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Review

Split defect and secondary fermentation in Swiss-type cheeses – A review

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Abstract – Split and secondary fermentation defects in Swiss-type cheese varieties are manifested as undesirable slits or cracks that may lead to downgrading of the cheese. Split defect is associated with an excessive production of gas or an unsuitable cheese body that cannot accommodate gas produced, or a combination of both factors. Secondary fermentation is the apparent production of gas after the desired propionic fermentation of the warm room has taken place. No consensus exists as to the definitive causes of the defects, but possible causes are reviewed under factors that are associated with rheological behaviour (including cheese manufacture, acidification, intact protein content and proteolysis, seasonality of milk supply and ripening or storage temperature and duration) or with overproduction of gas (including milk microflora, propionic acid bacteria (PAB) – in particular strains with high aspartase activity and ability to grow at low temperatures, lactic acid bacteria, interactions between starter bacteria, facultatively heterofermentative lactobacilli (FHL) and other sources of gas including butyric acid fermentation). The influence of other parameters such as copper concentration, air incorporation, salt content, rind formation and cheese wrapping materials is also considered. Methods to reduce the prevalence of the split defect and secondary fermentation include addition of water to improve elastic properties by the removal of unfermented carbohydrate and the use of FHL to control PAB activity to prevent the production of excessive gas.

Swiss-type cheese / split defect / secondary fermentation / eye formation

摘要 – Swiss 型干酪的开裂和后发酵作用——综述。由于后发酵作用引起 Swiss 型干酪出现的开裂和裂纹缺陷使得产品的等级下降。 开裂和裂纹与过量的气体产生和不适宜的干酪结构形成有关，不适宜的结构无法容纳产生的气体。 后发酵是指在温室中丙酸发酵后产生的气体。 至于缺陷产生的原因至今没有一个共识，但可能与干酪流变性 (包括干酪生产、酸化、完整蛋白质含量和水解、泌乳季节，以及干酪成熟或贮存温度和时间) 或与过度产气(包括牛乳的微生物菌群、丙酸菌特别是一些高天冬氨酸酶活性和低温生长的菌株、乳酸菌、发酵剂菌株之间的相互作用、兼性异型发酵乳酸菌和丁酸发酵产生的气体) 相关。 其他影响参数，如铜离子浓度、空气进入量、含盐量、外层表皮的形成和干酪包装材料的影响也需要考虑。 减少裂纹缺陷和抑制后发酵的方法有加水除去未发酵的碳水化合物以改善干酪的弹性，使用兼性异型发酵乳杆菌来控制丙酸菌的活性也可以防止产生过量气体。

Swiss 干酪 / 开裂缺陷 / 后发酵 / 眼状结构形成

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Résumé – Défaut de lainure et fermentation secondaire des fromages à pâte pressée cuite – une revue. Les défauts de lainure et de fermentation secondaire des variétés de fromage à pâte pressée cuite se manifestent sous forme de fentes ou fissures qui peuvent entraîner le déclassement du fromage. Le défaut de lainure est associé à une production excessive de gaz ou à une pâte fromagère inadaptée pour contenir le gaz produit, ou à une combinaison des deux facteurs. La fermentation secondaire est la production apparente de gaz après que la fermentation propionique désirée dans la cave chaude ait eu lieu. Il n'existe pas de consensus sur les causes définitives de ces défauts, mais des causes possibles sont présentées au regard de facteurs associés au comportement rhéologique (incluant la fabrication fromagère, l'acidification, la teneur en protéines intactes et la protéolyse, la saisonnalité de l'approvisionnement de lait, et la température et la durée de l'affinage et du stockage) ou associés à la surproduction de gaz (incluant la microflore du lait, les bactéries propioniques, en particulier les souches ayant une activité aspartase élevée et l'aptitude à croître à basses températures, les bactéries lactiques, les interactions entre les bactéries du levain, les lactobacilles hétérofermentaires facultatifs et d'autres sources de gaz incluant la fermentation butyrique). L'influence d'autres paramètres tels que la concentration en cuivre, l'incorporation d'air, la teneur en sel, la formation de la croûte et le matériel d'emballage du fromage est aussi examinée. Les méthodes pour réduire la prévalence du défaut de lainure et la fermentation secondaire incluent l'addition d'eau pour améliorer les propriétés élastiques par retrait des glucides non fermentés, et l'utilisation de lactobacilles hétérofermentaires facultatifs pour contrôler l'activité des bactéries propioniques pour prévenir la production excessive de gaz.

fromage à pâte pressée cuite / défaut de lainure / fermentation secondaire / ouverture du fromage

1. SWISS-TYPE CHEESE

Swiss-type cheese is a generic term for hard cheeses that were initially produced in the Emmen valley in Switzerland [31]. They are easily identifiable by characteristic round regular eyes that vary in size from 1 to 3 cm in properly produced cheese. The body of the cheese is hard to semi-hard. Examples of Swiss-type cheese include Gruyère, Jarlsberg, Comté, Maasdammer, Appenzeller, Leerdammer and the most commonly produced cheese with large eyes, Emmental (often referred to simply as "Swiss cheese") [31, 105], although it should be noted that French Gruyère contains a small number of eyes, while Swiss Gruyère is blind. There is no internationally recognised definition of Swiss-type cheeses that differentiates them from other varieties; however, a propionic acid fermentation that either occurs spontaneously or through the application of a culture of selected propionic acid bacteria (PAB) is characteristic [30].

Swiss-type cheeses are produced widely in Europe and in the US. Approximately 466 000 tonnes of Emmental are produced annually in Europe, with France and Germany as the biggest producers and consumers of this cheese. In 2004, France produced 252 700 tonnes making it the biggest producer of the Emmental cheese and accounted for ~ 25% of all French ripened cheese produced [122]. Germany produced 85 200 tonnes in 2004 [122], while in the US 142 200 tonnes of Swiss cheese were produced in 2007 making it the fifth most important cheese type in the US accounting for ~ 3.23% of the total cheese produced [112].

The biochemistry and microbiology of Swiss-type cheeses has been reviewed extensively [30, 76, 79, 105]. In summary, two major desirable fermentations by bacteria occur, lactic acid and propionic acid fermentations. Lactic acid fermentation occurs early in manufacture where lactose is converted to lactic acid. Swiss-type cheese starter cultures contain thermophilic species such as *Streptococcus thermophilus, Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis* and may also include mesophilic species such as *Lactobacillus lactis* subsp. *lactis* and *L. lactis* subsp.

cremoris [1, 30, 105]. Other Swiss-type cheeses such as Maasdam are made with mesophilic cultures only or with *L. delbrueckii* subsp. *bulgaricus* [48]. Lactose is metabolised by *S. thermophilus* to galactose and L-lactic acid during the first 24 h of ripening [29, 111]. The lactobacilli ferment the galactose to L-lactic acid and/or D-lactic acid depending on the species [111]. This lactic acid fermentation has a major influence on cheese quality due to its effects on pH, syneresis, the inhibition of other bacterial growth, removal of calcium and also in providing the substrate for propionic fermentation [31]. Thus, the selection of correct starter cultures for cheese production is vital to cheese quality [38].

Propionibacterium freudenreichii is the main species used as ripening culture in Swiss-type cheese. The propionic acid fermentation produces metabolites (propionic acid and acetic acid) that are essential contributors to the development of the characteristic nutty-sweet flavours of Swiss-type cheeses. The CO_2 produced is responsible for eye formation [31, 105].

2. SPLIT DEFECT AND SECONDARY FERMENTATION: AN OVERVIEW

Split defect manifests as an undesirable opening and appears as slits and cracks that are visible in the cut cheese loaf and may lead to downgrading of the cheese [91]. It occurs commonly in Swiss cheese and usually during the cooling period of the production process [46, 91]. Generally, split defect is due to increased gas production such as secondary fermentation and/or unsuitable texture within the cheese, which impacts negatively on its openness and elasticity of the cheese [91]. Secondary fermentation is linked with split defect as an apparent resumption of gas production and occurs during the cold room ripening (after the desired propionic acid fermentation of the warm room ripening), causing splitting and variation in eye size of the cheese. The result is a cheese, due to structural reasons and/or excess gas production, that cannot withstand the gas pressure and therefore cracks and split form [31, 46].

There is a certain ambiguity in the published literature due to the close relationship between secondary fermentation and split defect. Thus for this review, they are considered as two separate defects, but with an inference that secondary fermentation contributes to causing split defect.

There is no consensus on why or how a split defect occurs and how it can be avoided. Different studies have advanced the issue. Park et al. [82] demonstrated that gas splitting was dependent on the strain of PAB used; however, no obvious reason for this dependency was established.

Fröhlich-Wyder et al. [32] investigated PAB interactions between starter and non-starter lactic acid bacteria (NSLAB) and found that secondary fermentation is affected by the aspartase activity of PAB, the presence of facultatively heterofermentative lactobacilli (FHL) and the use of *L. helveticus* as a component of the starter. Jimeno et al. [51] showed that FHL inhibited the growth of PAB and reduced the prevalence of splits. White et al. [119] reported that the combination of PAB and *Lactobacillus* cultures used for the manufacture influenced split formation. Studies undertaken to establish a relationship between the defect and proteolysis by White et al. [119] were unable to provide a clear explanation as to the causes and control of the defect.

3. MECHANISMS OF OPENINGS

The formation of the characteristic eyes of Swiss cheese is coupled with the production of CO_2 during the warm room ripening stage [79]; the CO_2 gas pressure is the

mechanical means by which the curd is opened up to form eyes [91]. Propionic fermentation of lactate is the main source of CO_2 in Swiss-type cheeses, e.g. in traditional Emmental cheese lactate is transformed into propionate, acetate and CO_2. A number of factors are essential for proper eye formation.

3.1. The presence of nuclei

Nuclei are microscopic bubbles that are trapped in the curd structure and serve as sites into which CO_2 dissociates from solution to accumulate as gas, and thus to develop into eyes [72]. Originating from microscopic bubbles of air, formed from foam produced by milk handling treatments or associated with particulate material in the milk, they become attached to curd particles and contain nitrogen as the principal gas component as oxygen is removed by starter bacteria activity [72]. The number of eyes developed is determined by the extent of nucleation and the shape is determined by the cheese consistency with both dependent on gas production [88]. Physical openness and inhomogeneity within the curd lead to a greater number of eyes being formed. The number of eyes can be increased by manufacturing procedures such as thermisation and by applying a vacuum to the cheese during the filling and pressing processes. Accidental incorporation of H_2-producing microorganisms within the cheese may also contribute to increase the number of eyes [105].

Similar to the importance of nuclei for correct eye formation, it is also necessary that the dipping (pitching of curds under whey), draining and pressing processes undertaken during cheese manufacture should prevent air inclusion in the curd and unnecessary mechanical stress that could disturb the knitting of the curd should also be avoided [79, 91]. During Swiss-type cheese manufacture, the curds and whey may be pumped together into a special vat where the curds are allowed to settle and collect under the whey [79]. The curd is then pressed and the whey removed. Pressing should produce a knitted curd that is uniform and unbroken with no entrapped air within the structure [91].

3.2. Suitable conditions for the growth of PAB and adequate gas production

It is necessary to ensure that conditions (pH, temperature and a_w) of the curd are sufficient for preferential growth of PAB so as to achieve correct and adequate CO_2 production [85, 111]. In particular, curd pH at the time of transfer to the warm room greatly influences the eye formation in Swiss-type cheese [58]. A curd with a low pH (< 5.2) at day (d) 1 after manufacture decreases the probability of a correct subsequent eye formation due to lack of growth of PAB, whereas a high pH (> 5.4) increases the chances of excessive gas production [60]. A pH of 6.5–7 is the optimal growth range for PAB [94].

3.3. Gas formation and accumulation

In traditional Emmental cheese, eye formation occurs at the nuclei in about 20–30 days [110]. As concentrations of CO_2 increase, the gas will diffuse into any small nuclei present, and as gas production continues an overpressure (1–1.5 bar) will be established and small eye/holes formed in the curd [2, 79, 117]. Eye formation is dependent on the length of time CO_2 is present, the amount and intensity of CO_2 production and the pressure and diffusion of CO_2 within the cheese [105]. After ~ 50 days, CO_2 production has continued with a subsequent enlargement and increase in the number of eyes. The appearance

of new eyes decreases as the CO_2 production declines when cheese is placed in the cold room [105]. The pressure of gas is dependent on diffusion rates from the cheese and on the solubility of gas within the cheese loaf. The solubility of CO_2 is dependent on pH and temperature, with increases in both factors resulting in a decreased solubility of CO_2 [31]. In an 80-kg loaf, ~ 120 L of CO_2 gas can be produced before cheese is sufficiently ripened for consumption with ~ 60 L dissolved in the cheese paste, ~ 20 L present in the eyes and ~ 40 L diffused from the loaf [31]. The rind or packaging material functions as a physical barrier and, in eye development, it dictates the rate of CO_2 loss from the surface, and hence the loss of the CO_2 from the cheese to the surroundings, thereby influencing the CO_2 partial pressure within the cheese [31, 46].

3.4. Curd texture and its ability to accommodate the gas formed

It is necessary to have a curd that is elastic and pliable enough to accommodate the gas that is formed without any defects occurring. The relatively low production of acid before the point of drainage is the main reason for the elastic texture that is associated with Swiss-type cheese as a higher pH results in high mineral content of the curd and contributes to elasticity of the curd [59, 66]. High water and fat content also contribute to the soft elastic structure of Emmental, which helps in maintaining the integrity of the curd during eye formation [31].

Nuclei will more readily expand into holes if the biaxial elongation rate of the cheese curd is high, which will occur if the biaxial elongational viscosity is low, as there is less resistance against the extension of the hole. The onset of hole formation results in an initial fracture, as the overpressure in the nuclei is large and the hole grows relatively fast, after this the hole would ideally expand by flow and form a spherical eye [117]. Split formation in the cheese mass is likely if the elongational viscosity is high or/and the fracture stress is low (i.e. fracture occurring at low deformation) [66, 117, 123], particularly if the gas production rates are high.

4. FACTORS AFFECTING THE FORMATION OF EYES OR SLITS AND CRACKS IN SWISS-TYPE CHEESE: RHEOLOGICAL BEHAVIOUR

4.1. Manufacture parameters

Eye formation in Swiss-type cheese requires appropriate physicochemical and mechanical properties of the protein matrix which determine the structural properties of cheese thus influencing openness [79]. Physicochemical properties are related to cheese composition, while mechanical properties are related to both cheese composition and proteolysis [15, 79]. Swiss-type cheeses have high elastic properties, high deformability or high cohesion (fracture strain) and high fracture stress expressing high mechanical resistance [79].

4.1.1. Acidification during cheese manufacture

Acidification during manufacture of Swiss-type cheeses influences the cheese texture [66] due to its effect on bacterial growth, colloidal calcium phosphate (content and solubility) and its effect on pH and compositional parameters. Lower pH is associated with a greater probability of slit formation due to the propensity of a cheese to develop slits with shortness of the cheese texture [117]. The model of Horne [47] for casein interactions

suggests that the *para*-casein matrix is stabilised by crosslinking of different casein molecules and chains by hydrophobic interactions which is mediated by colloidal calcium [66]. Emmental contains ~ 1000 mg calcium/100 g of cheese [105] (~ 50% more than Cheddar cheese), and these high levels are mainly due to the higher pH at separation of curds and whey of Swiss-type cheese and confer an elastic texture to the cheese [66]. Berdagué et al. [6] reported a correlation between the occurrence of split defect in Comté cheeses with decreased calcium content at 20 h after manufacture.

Low acid development and thus higher cheese pH is associated with high cooking temperatures that are used during the manufacture of certain Swiss-type cheeses [91]. Syneresis is also influenced by acidification, with decreasing pH increasing the rate of syneresis within the curd [62, 121]. In turn, this determines the moisture content, proteolytic activity and other related constituents of the cheese, in particular the proportion of undissolved calcium associated with the casein matrix [60, 66]. Notz et al. [81] reported increased elasticity and decreased mechanical resistance in Comté-type cheeses with increased fat contents due to a higher moisture content resulting from reduced levels of syneresis. Acidification also influences casein hydration and electrostatic and hydrophobic interactions between casein molecules [119], and the cohesion and mechanical properties of the cheese are influenced by interactions between minerals, water and protein and particularly pH at 24 h which affects the structural state of the protein [79]. The activity of plasmin and chymosin is also dependent on pH (as well as on salt to moisture and casein to moisture ratios) [59].

Water may be added to milk or the curd/whey mixture (up to 20% of the milk weight for Emmental cheese and up to 35% for Masdaam cheese) during cheese manufacture to avoid producing a cheese with a low pH [30]. This dilution causes a decreased amount of fermentable lactose to be present and therefore a decrease in the overall lactic acid content of the curd. This results in cheese with a higher moisture content and a higher pH that is favourable to PAB growth, but also results in a more elastic texture and the levels of fracture stress and strain are increased [50]. Luyten [68] reported that at a given pH, the rheological stiffness of a cheese was decreased by increasing the moisture during maturation. Thus, the risk of late fermentation which is higher in cheeses with inappropriate elasticity may potentially be reduced by water addition during manufacture of Emmental cheese [50].

4.1.2. Influence of manufacture parameters on protein and proteolysis

α_{s1}-Casein is the primary casein involved in maintaining the elastic structure of Emmental and is vital to maintaining the integrity of the curd during eye formation as the elastic protein structure will bend and accommodate the accumulation of CO_2 [36, 119]. Emmental has a higher level of native protein (intact α_{s1} + α_{s2} + β-CN) than other Swiss-type cheese varieties such as Comté and Beaufort and as a result Emmental has higher elastic properties [42]. More native caseins in the protein matrix contribute to the cheese having increased firmness and deformability, properties that are appropriate to openness [79].

Research has not shown a strong correlation between degree of proteolysis and prevalence of the split defect [97, 119]. However, proteolytic action would be a logical factor that contributes to split defect. Fracture strain (longness) decreases as proteolysis increases [67] and shortening of cheese texture due to loss of elasticity by proteolysis may contribute to splitting occurring due to an inability of the texture

to cope with the gas pressure [32]. Berdagué et al. [6] and Grappin et al. [43] related split intensity to higher levels of secondary proteolysis as measured nitrogen levels soluble in phosphotungstic acid.

In Swiss-type cheese, plasmin and the proteinases and peptidases from starter, non-starter and secondary starter [34, 35] are the principal proteolytic agents [25, 93]. The activity of proteolytic enzymes in cheese is influenced by a_w, copper content, water content, lactic acid concentration, pH, storage temperature and time [105]. The greater role of plasmin in proteolysis in Swiss-type cheese is due to the greater conversion of plasminogen to plasmin, as inhibitors of plasmin activators and plasmin are inactivated or are lost in the whey by the use of relatively high cook temperatures, up to 55 °C [25]. Chymosin is associated with the initial shortening (reduction in yield force) of Cheddar cheese texture via hydrolysis of α_{s1}-casein [15]. However, the coagulant is inactivated to a degree dependent on the cooking temperatures used during manufacture of Swiss-type cheeses. Cooking temperatures during Emmental manufacture exceed 50 °C resulting in little residual coagulant activity [37, 90], while cooking temperatures of ~ 36–40 °C used in Maasdam/Leerdammer and Jarlsberg manufacture result in higher residual coagulant activities with greater hydrolysis of α_{s1}-casein or its breakdown products [75]. There is a greater degradation of α_{s1}-casein in cheese manufactured from raw milk which is attributed to the microflora of the raw milk and their associated enzymes [21, 23] and also possibly to the indigenous milk enzyme, cathepsin D [41]. Studies have also suggested a putative role for cathepsin D and for thermophilic lactobacilli [7, 33] in the hydrolysis of α_{s1}-casein during ripening of Swiss-type cheeses. Low activity of plasmin towards α_{s1}-casein compared to β- and α_{s2}-casein also enhances the role of α_{s1}-casein in Swiss-type cheese structure [5, 114].

4.2. Ripening parameters

4.2.1. Rheological profile during ripening

Flückiger [27] investigated changes in rheological properties during ripening of Emmental cheese using compression (and relaxation) tests on cheese samples at ripening and other temperatures. Those authors reported that elasticity decreased during the ripening period independent of temperature. Measurements of firmness were lowest in cheeses in the warm room, while those of fracture stress decreased sharply on entering the warm room. Fracture strain increased at the beginning of the warm room and then decreased throughout ripening with a rapid change observed on transfer to cold storage. Noël et al. [79] observed from that study that during the warm room phase of ripening when gas production was greatest, the cheeses had the lowest firmness and a higher elastic modulus supporting a higher deformation before breaking and showing a relative resistance to fracture, and thus attaining appropriate mechanical properties for eye formation.

Noël et al. [79] suggested that openness is related to local phenomena as CO_2 content in Emmental was observed to be heterogeneous [40]. Akkerman et al. [2] predicted that a slit would be formed instead of an eye if the local overpressure is higher than the local fracture stress. Ruegg and Moor [100] studied the size, shape and distribution of curd granules in some hard and semi-hard cheeses produced in Switzerland and reported that pressing of the cheese loaves flattened the granules. It was also reported that slit formation occurred in Raclette cheese late in maturation and slits followed the granule borders, but also cut through granules; eye formation occurred best where the curd granules were apparently completely fused together [100]. Grappin et al. [43] studied splits in Comté

cheese and reported that splits are usually parallel to the surface that is perpendicular to the press direction. Tests on the samples of cheese perpendicular to the press direction showed higher stress and strain values at fracture compared to the samples tested parallel. It was postulated that if there is a large difference between rheological results from two different directions of a cheese sample, the formation of eyes may result in a slit being formed instead of proper eyes [43].

4.2.2. Temperature and duration of ripening and storage

In general, the longer the cheese is stored the greater the prevalence of splits within a cheese prone to splitting [119]. Once the cheese has been transferred to the warm room, few measures can be taken to affect the quality of the cheese [91]. The optimum growth for PAB is between 25 and 35 °C [94] and temperatures of ~ 20–24 °C are used for the warm ripening to promote PAB growth and CO_2 formation [105]. The high temperature used during the warm room phase contributes to maintaining the soft elastic structure of the cheese and increases the ability of the cheese to support eye formation [31, 119]. As the temperature increases, the molecules, particles and strands in the cheese possess more thermal energy and a greater motion causing relaxation of protein interactions and change in the cheese towards a more liquid-like state [67].

For a cheese to stretch, casein molecules must interact with each other and be pliable enough to release stress (i.e. the pressure of gas is reduced by eye formation), but also maintain sufficient contact between the molecules to prevent rupture. Time during which the gas is evolved is important, if gas is produced slowly stretch properties may be retained, whereas if gas is evolved quickly the cheese is more likely to crack or split as the cheese is rapidly stretched [117, 119].

Cheese texture may change with the physical state of the fats depending on the storage temperature and time [22]. Crystallisation of milk fat influences its rheological properties as fat in cheese is composed of different triglycerides that have different melting temperatures spanning from approximately −40 to 40 °C [64]. Lopez et al. [64] monitored the thermal properties and solid fat content in Emmental cheese using differential scanning calorimetry and reported that crystallisation of fat occurred on cooling to about 15 °C. Thus, during ripening of Swiss-type cheeses, e.g. Emmental, the ripening temperatures (~ 22–4 °C) greatly influence the solid fat content, and thus the rheological properties of the cheese and its ability to accommodate further gas production.

Proteolysis takes place mainly in the warm room [109, 113]. Thierry et al. [109] studied the kinetics of the microbial and biochemical changes of the aqueous phase of cheese in three Swiss-type cheeses industrially manufactured and observed that levels of nitrogen soluble at pH 4.6 and in 12% TCA and levels of free amino acids (FAA) increased during ripening, at a rate dependent on the ripening room temperature. An increase in the ripening temperature increases proteolytic activity and promotes greater gas production within the cheese, with even a 1 °C increase in temperature resulting in a measurable difference [43]. Levels of FAA increased, but their relative proportion remained essentially constant, apart from amino acids catabolised by bacteria (Arg and Asp, in particular) [109].

After warm room ripening, cheese is transferred to maturation rooms at lower temperatures or to cold storage (2–13 °C) [91]. Cheese loses some of its elasticity as at lower temperatures the rheological properties of the cheese change resulting in a greater tendency to fracture upon increased CO_2 pressure (as fat is in a more solid state) [43, 46]. At low temperatures, the cheese

curd may also become supersaturated with CO_2. An unintended increase in temperature decreases gas solubility and this increases the CO_2 pressure possibly resulting in splitting of the cheese, due to the higher resistance to gas compression of the cheese mass. Therefore, it is imperative to ensure that temperature fluctuations be prevented [46, 86].

Reinbold et al. [92] varied the materials of the cheese storage container and temperature profiles and showed that rapid cooling produced uneven temperature in the cheese. This can affect moisture and bacterial activity within the cheese by forming localised areas of difference. The outer zone of the cheese cools down more quickly than the inner zone resulting in more proteolytic microflora in the outer zones of the cheese, and hence a shorter and firmer body [105]. It is therefore more desirable to have a uniform cooling and also to arrest propionic fermentation before excess gas is produced [92].

4.3. Seasonality of milk supply

Season of manufacture of cheese has been linked to variations in milk composition, cheese moisture, cheese yield and quality [12, 60]. Plasmin content of milk also varies with stage of lactation [93]. In countries with seasonal milk supply, cheeses manufactured from early lactation milk have poorer eye development and this may be partly attributed to a lower concentration of plasmin in the milk [60].

Fröhlich-Wyder and Bachmann [30] reported that cheese made from winter milk from cows fed on hay and silage has a slightly slower rate of acidification and shows a greater occurrence of secondary fermentation compared to grass-fed summer milk. Gilles et al. [38] reported that slower rates of acidification during the manufacture of Swiss-type cheese resulted in a higher moisture content in the final cheese. However, White et al. [119] reported that the production of cheese from summer milk resulted in a higher moisture content compared to cheese manufactured in the winter season and that the former had a greater tendency to split compared to the latter. A higher cheese moisture may result in higher levels of proteolysis, which could result in an altered texture and variable rates of release of small peptides and amino acids that could contribute to secondary fermentation [30]. Furthermore, cheese with an increased moisture content due to poor initial acidification may lead to an increased unfermented carbohydrate content after 24 h in the curd and, by subsequent fermentation, may result in low curd pH [29] resulting in undesirable texture and elasticity of the curd [111].

Rohm et al. [96] observed from large-scale experiments that Emmental cheese produced during the winter months showed a significantly firmer body, accelerated fermentations of lactate and propionate as well as reduced proteolysis compared to cheese produced in the summer season. Firmness was the parameter that was most heavily influenced by the season of production resulting in differences in fracture properties. This is consistent with the results obtained by Ginzinger et al. [39] for Austrian Bergkäse and may possibly have negative consequences in relation to split defect [69]. The change in firmness of the Emmental with season was attributed to the difference in milk fat composition with increases in liquid proportions in the fat phase of cheese increasing from winter to summer [96].

The diet of the cow (e.g. hay and fodder beet) can cause an elevated saturated fatty acid content in milk used for cheese manufacture and has been shown to be partly responsible for a hardening of the cheese [31]. The diet can be supplemented in order to increase the unsaturated fatty acid content of the milk and produce a softer cheese texture [13, 49, 76].

5. FACTORS AFFECTING THE FORMATION OF EYES OR SLITS AND CRACKS IN SWISS-TYPE CHEESE: OVERPRODUCTION OF GAS

5.1. Milk

Milk for the manufacture of Swiss-type cheese must be of high quality containing as few bacteria as possible to ensure optimum starter activity and prevention of secondary contamination [76, 91]. In Switzerland where Swiss-type cheeses are made from raw milk, strict measures and laws in relation to the milk quality are applied [91]. Somatic cell counts (SCCs) are a general indication of the quality of milk and vary depending on the period of lactation. Elevated SCC can correlate with a change in milk composition [52] and increased SCC has been associated with increased proteolysis during cheese ripening. Therefore, it is desirable to have a low SCC to produce quality cheese [14].

The heat treatment of the milk before cheese manufacture may impact on split defect. Swiss cheese is prone to split defect whether it is made from pasteurised or raw milk; however, no detailed work to compare both treatments in relation to this defect has been published. Higher numbers of lactobacilli (FHL), enterococci and coliforms are found in Bergkäse cheese made from raw milk compared with cheeses made from pasteurised milk [23]. Pasteurisation reduces the growth of microorganisms in general during ripening of Emmental [7]. A greater diversity of microorganisms exist in cheese made from raw milk, but this diversity declines during ripening [41]. Also, raw milk may contain wild PAB and NSLAB strains that may promote secondary fermentation [45]. As previously discussed, there is a greater degradation of α_{s1}-casein in cheese manufactured from raw milk attributed to the raw milk microflora, their associated enzymes [21, 23] and also possibly to cathepsin D [41].

Baer and Ryba [4] showed that the influence of raw milk microflora stimulated PAB growth through their proteolytic action, and Beuvier et al. [7] reported that cheeses made with raw milk show greater proteolysis and stronger propionic acid fermentation, although other studies have shown no difference in cheese texture between cheeses made from pasteurised and raw milk [9, 39]. Further research is required to determine if greater levels of proteolysis and enhanced propionic acid fermentation may promote the occurrence of split defect or secondary fermentation in cheeses made from raw milk in comparison to those made from pasteurised milks.

5.2. PAB

Selected PAB of the species *P. freudenreichii* are the main and desirable gas and eye formers in Swiss-type cheese [30]. However, it has been shown that excessive growth of PAB is a major factor that contributes to split defect [4]. Low numbers of *P. freudenreichii* may gain access to the milk via the environment; however, nowadays they are usually added intentionally [30]. Inocula of PAB in cheese milk range from 10^3 to 10^6 cfu·mL^{-1} [79, 86]. When the desired eye formation has been attained, the PAB have often reached levels of 10^8–10^9 cfu·g^{-1} in cheese [105]. White et al. [119] showed that the selection of the PAB subspecies and strain is vitally important, as the strain of PAB used for manufacture had the most obvious effect on split formation.

The metabolism of PAB involves several complex pathways and is not fully understood [84]. PAB can utilise a variety of substrates; however, lactate is the main energy source for propionic fermentation. The classical metabolic pathway of propionic acid fermentation in Swiss-type cheese is where

lactate is fermented to propionate, acetate, CO_2 and H_2O [30]:

$$3 \text{ lactate} \rightarrow 2 \text{ propionate} + \text{acetate} + CO_2 + H_2O + \text{ATP}.$$

However, the results of these theoretical equations are rarely found in Swiss-type cheese and the relative concentrations of propionate, acetate and CO_2 may be different [84].

5.2.1. *PAB with high aspartase activity*

The enzyme aspartase, which catalyses the deamination of aspartate, is found in PAB and aspartase activity varies widely from strain to strain of PAB [31]. Lactate is coupled with the fermentation of aspartate, if present, to acetate, CO_2, succinate and NH_3 with no propionate produced [30].

$$\text{lactate} + 2 \text{ aspartate} \rightarrow \text{acetate} + CO_2 + 2 \text{ succinate} + 2 \ NH_3 + \text{ATP}.$$

This would lead to an increased production of CO_2 during lactate utilisation [84]. L-Lactate is again preferentially used as the aspartate is metabolised [32]. More lactate is fermented to acetate and CO_2 as opposed to propionate in cheese where strains with a high aspartase activity are used. Aspartase activity is therefore important as strains with high aspartase activity have a greater tendency towards secondary fermentation [120]. Piveteau et al. [85] monitored changes in the amino acid composition of whey during PAB fermentation and reported that succinate was only produced when aspartate was present. Comparison of 30 Swiss-type cheese samples revealed that the deamination of aspartate to succinate by PAB increased the risk of secondary fermentation [3]. This was attributed to CO_2 production by aspartate metabolism and fermentation of lactic acid. A careful selection of PAB strains is necessary as high aspartase activity may result in secondary fermentation, but a low aspartase activity may result in negative effects in relation to openness and flavour intensity and prolong ripening times [120].

However, it has still not been proven conclusively whether aspartase activity is the cause or merely an indicator of secondary fermentation [32].

5.2.2. *Ability of PAB to grow at low temperatures*

Strains of *P. freudenreichii* were observed to grow at 3.8 °C in sodium lactate broth [56]. Park et al. [82] demonstrated that some strains of *P. freudenreichii* grew well at 7.2 °C, a temperature used for the storage of Swiss-type cheese after warm room ripening. Cheeses made with these strains showed a larger eye volume and a greater tendency to split compared to those made with strains that were inhibited more at low temperature [46]. From experimentation on two PAB cultures, it was shown at 24 °C that there was little difference in the growth rate between the two strains, whereas at 12–14 °C only one strain was capable of growth at the lower temperatures and this was the strain most prone to causing secondary fermentation [4]. The ability of gas-producing organisms to grow at low temperatures is a crucial parameter in the cause of secondary fermentation, and this should be taken into consideration when selecting a strain of PAB [4].

5.3. Lactic acid bacteria

Before whey drainage and brining, the primary acid-producing starter (*S. thermophilus*) has the greatest influence on pH [60]. *Lactobacillus helveticus* theoretically

releases a racemic mixture of D- and L-lactic acid in a 1:2 ratio [30]. D- and L-lactate are the substrates for propionic fermentation [94]. PAB utilise L-lactate preferentially, and this is explained by the fact that L-lactate metabolism results in high intracellular pyruvate concentration that has a stronger inhibitory effect on D-lactate dehydrogenase activity than on L-lactate dehydrogenase activity [84]. Berdagué et al. [6] reported a correlation between occurrence of split defect in Comté cheeses with increased D-lactate levels at 20 h after manufacture.

Acidification due to lactic acid bacteria (LAB) during cheese manufacture influences bacterial growth, calcium associated with the casein matrix, syneresis, pH and composition and enzyme activity. As previously described in Section 3.2, correct pH is vital to eye formation, while lower pH is associated with a greater probability of slit formation due to shortness of the cheese texture [117].

5.3.1. Interactions between starter LAB and PAB

The interactions and effects of mixed cultures of some strains of lactobacilli and PAB are well documented [82, 110]. White et al. [119] reported that the incidence of splits can be controlled by a correct selection of *L. helveticus* and *P. freudenreichii* cultures. Different strains of LAB and PAB can have stimulatory effect on the propionic fermentation [85]. Using a whey medium, strains of *L. helveticus* and *S. thermophilus* have been shown to increase the PAB growth with an increase in biomass and fermentation products; in particular, there was a large increase in propionic acid produced [18]. Research on the interactions between *L. helveticus* and *P. freudenreichii* showed that fermentative capability of both microorganisms was enhanced when grown as mixed cultures in a complex medium [83]. Piveteau et al. [85, 87] reported that when the growth rate of *P. freudenreichii* increased as a result of the stimulation by *L. helveticus,* a greater conversion of lactate to propionate and acetate was observed, and thus presumably a larger CO_2 production. Thierry et al. [110] reported thermophilic LAB to influence PAB growth with significant differences observed in doubling times and lactate consumption by the PAB, to a degree dependent on strain when grown on an Emmental juice-like media [101].

Piveteau et al. [85] studied the interaction between 14 strains of LAB with 4 strains of PAB in whey and showed that none of the LAB strains had an inhibitory action on PAB activity. Stimulation of PAB strains by strains of LAB varied widely from strain to strain and strain pairing used with some showing no apparent interaction. All except one of the LAB strains used stimulated at least one of the four strains of PAB. It was reported from that study that *L. helveticus* was the most consistent stimulator of PAB as the four strains of PAB were stimulated by the three strains of *L. helveticus* used. However, Kerjean et al. [53] reported that the greatest stimulation of five strains of PAB, as measured by the production of propionic acid in a whey-model system, was achieved by four of six strains of *L. delbrueckii* subsp. *lactis* tested and that the lowest level of stimulation was achieved by one of five strains of *L. helveticus*. In general, those authors reported that stimulation of PAB was higher with *L. delbrueckii* subsp. *lactis* than with *L. helveticus,* although variations between strains occurred.

5.3.2. Interactions between starters LAB and PAB: proteolysis

LAB are generally weakly proteolytic; however, they do possess an extensive proteinase/peptidase system and are capable

of proteolysis [103]. *Lactobacillus helveticus* is recognised as possessing efficient protease and peptidase activities with respect to milk proteins [26, 73]. *L. helveticus* autolyses rapidly during ripening and releases active peptidases in the cheese [35, 113], and increases in the levels of amino acids and peptides in whey and most amino acids produced were shown to be utilised by the PAB [85]. Strains of *L. helveticus* used for cheese manufacture have been shown to have an influence on secondary fermentation [30]. White et al. [119] studied split formation caused by two different strains of *L. helveticus* and found that one strain produced more splits. The reason for this was not ascertained as the degree of measured proteolysis for both strains was the same, but it was postulated that a difference may have occurred in small peptides and/or in the levels of amino acids produced by the starter cultures.

PAB possess proteolytic enzymes [57]; however, most of them are intracellular and are poorly released within the cheese. Fröhlich-Wyder et al. [32] proposed that proteolysis and amino acid catabolism may result in a higher pH, and thus better growing conditions for the PAB, and hence a greater gas production. Amino acid catabolism may also contribute to further gas production as decarboxylation produces additional CO_2 [105].

Research by Baer and Ryba [4] reported that under the experimental conditions used for their study, stimulation of PAB by LAB was due to FAA. If FAA are present, they could result in increased growth rates and as a result more CO_2 being produced, which is correlated to an increased risk of secondary fermentation. From experimentation on 30 Emmental cheeses, it was found that there was a correlation between FAA levels in the cheese and secondary fermentation as assessed by a professional panel [4]. Aspartate, alanine and serine can be used by PAB [16], but aspartate is the main amino acid used by PAB and the greatest stimulator of growth [85]. Crow [16] demonstrated that aspartate was the amino acid that was most readily metabolised in a Swiss-type cheese environment by PAB. The addition of aspartate had an apparent stimulatory effect on the growth of *P. freudenreichii* in control whey and reduced the amount of lactate converted to propionate, but not the amount of lactate converted to acetate. It is thought that this may have importance as aspartate may be released due to proteolytic activity [85].

Conversely, Piveteau et al. [87] showed in a simple whey-based model that tetra-, penta- and hexa-peptides produced by proteinases associated with the cell wall of *L. helveticus* from α_s- and β-casein had a greater stimulatory effect on the growth of *P. freudenreichii* than amino acids. In this study, no evidence was found that the amino acids produced (proline and alanine were found in the greatest quantities) had any stimulatory effect on PAB. Attempts to purify the stimulant were unsuccessful. However, nine peptide peaks were obtained from HPLC of which six were stimulatory for *P. freudenreichii,* the stimulation was less than the stimulation observed in the unfractioned material, which implies that more than one peptide may be involved. Thierry et al. [110] reported that propionibacteria were stimulated by high peptide levels and low levels of FAA as well as low salt levels, low proportions of L(+)-lactate and other undetermined factors.

5.4. FHL

The NSLAB in Swiss-type cheese are mainly composed of FHL that begin growth at the beginning of ripening [35] and eventually reach ~ 10^8 cfu·g^{-1} [109]. In a study by Demarigny et al. [21], *L. paracasei, L. plantarum, L. rhamnosus* and *L. brevis* were all detected in Swiss-type cheese,

but as the cheese ripened *L. paracasei* began to dominate the NSLAB flora.

In artisanal cheese manufacture in Switzerland, FHL are often inoculated into the milk to control PAB activity and reduce secondary fermentation [4]. The suppression of the PAB growth by FHL may be explained by the fact that both these microorganisms compete for some of the same nutrients, which results in less nutrients available to PAB for growth [118]. However, Jimeno et al. [51] attributed the inhibition of the PAB by FHL to citrate metabolism in Emmental cheese. Martley and Crow [72] demonstrated citrate utilisation by *L. plantarum* in Emmental and showed in cheese with citrate-fermenting organisms that less lactate was fermented and less propionic acid was formed compared to cheese manufactured without citrate-fermenting organisms. Weinreichter et al. [118] reported that propionibacteria developed more readily in cheeses made with citrate negative strains of FHL in comparison to citrate positive strains.

Jimeno et al. [51] showed that FHL strains such as *L. rhamnosus* and *L. casei* with high peptidolytic activity and fast autolysing nature are present in significant numbers in Swiss-type cheese, and inhibited the growth of PAB and reduced the prevalence of splits. All citrate initially present was consumed during the growth of added *L. rhamnosus*. Competition for citrate by itself did not account for the inhibition of PAB growth, as PAB do not utilise citrate directly. FHL metabolise citrate to acetate, formate and CO_2, acetate and formate appear to have an inhibitory effect on PAB growth. Other factors may also contribute to this inhibition; a higher copper level, previously chelated by citrate, was found in juice extracts from cheese manufactured with FHL, and it was observed that the ratio of copper to citrate played an important role in PAB inhibition. Diacetyl inhibits the growth of PAB and is produced by *L. rhamnosus* with citrate as the sole energy source and inhibition was shown at a value of 0.5 mmol L^{-1} of milk, which is a level observed in the cheese during FHL growth [51].

However, higher concentrations of formate, diacetyl and acetate compared to those found in Swiss-type cheese are required in defined growth media for minimal inhibition of PAB [4]. Cheese manufactured with FHL had less intensive proteolysis, as measured by the levels of FAA, compared to cheese manufactured without these microorganisms, possibly due to an inhibitory effect on the proteolytic activity of lactobacilli [4]. Conversely, Jimeno et al. [51] noted that aspartate was present in the cheese manufactured with FHL in much greater quantities (three times higher than the cheese manufactured without FHL) and that the inhibition effect of FHL on PAB was more pronounced in PAB with low aspartase activity. The reason for this observation is unknown [4].

5.5. Other sources of gas

Flückiger [27] reported that 70–80% of the CO_2 gas produced in Emmental cheese is due to the propionic fermentation during warm room ripening. However prior to warm room ripening, lactic acid fermentation by native milk and starter microflora may contribute 10–15 mL of CO_2/100 g of cheese [27]. In other cheese varieties with eyes but without propionic acid fermentation, e.g. Edam, Gouda and Herrgardsöst, CO_2 is produced from citrate by mixed strain mesophilic starters and *Lactococcus* sp. [91]. CO_2 can also be formed from other substrates such as citrate, lactose, urea and amino acids [72]. Martley and Crow [71] demonstrated that citrate metabolism can occur in Swiss-type cheese resulting in the complete utilisation of the citrate; it was suggested that non-starter Cit^+ lactobacilli may be responsible. Certain pathways of

amino acid degradation can result in CO_2 production [57]; decarboxylation of amino acids results in the liberation of CO_2 and the conversion of amino acids to amines [17]. For example, decarboxylation of glutamic acid to γ-aminobutyric acid by mixed strains of thermophilic cultures is the main source of CO_2 in some Gouda-type cheeses [123].

5.5.1. *Other sources of gas: butyric acid fermentation*

Butyric acid fermentation in cheese can lead to the production of CO_2 and H_2 gas [31]. H_2 is only sparingly soluble in the cheese compared to CO_2; therefore, a small production of H_2 can lead to large openings and blowing of cheese [72]. Germination of spores from spore-forming anaerobic bacteria such as clostridia, which contaminate milk mainly via silage, can cause this defect [19]. The most common species involved are *C. tyrobutyricum* and *C. butyricum*; however, research also points to an association of *C. beijerinckii* and *C. sporogenes* in contributing to and promoting the defect. Recent research by Le Bourhis et al. [61] showed the ability of *C. beijerinckii* to grow in cheese and suggested that the species could have implications in relation to split defects.

Preventative measures to avoid contamination with *Clostridium* spores include prohibition of milk from silage fed herds, e.g. as is practised in Switzerland [105, 115]. Bactofugation can remove 95–99% of spores prior to cheese production [116]. Addition of nitrate and lysozyme to cheese milk prevents the growth of butyric acid bacteria [116]. Germination and growth of the spores can also be inhibited by ensuring that correct brining is carried out and a low ripening temperature used [108]. A biological control system may serve as a biological barrier to control and inhibit the proliferation of *Clostridium* spores [11, 80].

5.6. Other selected factors related to overproduction of gas

5.6.1. *Copper*

Manufacture of cheese in copper vats results in cheese with a copper concentration of up to 17 ppm, while cheese made in stainless steel vats contains concentrations of 0.5–1.5 ppm of copper [77]. Copper helps to control PAB activity in the manufacture of artisanal Swiss-type cheese [31]. It influences propionic fermentation as it inhibits lactate dehydrogenase of LAB and PAB, particularly of the latter. A relative increase in D(−)-lactate occurs as copper inhibits L(+)-lactate dehydrogenase to a greater extent [54, 55]. An increase in copper concentration depressed the rate of CO_2 formation and growth of three strains of PAB used in a study by Mueller et al. [77]; however, total CO_2 production after 13 days was approximately the same for all levels of copper used. Increasing copper concentrations with cupric sulphate solution in Swiss-type cheese increased the levels of proteolysis, but decreased gas production by the PAB, which resulted in a longer stay in the warm room [74]. Mueller et al. [77] reported that a copper content of 18 ppm in cheese had undesirable effects on cheese quality, particularly in relation to body, flavour and eye formation.

5.6.2. *Salt*

Swiss-type cheeses are salted by immersion in brine and brine times are adjusted to achieve desired salt/salt-in-moisture (S/M) levels [44]. Salt is added to control microbial growth (including LAB), enzymatic activity, syneresis, and it also influences cheese texture through physical changes in cheese proteins [44]. PAB are sensitive to salt [30], and inhibition of *P. freudenrichii* is expected in Emmental cheese even though salt levels are low;

~ 0.3–0.7% w/w. Growth of PAB at pH ~ 5.3 is strongly inhibited above a level of 3% [95]. Increased S/M levels also result in reduced production of CO_2 as a result of reduced propionic acid fermentation [95]. Similarly, lower levels of acetic and particularly propionic acid were reported in brine salted cheeses with higher salt concentrations [43].

Richoux et al. [95] suggested that strains of PAB which are more tolerant to salt would be more prone to secondary fermentation than strains that are sensitive to salt due to greater activity of the salt-tolerant strains at a given salt concentration. These authors suggested that the defect may be controlled more effectively by the use of the PAB with defined salt tolerances.

Brining also results in a firm dry rind that inhibits diffusion of CO_2 from the loaf [30] and in variations in a_w values between different regions of the cheese (with a_w values usually higher near the centre [102]). This can result in zonal variations in cheese composition and proteolysis within the cheese, which can result in differences in the textural and functional properties of the cheese [44, 67].

5.6.3. Wrapping materials and rind formation

In Swiss-type cheese manufacture where a permanent rind is produced, the firm dry rind inhibits the loss of CO_2 from the loaf and provides physical protection [30]. In rindless block Emmental, wrapping material is required. In an experiment to study the effects of four different wrapping materials, Hettinga et al. [46] found that impermeable wrapping (or equivalent rind) would produce a block Swiss-type cheese that has a greater disposition to split formation as CO_2 would not be able to diffuse out of the cheese. Therefore, selection of a wrapper with permeability sufficient only to stop mould growth and give proper eye formation is required. Handling of the cheese is also important as unprotected blocks may split and distort the eye shape if handled carelessly [91].

6. TECHNIQUES FOR THE MEASUREMENT OF CHEESE MICROSTRUCTURE, EYE FORMATION, CO_2 CONTENT AND POTENTIAL INDICATOR COMPOUNDS

6.1. Measurement of the microstructure of cheese

The microstructure of cheese significantly affects its processing characteristics and texture properties, and thus may provide understanding and a means of controlling eye formation and other cheese properties [64].

Ruegg and Moor [100] examined the size distribution, shape and junction pattern of curd granules in cheeses using light microscopy and digital image analysis. The micrographs obtained facilitated visualisation of the fine structure around eyes within the cheese. Confocal laser scanning micrography may be used to observe the fat and protein microstructure by staining the protein and fat network of thin slices of cheese [20]. Lopez et al. [63, 65] used confocal laser scanning microscopy to monitor the organisation of fat and disruption of the milk fat globule membrane during the manufacture and ripening of Emmental cheese and also to observe the curd junctions formed in Emmental and the changes in curd structure during the cheese development. Rousseau et al. [99] studied the structure of Emmental cheese using scanning electron microscopic techniques. Casein and fat globule structure were monitored during manufacture and curd granule junctions, gas microbubbles and microcolonies of internal cheese flora were also observed.

6.2. Measurement and detection of eye formation or slits and cracks

A number of techniques may offer potential for the analysis and detection of both eye formation and defects associated with eyes such as split defect in cheese. Rosenberg et al. [98] investigated the use of magnetic resonance imaging (MRI) to evaluate eye formation in Swiss-type cheese. A sample cheese was subjected to MRI prior to evaluating the cheese in a traditional manner by cutting the cheese and photographing the cross-section. Both techniques were compared, and the MRI analysis was shown to be an effective non-destructive, high-resolution tool for the evaluation of structural features of eye formation. Caccamo et al. [10] photographed the slices of cheese using a digital camera and measured the surface area of the cheese slice and the area occupied by gas holes developed using image analysis software. Eskelinen et al. [24] proposed that ultrasonic methods are capable of monitoring gas-solid structure of the cheeses and providing a detection method for eye formation and associated eye defects within the cheese. The advantages of this method are that it is non-destructive, relatively inexpensive and could aid in quality control. However, it has limitations for use as there are drawbacks in relation to penetration depth and speed in scanning the entire cheese volume.

6.3. Measurement of cheese CO_2 content

An accurate method for the determination of the CO_2 content of cheese would facilitate determination of whether correlations exist between the CO_2 content of cheese and split defect by comparing cheese with a proper eye formation to those cheeses that have a split defect. Previously CO_2 produced by PAB was quantified by determination of the intermediate compounds and final products of metabolism; however, this only gives a very rough estimate of the CO_2 levels [45]. Girard and Boyaval [40] measured the CO_2 content in a Swiss-type cheese by treatment of the sample by the method outlined by Bosset et al. [8] where samples were blended under a partial vacuum, sulphuric acid was added and the liberated CO_2 was analysed by GC. Enzymatic techniques may also be used to determine CO_2, but only small quantities of cheese sample can be used, which may not be representative of the whole cheese due to variations in CO_2 concentration within the cheese [40].

Nelson et al. [78] determined the CO_2 content of Cheddar cheese by a modification of the method described by Ma et al. [70] using a method of standard addition, i.e. determination of the CO_2 concentration of the unknown cheese sample by comparison to a set of samples of known concentration. Cheese samples were blended and degassed purified water and sulphuric acid added in an airtight blender. The head space was sampled by a needle connected to an infrared CO_2 analyser. This technique was repeated a number of times, using increasing increments of sodium bicarbonate standard solution that increases the CO_2 content, thus facilitating the formation of a method of standard addition linear regression equation. The CO_2 content of individual samples could be determined by extrapolation of the equation.

6.4. Potential indicators of split defect or secondary fermentation

No reliable indicators have been determined to predict the occurrence of split defect, although some studies have shown correlations between levels of certain biochemical parameters and cheeses with secondary fermentation defect. Lower levels of D-lactic acid and overall lactic acid were observed by Steffen et al. [104] in cheeses

with secondary fermentation, and higher contents of acetate, succinate, glutamate and *p*-benzoquinone. It was suggested that proteolysis and propionic fermentation were more intensive in cheese with secondary fermentation [106]. Steffen et al. [104] reported that a combination of *p*-benzoquinone and acetate could be used to differentiate between good quality cheese and those with secondary fermentation. Steffen et al. [107] reported that Emmental cheese susceptible to secondary fermentation had high aminopeptidase and low lactate dehydrogenase activities and had elevated acetate, succinate and glutamate levels. White et al. [119] found no direct correlations between split defect/secondary fermentation and moisture and fat contents, protein, pH or D/L lactate ratio.

7. CONCLUSIONS

Split defects are associated with excessive production of gas or an unsuitable cheese body that cannot accommodate the gas produced, or a combination of both factors, resulting in a cracking or splitting of the cheese. Secondary fermentation is an apparent resumption of gas production during cold room storage of the cheese. The interrelationship between split defect and secondary fermentation has led to a degree of ambiguity in the published literature concerning both defects. The causes of secondary fermentation are implicated with the causes of split defect as gas production in the cold room is associated with split defect, whereas the causes of split defect not only encompass secondary fermentation, but also inadequacies in the texture and body of the cheese.

Rheological and structural properties that influence eye or slit and crack formation in cheese are dependent on the appropriate physiochemical and mechanical properties of the cheese protein matrix. Acidification during cheese manufacture and its effect on the levels of calcium associated with the casein matrix and on interactions between minerals, water, protein and curd pH at 24 h influence casein hydration, electrostatic and hydrophobic interactions between casein molecules, and thus cohesion and mechanical properties of the cheese. Similarly intact casein is important in maintaining the elastic structure of the cheese and in accommodating eye formation after accumulation of CO_2. Although a strong correlation between proteolysis and prevalence of the split defect in Swiss-type cheeses has not been reported, a shortening of cheese texture and a loss of elasticity due to proteolysis may potentially contribute to split defects due to an inability of the texture to cope with the gas pressure, and one report has linked increased slit intensity with increased levels of secondary proteolysis. Season of manufacture produced a variable response; a firmer body was observed in cheese made during the winter months, and secondary fermentation in cheeses was linked to slower rates of acidification in winter milk, but split defects were also linked to higher moisture levels in cheeses made from summer milk. Rheological properties of the cheese in the warm and cold rooms are also of great importance. The high temperatures used during the warm room phase contribute to maintaining the soft elastic structure of the cheese and increase the ability of the cheese to support eye formation; however if gas is developed at a high rate, the cheese may be more prone to split. On transfer to the cold room, cheese loses elasticity and may also become supersaturated with CO_2; an unintended increase in temperature at this point decreases gas solubility, increases the gas pressure and may result in splitting of the cheese.

Overproduction of gas is the other primary influence in the formation of eyes or slits and cracks in Swiss-type cheeses. Heat or non-heat treatment of cheesemilk influences milk microflora, their proteolytic

activity and their influence on subsequent propionic acid fermentation and production of CO_2 in cheese. The strains of PAB used are of great importance and in particular their level of aspartase activity and their ability to grow at storage temperatures. Similarly, stimulation of PAB by some LAB may be due to the release of peptides and amino acids resulting from proteolytic activity. This process is very strain specific and the exact mechanisms of this stimulation process are unclear. It has been reported that the artisanal cheese industry in Switzerland has controlled this defect by the use of FHL. FHL inhibit the activity and growth of PAB resulting in the production of lower levels of CO_2 possibly due to: citrate metabolism, the inhibitory effects of acetate and formate towards PAB, reduction in proteolytic activity and/or by competition for nutrients with PAB; however, a definitive explanation has not yet been established. Other sources of gas have been considered including that from butyric acid fermentation, while parameters that also influence CO_2 production also include copper concentration, air incorporation, salt content, rind formation and cheese wrapping materials.

Development of secondary fermentation and/or split defects in Swiss-type cheeses is a multi-factorial issue where many of the causes of the defect and factors influencing the quality of the cheese are interconnected. Further research is required in particular to determine if proteolytic activity that has been postulated as a cause for split defect is responsible and through which mechanism. Similarly the exact mechanism of control of the defect by use of FHL is still unknown. At an industrial scale, research is required to elucidate why some cheeses from the same day of production may be free from the defect, while others may not. In conclusion, although much research has been undertaken in this field, further research is required to elucidate the mechanisms leading to this defect and the development of recent and more sophisticated analytical techniques may offer opportunities to achieve this.

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