Pink discolouration defect in commercial cheese: a review

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REVIEW PAPER

Pink discolouration defect in commercial cheese: a review

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Abstract Development of a pink color defect is an intermittent but persistent defect in a wide range of ripened cheese varieties (Swiss, Cheddar, Grana, and Italian types) which may or may not contain an added colorant, e.g., annatto. Pink discoloration results in downgrading or rejection of a product with consequential economic loss to producers. Pink discolorations can manifest in a number of ways, e.g., patches at the surface or within the cheese block, or as a uniform pink border occurring below the surface of cheese blocks. Little consensus exists as to what the discolorations and their underlying causes are. This review seeks to provide an overview and interpretation of the underlying factors associated with the defect for both research and commercial audiences. In cheeses without added colorant, pink discoloration has been associated with: certain strains of thermophilic lactobacilli and propionic acid bacteria, Maillard reactions, and microbial pigments (e.g., carotenoids and phenolic compounds), which may be responsible for development of pink-brown or dark brown discoloration. In cheeses with added colorant (usually annatto), the development of pink discoloration has been associated with: alteration of annatto colorant due to factors such as varying pH levels within the cheese matrix (particularly < pH 5.4), oxidation of bixin in storage under high intensity fluorescent lights in display cabinets, presence of oxygen, variations in redox potential, interactions between nitrates and annatto present in plastic surface coating and also due to interactions between colorants, and the use of high heat treatment during processed cheese manufacture.

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商业干酪中粉红色褪色问题:综述

摘要 ： 些含添加或不添加色素(如胭脂红)的成熟干酪(瑞士、切达、grana和意大利干酪)中出现的粉红色褪色问题是这类干酪经常出现的问题。粉红色褪色导致干酪产品质量下降,同时给生产商造成重大经济损失。粉红色褪色有多种表现形式,如干酪表面出现斑块、内部有断块,干酪断块表面下部边缘出现不均匀的粉红色。关于褪色以及褪色的原因目前还没达成共识。本文概述并诠释影响粉红色褪色的一些相关因素。对于未添加色素的干酪,粉红色褪色与某些嗜热乳酸菌、丙酸菌;美拉德反应和微生物色素(例如类胡萝卜素和酚类化合物)有关,这些因素可能是引起粉褐色或暗褐色褪色的原因。对于添加色素(如胭脂红)的干酪,粉红色褪色与胭脂红颜色的变化有关,而引起该变化的因素有干酪基质pH值的变化(特别是pH值<5.4) ；干酪在存储和销售过程中高强度荧光造成的胭脂素氧化作用;氧气的存在;氧化还原电位的变化;硝酸盐和胭脂红在塑料包装纸下的相互作用,也可能是由于色素间的相互作用以及干酪生产过程中的高温处理而引起的。

Keywords Pink · Discoloration · Cheese · Defect · Annatto · Pigment

关键词 粉红色 · 褪色 · 干酪 · 质量缺陷 · 胭脂红 · 色素

1 Introduction

Sporadic inconsistencies in cheese appearance may result in a downgrading of cheese and a consequential economic loss to producers. Pink discolorations have been reported intermittently across a wide geographical area (e.g., Europe, USA, and New Zealand) in a range of ripened cheeses employing differing manufacturing processes, including Swiss, Cheddar, Grana, and Italian types (Carini et al. 1979; Giuliano et al. 2003; Martley and Michel 2001; Park et al. 1967; Pelaez and Northolt 1988; Shannon et al. 1968), and also in ripened cheeses colored with annatto (Hong et al. 1995a, b; Mortensen et al. 2004; Mortensen et al. 2002; Shumaker and Wendorff 1998; Andersen et al. 2006). Little data is available to assess its extent or severity; however, it has been reported that 2 to 5% of Cheddar and Colby in supermarkets in US were affected with pink discoloration (Anonymous 2000). Pink discoloration defect can be manifest in a number of ways depending on cheese-type: at the surface of the cheese block (in patches or all over the block surface), sporadically distributed within the cheese block, or as a uniform pink border occurring below external surfaces of the cheese block conferring a pink ring appearance.

There is much ambiguity in literature relating to the pink discoloration defect and although referred to as a single defect, it is probably a number of individual defects covered under a broad term. In addition, the underlying causes of the defect(s) may be multifactorial and the defect(s) may only become manifest when the necessary factors occur together. Individual pink defects are probably caused by physicochemical (Govindarajan and Morris 1973; Hong et al. 1995a, b; Martley and Michel 2001; Paramita and Broome 2008; Piergiovanni and Prati 1983; Shumaker and Wendorff 1998) factors and/or microbial (Betzold 2004; Bottazzi et al. 2000; Park et al. 1967; Shannon et al. 1968, 1969) factors. In recent years, commercial cheese producers have manipulated manufacture processes and have increased diversity of culture combinations to diversify the types of varieties produced on existing commercial plant and to increase the diversity of cheese characteristics and flavor production in cheese manufacture (Sheehan et al. 2007a,b; Wilkinson et al. 2000). Such changes

often result in an incomplete knowledge of the full impact on the cheese ripening process leading to factors which may also contribute to the manifestation of pink defects.

In the absence of any definitive overview of pink discoloration in cheese, this review seeks to collate and interpret information from all previous studies and to provide a comprehensive understanding of the underlying factors related to the defect for both researchers in the area and also for commercial cheese producers. For the purposes of this review, the term pink discoloration will encompass all pinking defects; however, it will consider separately development factors which may contribute to the pinking defect in cheeses with and without added colorant (e.g., annatto).

2 Pinking in cheese with added colorants

2.1 Colorant characteristics

Colorants (e.g., β-carotene and annatto) are often used in cheese manufacture such as Cheddar and other territorial-type cheeses to achieve desired variations in cheese color. Annatto, an apocarotenoid is widely used in cheese manufacture and produces orange to red cheese colors. The major pigments of annatto are bixin and norbixin which occur naturally in *cis* isomers forms but can be converted to the more red *trans* form by the influence of heat and light (Kang et al. 2010; Smith 2004). Annatto is extracted from the seeds of a tropical fruit using different commercial processes (Kang et al. 2010). Extraction with either oil or solvents yields mainly bixin (80% of pigment), while extraction with aqueous alkali saponifyies bixin's methyl group (predominantly used to produce the colorant for dairy application) results in water-soluble norbixin as the predominant colorant (Britton et al. 1996; Delgado-Vargas and Paredes-López 1996; Giuliano et al. 2003; Hong et al. 1995a; Mortensen et al. 2002).

Studies have linked pink discoloration in cheese to alteration of the annatto constituents with associated factors including oxidation, precipitation in acidic conditions, annatto composition, display of cheese under fluorescent lighting, temperature, and pH levels (Barnicoat 1937, 1950; Govindarajan and Morris 1973; Hong et al. 1995a, b; Moir 1933; Morgan 1933; Najar et al. 1988). Addition rates of annatto to cheese in the UK range from 23.7 to 37.5 $mg.kg^{-1}$ of cheeses with a maximum of 50 mg of norbixin per kilogram of red Leicester cheese (Scotter et al. 2002). There is no legal maximum level of annatto in cheese in the United States (Kang et al. 2010).

2.2 Oxidation of colorants in natural cheese

Annatto, as a carotenoid, is sensitive to oxidation in foods (Smith 2004). As bixin and norbixin have highly conjugated structures, this leads to susceptibility to oxidation (Giuliano et al. 2003; Smith 2004). Pink discoloration may occur in cheeses during exposure to high-intensity fluorescent lighting in retail display cabinets caused by photooxidation; this process is affected by type and intensity of light, storage temperature, exposure time, type of coloring agent present, and cheese pH (Anonymous 2000; Mortensen et al. 2002, 2004). Cheeses stored under cool white fluorescent lighting showed a faster rate of pinking compared to cheese stored under

soft white lighting; however both types of light induced pink discoloration. An increased light intensity resulted in faster development of pink discoloration over the first 48 h of exposure only; thereafter, there was no difference in the incidence of pinking.

Hong et al. (1995b) have reported that pink discoloration intensity is dependent on the ratio of redness to yellowness remaining in the sample as measured by the Hunter LAB system. As cheese pH values were reduced from 5.4 to 4.8 and with samples displayed under fluorescent light, the *b** values (yellowness) were reduced at a faster rate compared to *a** values (redness) resulting in the formation of a pink color (Hong et al. 1995b). It was also suggested that producing Cheddar and Colby cheese within a pH range of 4.8–5.1 is optimum to reduce pink discoloration in cheese under storage in display cabinets (Anonymous 2000).

The influence of cheese packaging materials on the rate of formation of pink discoloration in annatto-colored cheeses displayed under high-intensity fluorescent lighting was evaluated. Hong et al. (1995a) have reported that use of film with higher oxygen transmission rate yielded a more bleached cheese appearance and resulted in greater lipid oxidation but did not influence Hunter *a** values (redness). Vacuum-packaged Cheddar cheese showed a definite increase in Hunter *a** values (redness) during light exposure, thereby exhibiting a greater tendency for pink discoloration (Hong et al. 1995a). Storage of shredded Cheddar in an atmosphere of 100% CO_2 under fluorescent lighting resulted in color bleaching with reduction of yellowness and redness values. It was proposed that an interaction between CO_2 and the high-intensity light may have resulted in the oxidation of bixin and in a change of cheese color (Colchin et al. 2001). Use of UV-blocking sleeves did not reduce pink discoloration in annatto-colored cheeses exposed to fluorescent lighting in comparison to those without protective shields, while aluminium foil laminate was found to provide the greatest stability in color in cheese stored under high-intensity fluorescent lighting (Hong et al. 1995a).

2.3 Influence of physicochemical factors on pink discoloration

Govindarajan and Morris (1973) have undertaken UV spectra and polyacrylamide gel electrophoretic analysis of pink material isolated from defective Cheddar cheese. This material was found to consist of norbixin associated with phospholipid, β-casein, and three other unidentified peptide components. Model studies indicated that decreased pH (possibly due to hydrogen sulphide production) resulted in a microfine precipitation of norbixin. It was proposed, although unproven, that the phospholipid may have prevented resolubilization of norbixin which appeared as pink zones in the cheese (Govindarajan and Morris 1973). No further research has been reported on this mechanism within cheese to date.

Recent model studies on whey expelled from cheese curd with added colorant (annatto) have described pink precipitations occurring. It was suggested that the negatively charged norbixin associates with positively charged sites of whey and casein proteins, and also that norbixin (dissociated in alkaline solution) can bind with divalent cations (calcium) and form salts possibly leading to a mass association and development of a pink precipitate. It was proposed that a similar reaction could cause pink discoloration in cheese. It was reported that as the pH levels were lowered in the

curd/whey model system, a greater incidence of the pink discoloration precipitation occurred. The increased precipitation may possibly be due to increased solubilization of calcium (phosphate), resulting in a higher availability of dissociated calcium (personal communication of unpublished data from work carried out by Cyber Colors, Co. Cork, Ireland). No research has been carried out to prove this mechanism in a cheese matrix.

2.4 Interactions between nitrate, annatto, and increased pH levels

Pink discoloration was reported at the surface of Gouda cheese which contained nitrate to prevent late blowing due to clostridia and which was coated with a plastic emulsion containing annatto. This was attributed to an interaction between high concentration of nitrite in the cheese rind (due to the presence of surface bacteria with a high nitrate-reducing capacity), the colorant annatto, and an increase in pH at the cheese surface resulting in a pink color formation (Pelaez and Northolt 1988).

2.5 Processed cheese with added annatto or colorant

Pink discoloration in processed cheese is manifest throughout the cheese block and not just at the surface (Shumaker and Wendorff 1998) and has previously been associated with a decomposition of colorant (Kosikowski 1982). Alkaline extracts of annatto used in processed cheese manufacture have shown a higher propensity to cause pink discoloration compared to other annatto extracts. It was also reported that emulsion-based annatto colorants in the processed cheese formulation were more susceptible to pink discoloration compared to suspension-based annatto colorants (Shumaker and Wendorff 1998; Zehren and Nusbaum 2000). Processed cheese has a greater tendency to develop pink discoloration when an increase in the amount of colored natural cheese (cheese containing annatto) and an increase in ratio of aged cheese (which has undergone extensive proteolysis) was used in the process blend (Shumaker and Wendorff 1998).

In addition and due to the relatively high temperatures involved in production, processed cheese is susceptible to Maillard browning and possible alteration of cheese color. Maillard browning in processed cheese is accelerated by high pH levels in the final product (>5.9) and storage at relatively high temperatures (35 °C) (Thomas 1969; Kristensen et al. 2001). The characteristics of the natural cheese blend used for manufacture will also influence the browning of processed cheese made from it. In particular, high levels of residual galactose and lactose in Cheddar cheese of high salt-in-moisture levels will result in increased levels of browning in the processed cheese (Bley et al. 1985; Thomas 1969).

3 Pinking in cheese without added colorant

There may be an overlap in factors associated with pinking in cheeses with and without added colorants; however, as no pigments (e.g., annatto) have been added, it is probable that only low levels of pigmented compounds are present which may

result in a pink color in cheese. Many factors associated with the development of pink discoloration in cheese without added colorants have been proposed; however, no study has identified any compound or structure which results in the pink color. A number of potential factors are reviewed as follows.

3.1 Manifestation of pink defect in cheese without color addition

Pink discolorations have been reported in Italian cheese varieties such as Romano, Provolone, Asiago, and Fontina cheese (Shannon and Olson 1969; Shannon et al. 1968, 1977). Pinking was observed as early as 2 months after manufacture in some Romano cheeses, manifesting as a pink band 2.5 cm beneath the surface which may migrate throughout the cheese block (Shannon et al. 1968). Pink discoloration was observed in packaged 3-month-old shredded and block Mozzarella cheese produced by a number of different producers in the midwest United States (Betzold 2004). Bottazzi et al. (2000) have described a pink discoloration in Grana cheese occurring as an intense ring under the cheese rind. Pinking was also observed at 1 to 2 cm under the surface of Swiss-type cheese blocks and around trier holes and around other mechanical openings within the cheese (Chang et al. 1985; Park et al. 1967). In ripened New Zealand Cheddar cheese without added colorant pink, discolorations occurred as patches on and near the surface of cheese blocks (Martley and Michel 2001).

In studies on pinking of cheese without added colorant, it is generally reported that the pink discoloration fades within 24 h when defective areas are removed from packaging and exposed to air (Betzold 2004; Martley and Michel 2001; Shannon et al. 1968). It is also suggested in a number of reports that a critical level of oxygen is required for the defect to occur, as the pink discoloration generally occurs at the surface or close to the surface of the cheese block or around plug holes (where samples had previously been withdrawn from the cheese) (Martley and Michel 2001; Park et al. 1967).

3.2 Cheese composition

No correlation has been observed between cheese compositional profiles and development of the pink defect. Betzold (2004) reported that no processing differences were evident between cheese production plants producing cheese with and without the defect. No differences in compositional parameters (moisture, fat-in-dry matter, salt-in-moisture, and pH) between defective young Cheddar cheeses and cheeses free of the discoloration were identified (Betzold 2004; Martley and Michel 2001).

Similarly, no correlation was found between variations in compositional properties of Italian cheese varieties including pH and contents of moisture, salt, fat, and levels of soluble nitrogen or in cheese sensory quality and development of pink discoloration (Shannon et al. 1966). Salt penetration was not related to color development, as when cheese blocks expected to exhibit the defect were cut in half, pink discoloration developed under this newly exposed center surface where no salt had been applied (Shannon et al. 1968). Chang et al. (1985) reported development of a dark pink color evident as a band or stripe through wheels of Swiss-type cheese. However, no

correlation was found between levels of histamine, tyramine, tryptamine, histidine, tyrosine, and tryptophane levels in the cheese and the presence of the pink discoloration. Martley and Michel (2001) considered levels of lactose, glucose, citrate, acetate, nitrate, and D- and L-lactate in ripened Cheddar cheeses with pink discoloration defect to be of normal composition, except for increased levels of galactose in 66% of defective cheeses.

3.3 Chemical nature of discoloration

The pink discoloration in cheeses without added colorant was consistently found to be associated with the nondialyzable fat-free, water-insoluble protein fraction of the cheese. Attempts at extracting compound(s) responsible for the pink discoloration in Cheddar-type cheese using a wide range of solvents (HCl, NaOH, citrate solution, ethanol, methanol, water, acetone, dichloromethane, ethyl acetate, and dimethylsulfoxide; alkaline urea sample buffer) and under conditions to minimize the effect of oxygen (in nitrogen atmosphere, by use of reducing agents, β-mercaptoethanol, 2,6-dieterbutyl,4-methyl phenol, and ascorbic acid) were unsuccessful (Martley and Michel 2001). Enzymatic digestion with pepsin, trypsin, and peptidase did not release the pink compound into solution (Martley and Michel 2001). Attempts at extraction of the pink discoloration from the protein fraction in Italian-type cheese using solvents (2-butanone, ethyl acetate, carbon tetrachloride, chloroform, propanol, 2-propanol, methanol, ethanol, petroleum ether, and ethyl ether) dispersing agents (urea and potassium thiocyanate) and by digestion using acid and pepsin were also unsuccessful (Shannon et al. 1968).

Addition of tyrosine (a free amino acid), pyruvate, ascorbate (reducing agents), and fumarate to cheese curd prior to hooping of Italian cheese-type was carried out to determine their influence on development of pink discoloration. Tyrosine increased the intensity of the pink discoloration, while pyruvate (a product of glycolysis), ascorbate, and fumarate accelerated the rate of development of the defect. However, these reagents alone did not bring about the defect and it was proposed that they may mediate the rate of pigment formation and, in the case of tyrosine, may be a precursor of a pigment produced in the cheese, e.g., conversion of tyrosine to melanins (pink to orange in color) by tyrosinase (Shannon et al. 1977).

3.4 Influence of cheese starter cultures on pink discolorations

It has been widely reported that certain strains of starter cultures used for cheese manufacture have a greater tendency to produce pink discolorations in cheese (Bottazzi et al. 2000; Park et al. 1967; Shannon et al. 1969); most notably lactobacilli and propionic acid bacteria.

3.4.1 Lactobacilli

Certain strains of lactobacilli when used for manufacture of Romano cheese had a greater tendency to produce pink defective cheese, while other strains were not associated with appearance of pink discoloration (Shannon and Olson 1969). Cheese produced with lactobacilli cultures associated with the discoloration had a higher

oxidation–reduction potential, as indicated by neutral red indicator, 48 h after manufacturing than those made from strains not associated with the defect (Shannon et al. 1977). Development of the pink discoloration was accelerated by higher ripening temperatures (15.5 °C) and by enhanced penetration of O_2 into the cheese but not by the absorption of NaCl by the cheese (Shannon et al. 1969). Shannon and Olson (1969) developed a screening test to evaluate cultures tendency to produce pink discoloration. Single-strain cultures of streptococci and lactobacilli, which differed in their ability to produce pink discoloration in cheese, were incubated in a calcium carbonate–skim milk medium and phosphate–skim milk medium separately. Observations were made for color development after incubation (for 10 days at 37 °C (Shannon and Olson 1969). Both of the test media were found to be indicators of potential defect-producing cultures; starter cultures which developed discoloration in cheese gave positive reactions (i.e., a color change in the media after incubation) in the screening test, while cultures which were not associated with the pink defect gave a negative result (no color change). This study also proposed that *Lactobacillus* species are capable of producing the defect without interactive effects of other microorganisms (Shannon and Olson 1969). Betzold (2004) used this method by Shannon and Olson (1969) to screen starter cultures used for Mozzarella manufacture. These authors have shown a low correlation between this predictive assay and presence or absence of pinking in Mozzarella cheese was reported. Betzold (2004) postulated that all rod cultures may have the means to impart the characteristic defect to some degrees and that a higher rod to coccus ratio in the cheese may result in a greater chance of the defect occurring.

Shannon et al. (1977) have compared the oxidative metabolism of specific strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus helveticus* (strain which have previously been linked with a greater incidence of pinking) to a specific strain of *L. delbrueckii* subsp. *lactis* (not known to produce pink discoloration defects). Hydrogen peroxide was produced by the growth of *L. lactis* in various media but hydrogen peroxide did not accumulate during growth of the two lactobacilli strains with a greater tendency to produce the defect. Suspensions of *L. lactis* consumed oxygen more rapidly in the presence of glucose and lactate compared to the two strains (*L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*) which were only moderately active.

Bottazzi et al. (2000) have also associated the pinking with the biochemical behavior of a specific *L. helveticus* (isolated natural whey culture) used as starter culture in Grana cheese production. All the cheeses made with this starter culture were decisively pink-colored and with a more flavorful taste after 8 months of ripening compared with cheese produced using other natural whey cultures. Cheeses produced with the strain prone to pinking had higher levels of soluble nitrogen, every free amino acid, and white tyrosine crystals, thus demonstrating a possible correlation between proteolytic activity and pink discoloration. The enzymatic activity developed by the *L. helveticus* strain used as single-strain starter culture appeared to operate in a different way from that of the same strain when grown together with other strains, as occurs in the natural whey culture. A detailed study of the biochemical activity and the technological properties of potential strains of thermophilic lactobacilli was recommended prior to their utilization in Grana cheese production (Bottazzi et al. 2000).

3.4.2 Propionic acid bacteria

During a study of eye formation Swiss-type cheese, Park et al. (1967) observed pink discoloration. In this study, two different propionibacteria were compared, *P. shermanii* and *P. arabinosum*; in cheese made with the strain of *P. shermanii*, there was a significantly greater incidence of pinking (95% of the *P. shermanii* cheese had pink zones) compared to cheese made with *P. arabinosum* (2% of cheese had occurrence of pinking). However, no mechanism relating to the development of the pink discoloration was suggested.

3.5 Maillard browning

Pink discolorations were reported in ripened New Zealand Cheddar cheese manufactured without added colorant (Martley and Michel 2001). Starter cultures used consisted of a *Streptococcus thermophilus* and a *Lactococcus lactis* subsp. *cremoris* strain and did not contain any lactobacilli starter strain. Microbial analyses did not establish any significant difference in NSLAB populations or differences in other microflora which may be linked to pink color formation. However, analyses carried out on ripened cheese composition indicated increased levels of galactose in 66% of defective cheese (Martley and Michel 2001).

Martley and Michel (2001) have postulated that Maillard reactions may be involved in the formation of the defect, as higher levels of galactose were detected in defective cheese and that at least three factors are required for development of pinking in Cheddar type cheese. First, galactose must be present during ripening, which probably accumulated due to the metabolism of *Streptococcus thermophilus*; second, low molecular weight nitrogen compounds (peptides, amino acids) are formed due to proteolysis processes; and finally, the establishment of a critical oxygen level within the cheese reflecting the oxygen permeability of the packaging material. The requirement for a critical level of oxygen was made from observations that the pink discoloration occurred at the surface of the cheese and developed around plug holes where samples had been withdrawn (Martley and Michel 2001).

Generally, Maillard reactions require higher temperatures than those encountered in cheese production; however, Paramita and Broome (2008) have proposed that pink discoloration (in Romano-type cheese) could be due to the presence of α-dicarbonyl compounds which are intermediate products of the Maillard reactions. These compounds such as glyoxal, methylglyoxal and diacetyl can be produced by lactic acid bacteria and thus the high temperatures normally associated with the Maillard reactions may not be required to produce a pink defect. Although unproven in cheese, these authors have demonstrated a pink/brown color development in UHT milk at 15 °C with added diacetyl. Lo et al. (1994) have proposed that two reactions could cause a color formation; the first, with free arginine amine group (resulting from Strecker degradation) or with the guanidino group of arginine and the second, an irreversible reaction of diacetyl with arginine residues in milk proteins or peptides. A further self-condensation reaction may also occur over a longer period of time (Paramita and Broome 2008). However, this proposed pathway has not yet been demonstrated to produce pinking in a cheese system and the source and stability of

sufficient quantities of the intermediate compounds (diacetyl) in cheese has yet to be elucidated.

4 Other types of cheeses with pink discolorations

Studies of the influence on photooxidation of cheeses stored under Modified Atmosphere Packaging have shown that both flavor (lipid oxidation) and color are both affected by light exposure and packaging atmosphere (Colchin et al. 2001; Juric et al. 2003; Kristensen et al. 2000; Trobetas et al. 2008). Sliced Havarti cheese stored in modified atmosphere packaging (25% CO_2 and 75%N_2) exposed to light, showed a slight but significant increase in a^* (redness) values compared to cheese protected from light (Kristensen et al. 2000). In a similar study, light exposure resulted in significantly decreased yellowness and increased redness during storage of sliced Samsø cheese packaged in 0%, 20%, or 100% CO_2 (Juric et al. 2003).

5 Cheese pigments production by microbial sources

A wide range of pigments may be produced by fungi, yeast, and bacteria (Dufossé 2009). Phenolic compounds may be responsible for discoloration in a number of cheese varieties. Contaminating microbes (Pseudomonads, Enterococci), primary starters (*L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *helveticus*) and secondary cultures (e.g., *Candida* species, *Yarrowia lipolytica* and *Brevibacterium linens*) have been implicated in the production of pink/red discolorations in cheese (Asperger 1986; Carini et al. 1979; Dincheva 1979; Forge et al. 1977; McSweeney 2007; O'Connell and Fox 2001). The development of pink-brown or dark brown patches, "Bankrot," on mould-ripened cheeses such as Gorgonzola, Camembert, and Brie and also nonmould ripened cheeses such as Emmental, Provolone and Romano, Tilsit, Grana, and Parmesan may be attributed to the production and/or the assimilation of compounds, e.g., oxidation of tyrosine to melanins (pigments which can be pink to orange in color) by tyrosinase (Asperger 1986; Carreira et al. 1998; O'Connell and Fox 2001). The reaction is dependent on the concentration of tyrosinase, pH, moisture content, redox potential, oxygen, free fatty acid concentration, and free tyrosine level (i.e., proteolysis) (O'Connell and Fox 2001).

In red-smear ripened (surface-ripened) soft cheeses, the production of red pigments which are desirable is generated by smear bacteria at the surface of cheeses. This cheese surface coloration is a complex process involving physical, chemical, and microbial interactions. Physical and chemical parameters, such as temperature, dissolved oxygen, pH, and growth substrates are important for bacterial development and pigment production (Ferchichi et al. 1986). In general, surface ripening begins with the growth of yeasts, which metabolize lactic acid and increase pH and produce growth factors useful to bacteria. When pH values are greater than six, smear bacteria begin to grow on the cheese surface (Bockelmann 1997; Eliskases-Lechner and Ginzinger 1995. The microflora in red-smear cheeses consists of a mixed population composed of bacteria belonging to the *Brevibacterium linens*, *Arthrobacter, Corynebacterium, Microbacterium*, and *Rhodococcus* groups (Bockelmann 1997; Galaup et

al. 2005; Gavrish et al. 2004; Valdés-Stauber et al. 1997). Piantanida et al. (1996) showed that the pink-red uniform color on Taleggio cheese surface indicated the presence of *B. linens* but it is unlikely that this organism is solely responsible for the color of the smear-ripened cheese (Brennan et al. 2004; Eliskase-Lechner and Ginzinger 1995). Research by Seiler (1986), Bockelmann and Hoppe-Seyler (2001), and Leclercq-Perlat et al. (2000) have highlighted the influences of the interactions between the bacteria and/or yeasts (e.g., *Debaryomyces hansenii*, *Brevibacterium*, and *Arthrobacter* species) on the pigmentation color of smear cheeses. The pigments produced by *B. linens* were identified as carotenoids (Kohl et al. 1983; Galaup et al 2005). Carotenoids are yellow to red pigments which are tetraterpenoids usually consisting of eight isoprene units and occur widely in nature with more than 650 separate structures identified (Dufossé and de Echanove 2004). *Rhodotorula* yeasts which have been isolated from cheese are also capable of producing carotenoids, such as β-carotene; however, involvement in pink discoloration defect in cheese is not reported (Aksu and Tuğba Eren 2005; Gabier et al. 2005).

6 Conclusions

Pink discoloration in cheese can be divided into two categories: (1) in cheese where colorants such as annatto are added and (2) in cheese without added colorant. In cheese with added annatto, the factors associated with pink defect generally alter the annatto and as a result, give a pink color to the cheese. It has been reported that pinking occurrence/incidence increased due to decreases in cheese pH (particularly pH <5.4). Decreased pH may result in precipitation of norbixin (a component of annatto) which associated with phospholipid; this precipitate is incapable of resolubilization, thus causing a pink discoloration. A reduction in pH may also result in norbixin binding with divalent cations such as calcium and form salts leading to a mass association and development of a pink precipitate. Both mechanisms remain unproven in a cheese system. Light-induced photooxidation has been shown to increase the redness values in cheese and may be minimized by limiting exposure to fluorescent lighting and heat, use of foil packaging materials, and attainment of correct pH. An interaction between bacteria with high nitrate reducing capacity and plastic coatings containing annatto was proposed as a possible reason for surface pinking in Gouda cheese. In processed cheese, the use of annatto or natural cheese (used in the process blend) containing high levels of norbixin, use of high heat treatment and storage temperatures, a long processing duration, use of aged cheese which has undergone extensive proteolysis, and a low pH have been shown to promote pink discoloration in cheese.

In ripened cheese without added colorant, a large knowledge gap remains in relation to the underlying causes of the defect. The pinking of noncolored cheese can manifest in different locations within the block or wheel dependent on cheese variety; however, similar observations have been reported: fading of the pink discoloration when cheese is exposed to air (oxygen), development of pink discoloration in openings (e.g., around plug holes) or areas towards the surface of the cheese, association of the pink discoloration with the water-insoluble protein fraction, and unsuccessful attempts at extracting the color compound for identification. No study

has definitively elucidated what the pink discoloration is and how it is formed. Some research groups have proposed that the pink discoloration could be due to Maillard browning or a low-temperature Maillard browning-type mechanism in which α-dicarbonyl groups may act as intermediates. Another study has suggested that the enzymatic conversion of tyrosine to melanin pigments due to the action of tyrosinase may result in discoloration in cheese.

The majority of studies have focused on cheese starter cultures, particularly, thermophilic lactobacilli, as certain strains of lactobacilli have a greater tendency to produce pink defective cheese, while other strains are never associated with appearance of pink discoloration. Similarly, specific strains of propionic acid bacteria used for Swiss-type cheese manufacture are also associated with the pink discoloration. However, no correlation has been shown thus far between compositional and microbial properties of these cheese-types and development of pinking. Grana and Italian varieties produced with starters with a greater tendency to produce the defect showed higher proteolysis levels (in Grana cheese) and a higher oxidation reduction potential (in the Italian varieties); however, comparing strains in such a manner is problematic as they may confer different microbial and physiochemical attributes to the cheeses which may or may not be related to the pink defect. Ultimately, it remains unclear as to whether these strains are simply supplying the correct conditions for the defect to manifest, or if the strains themselves are conferring the pink discoloration by some unknown mechanisms.

Pink discoloration defect is a complex issue and it is more likely that a set of conditions rather than one single factor is required for its development. Further research is required to elucidate the underlying factors, not least the role of oxygen content, oxidation reduction potential, cheese microbial flora, and their interaction with the cheese manufacture process in the development of the defect.

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