in Table 3 and Fig. 3a).

It can thus be confirmed that the eye formation in the semi-hard cheeses had started before the cheese body was saturated with CO_2. However, at the medium-fat level, CO_2 production and solubility were balanced, and for partially skimmed and skim cheeses, the capacity of the cheese body to solubilise CO_2 was significantly lower than the amount of CO_2 produced (Fig. 3a,b). The relative eye volume reached its maximum in the partially skimmed cheeses (Fig. 2a), revealing the importance of not only considering CO_2 formation but also CO_2 solubilisation in cheese. A linear regression analysis confirmed the significant influence of the CO_2 produced, the fat content and the salt content in the aqueous phase on eye formation in cheese (adjusted R-squared = 0.949 with $p = 0.002$). In contrast, the water content in this regression model at the defined temperature of 10–12 °C did not have a significant impact.

In the hard Swiss-type cheeses, CO_2 production and solubility were already balanced at the high-fat level (~30 mmol kg^{-1}; Fig. 3b); in comparison with the semi-hard cheeses, there was no variant with a lower saturation capacity than the amount of CO_2 formed. This means that there was always excess CO_2, considering that eye formation probably begins at ~18 mmol kg^{-1}. In Fig. 3a,b, both the CO_2 production and the solubility are compared with eye formation in cheese, expressed as relative eye volume. As can be seen, CO_2 formation and CO_2 solubility develop in opposite directions with changing fat levels. The eye volume increased with increasing CO_2 formation. In cases when CO_2 production and solubility were balanced (MF and HF cheeses for semi-hard and hard cheeses, respectively), a substantial volume and number of openings were already recognisable, as seen in Fig. 2a,b. In other words, eye formation was also observed at CO_2 concentrations below cheese saturation.

4. Conclusions

To achieve the defined fat levels in the cheeses, water and protein content had to be adjusted. With decreasing fat content, the water content, content of protein and pH value mostly increased, whereas moisture in the fat-free basis decreased. A higher water content and pH value not only led to stronger fermentation processes but also to more proteolysis.

The direct influence of fat content on eye formation could be clearly distinguished from the effects of an altered cheese composition on fermentation pathways, and thus on CO_2 production. The CO_2 formation on the basis of fermentation products and the capacity of the cheese body to dissolve CO_2 were calculated. These values were compared with the relative eye volume (%) measured by CT. This study shows that an increasing fat content had a decreasing effect on eye formation in semi-hard cheeses. Although the saturation concentration of CO_2 in the full-fat cheese had not been reached, some eye formation still occurred. In contrast, more CO_2 was released from propionic acid fermentation in the hard Swiss-type cheeses than could be dissolved, and diffused into eye nuclei to form more and larger eyes at all fat levels. For hard Swiss-type cheeses, the effect of more eye formation with decreasing fat content was strong.

In conclusion, changing fat content in cheese always influences eye formation, as a consequence of altered fermentation processes and/or of the different capacity of the cheese body to dissolve CO_2. These findings explain observations made at cheese factories, and support the industry in controlling eye formation in low- and high-fat cheese varieties requiring eye formation.

Declaration of competing interest

None.

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