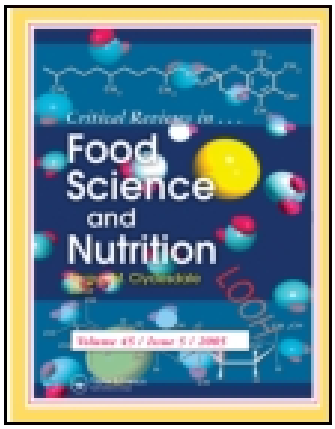

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Cheddar Cheese Ripening and Flavor Characterization: A Review

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Cheddar cheese is a biochemically dynamic product that undergoes significant changes during ripening. Freshly made curds of various cheese varieties have bland and largely similar flavors and aroma and, during ripening, flavoring compounds are produced that are characteristic of each variety. The biochemical changes occurring during ripening are grouped into primary events including glycolysis, lipolysis, and proteolysis followed by secondary biochemical changes such as metabolism of fatty acids and amino acids which are important for the production of secondary metabolites, including a number of compounds necessary for flavor development. A key feature of cheese manufacture is the metabolism of lactose to lactate by selected cultures of lactic acid bacteria. The rate and extent of acidification influence the initial texture of the curd by controlling the rate of demineralization. The degree of lipolysis in cheese depends on the variety of cheese and may vary from slight to extensive; however, proteolysis is the most complex of the primary events during cheese ripening, especially in Cheddar-type cheese.

Keywords Cheddar cheese, ripening, biochemical changes, glycolysis, lipolysis, proteolysis, texture, flavor

INTRODUCTION

Cheese is a form of milk preservation and offers a diversity of flavors, textures, and forms (Fox et al., 2000; Singh et al., 2003). Its sales have shown steady growth, mainly because of the increased use of cheese as an ingredient in a number of prepared foods (Ye et al., 2009) and it is made in almost every country all over the world to offer an excellent source of protein, fat, and minerals (Pi et al., 2009).

Cheddar is a hard, ripened cheese produced by acidification and concentration of milk following gel formation with rennet (Banks, 2002). It forms a significant proportion of international trade in cheese and, as a result of new world trade agreements, the volume of cheese trade is likely to increase. It has high nutritional value due to its high concentration of casein, which contains various levels of all essential amino acids (Hughes and Willenberg, 1993). It also contains fat and small amounts of other nutrients such as vitamins A, B_2, B_6, and B_{12}. Because of its high-protein and calcium contents, cheese in moderation is an important component of balanced diet (Considine, 1982).

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A mixture of moisture, fat, salt, peptides, amino acids, micro flora, minerals, and other minor constituents occluded within a casein matrix combine to make Cheddar a complex food (Maarse et al., 1994). The component balance theory states that a mixture of the right chemicals at the appropriate levels would produce a Cheddar aroma and texture (House and Acree, 2002) because the cheese aroma is considered to be the result of various volatile components, which individually do not reflect the overall odor (Adda, 1984), and the stimulus responsible for Cheddar cheese flavor is widely believed to be type specific and involves concentration of numerous aromatic and nonvolatile compounds (O'Riordan and Delahunty, 2001).

Unlike many processed food products, for which stability is the key criterion, cheese experiences considerable changes during ripening. Freshly made curds of various cheese varieties have bland and largely similar flavors and aroma and during ripening, flavoring compounds are produced that are characteristic of each variety (McSweeney and Sousa, 2000).

The biochemical changes occurring during ripening may be grouped into primary events that include the metabolism of residual lactose and of lactate and citrate (often, although erroneously, referred to collectively as glycolysis), lipolysis, and proteolysis. Following these primary events, secondary biochemical events like metabolism of fatty acids and amino acids

are very important for the production of variety of secondary metabolites, including a number of compounds which are necessary for flavor development (Hill and Ross, 1998; McSweeney, 2004).

The acceptability of Cheddar cheese depends largely on the flavor formed during ripening (Banks, 2002). The flavor profiles of cheese are complex and influenced by many substances like organic acids, sulfur compounds, lactones, methyl ketones, alcohols, and phenolic substances (Seitz, 1990; Urbach, 1993). Organic acids play an integral role in cheese quality because they are important flavor compounds formed as a result of carbohydrate catabolism and hydrolysis of milk fat, normal ruminant metabolic processes, bacterial growth, or addition of acidulants during cheese making (Llano et al., 1996; Akalin et al., 2002; Izco et al., 2002; Murtaza et al., 2012).

CHEDDAR CHEESE MANUFACTURING

Milk Composition

Milk composition affects the cheese yield and quality. Casein is the protein, which we use when making cheese. Most of the whey proteins, which constitute about 20% of the proteins, will be lost with the whey during cheese making. The molecular components in casein are αS1-, αS2-, β-, and κ-casein differing in amino acid composition, phosphorylation, and glycosylation (Walstra et al., 2006). Coagulation time depends on the protein (casein) contents. Increase in milk protein (>3%) results in slight increase in gelation time. A minimum protein level of 2.5–3.0% is necessary for gel formation in cheese manufacturing (Fox et al., 2000). Increasing fat content in the range 0.1–10% while maintaining the protein level constant (3.3%) enhances the coagulation properties of rennet, as reflected by the decrease in coagulation time and set-to-cut time and higher values for firming rate (S_{mas}) and firmness (G') of the curd (Fox et al., 2000).

The composition of milk changes markedly during lactation with regard to its fundamental components, micellar structure, and salt equilibria. Its technologic and physicochemical properties vary as well (Coulon, 1994), with consequent effects on the yield and the quality of cheese (Kefford et al., 1995; Lucey, 1996). Early-lactation milk tends to have a good reactivity with rennet (White and Davies, 1958). Late-lactation milk, on the other hand, is considered less suitable for the manufacture of several cheese varieties, mainly because of defects in syneresis of the curd (O'Keeffe, 1984). Data concerning the trend of the rheological properties of the milk in the course of lactation are limited and, sometimes, controversial (Bonato et al., 1987; Coulon et al., 1998; Okigbo et al., 1985). Therefore, milk used for cheese making is generally standardized for fat and casein values.

As compared with cow milk, buffalo milk is richer in fat, lactose, protein, total solids, vitamins, and minerals, such as calcium, magnesium, and inorganic phosphate (Fundora et al., 2001; Ahmad et al., 2008). Buffalo's milk is ranked second in the world after cow's milk, constituting more than 12% of the world's milk production (CNIEL, 2002). In India and Pakistan (both producing approximately 80% of the world's production of buffalo milk), this milk is used for making different dairy products including Cheddar cheeses (Ahmad et al., 2008).

Murtaza and colleagues (2008) concluded that the nutritional value and acceptability of Cheddar cheese manufactured from buffalo milk is much superior to that from cow milk. Therefore, buffalo milk, because of its chemical composition, offers excellent opportunities for the development of Cheddar type cheese.

MANUFACTURING PROCESS

The basic steps in the transformation of milk into cheese consist of coagulation, draining, and ripening (Azarnia et al., 2006). During the coagulation step, modification of casein micelles occurs under the action of proteolytic enzymes or lactic acid. Biochemical modifications of the curd components occur in the ripening stage (Spreer, 1998; Mulvihill and Ennis, 2003).

Cheddar curd manufacture commences with the selection and pretreatment of milk of high microbiologic and chemical quality. Most Cheddar cheese milk is now pasteurized just before use, but raw milk is still used in both commercial and farmhouse cheese making. In general, cheese made from raw milk develops the characteristic Cheddar flavor more rapidly, reaching its best flavor at three to six months (Price and Call, 1969). On the other hand, cheese made from pasteurized milk takes twice as long as that made from raw milk to develop the same flavor intensity and ripens more slowly than raw milk cheese (Fox, 1993). McSweeney and colleagues (1993) compared the quality of Cheddar made from raw, pasteurized, or microfiltered milks. The cheeses from pasteurized or microfiltered milk were of good and equal quality, but raw milk cheese was downgraded because its flavor was unusual, much more intense, and developed much faster than that of the other cheeses.

Pasteurization of milk causes very limited heat-induced interaction of whey proteins with casein and results in the retention of additional whey proteins in cheese beyond the normal amount which is soluble in the aqueous phase of cheese. The presence of heat-denatured whey proteins in cheese may influence the accessibility of caseins to proteinases during ripening (Lau et al., 1991).

Acidification during cheese manufacture is one of the primary events in the manufacture of most, if not all, cheese varieties and involves the fermentation of lactose to lactic acid by selected lactic acid bacteria (Fox et al., 1996). The most suitable and widely used starter cultures for Cheddar cheese manufacturing are *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* (Michel and Martley, 2001). The rate and point of the process at which lactic acid is principally produced is characteristic of the variety. In Cheddar type cheese, most acid

is produced before molding, while in most other varieties, it occurs mainly after molding. Acid production affects almost all aspects of cheese manufacture and, therefore, cheese composition, texture, and flavor. The amount of acid has a marked effect on the level of proteolysis and other reactions in the resulting cheese. The activity of the coagulant during manufacture and the retention of coagulant depend on the amount of acid produced during the initial stages of manufacture (Rehman et al., 2004).

The role of pH in cheese texture is particularly important, because changes in pH are related directly to chemical changes in the protein network of the cheese curd. As the pH of the cheese curd decreases, there is a concomitant loss of colloidal calcium phosphate from the casein micelles and, at less than pH 5.5 or thereabout, a progressive dissociation of the sub-micelles into smaller aggregates occurs (Lawrence et al., 1987). The solubilization of colloidal calcium phosphate, among other factors, affects curd (cheese) texture, stretchability, and meltability.

The manufacture of Cheddar continues with coagulation of the milk by rennet. Rennet coagulation of milk is a two-step process. The first step involves the enzymatic hydrolysis of κ-casein, and the second involves the coagulation of casein by Ca^{2+} at temperatures $>20^{\circ}$C. Chymosin in rennet specifically cleaves κ-casein at Phe105-Met106, which leads to the release of the hydrophilic caseinomacropeptide (k-CN (f106–169) part of κ-casein, located at the surface of the casein micelles. When intact, the micelles are kept colloidally dispersed in milk by steric and electrostatic repulsion involving the negatively charged casein omacropeptide part of κ-casein. The casein micelles become unstable following the removal of these hydrophilic peptides; then, at an appropriate temperature (e.g., 30°C) the milk coagulates under the influence of Ca^{2+} in the medium (Dalgleish, 1993).

A rennet milk gel is quite stable if maintained under inactive conditions, but if it is cut or broken, syneresis occurs rapidly (Fox, 1993). During practical cheese making, cutting the curd into small pieces gives faster (initial) syneresis which is proportional to the area of the surface exhibiting syneresis. The rate and extent of syneresis are influenced by milk composition, particularly calcium level, casein concentration, pH of the whey, cooking temperature, rate of stirring of the curd–whey mixture, and time (Walstra et al., 1987).

In Cheddar manufacturing, after cooking and whey drainage, the curd is allowed to rest for a considerable time to develop sufficient acidity, after which the coherent curd mass, is cut into fairly small pieces (milling), salted, molded, and pressed. During several of these processing steps, the curd may lose considerable moisture (Walstra, 1993). In Cheddar-type cheese, during cheddaring, the drained mass of curd is allowed to spread laterally for a considerable time, which helps to retain higher moisture content (one to two percent more water). The main cause of the differences is presumably that the flow of curd promotes deformation of curd grains, thus closing pores and hindering drainage of any moisture still leaving the grains due to syneresis. The composition of the finished cheese is, to a very large degree, determined by the extent of syneresis and, because this is readily under the control of the cheese-maker, it is here that the differentiation of individual cheese varieties really begins (Fox, 1993).

Salting is carried out in Cheddar by mixing dry salt with broken or milled curd at the end of manufacture. Salt is reasonably uniformly distributed with the milled-curd. However, complete equilibrium is rare (Sheehan et al., 2009). Cheddar cheese usually contains 1.4–1.5% salt. Concentrations higher than 2% makes the cheese too dry and slows down the ripening; causing underdeveloped body and flavor (Kosikowski and Mistry, 1997a).

Salt exercises one or more functions as it affects the cheese ripening and, therefore, influences the production of organic acids, directly modifies flavor, promotes curd syneresis, reduces water activity, and influences the activity of rennet, starter and non-starter lactic acid bacteria (NSLAB) and of their enzymes, and indigenous milk enzymes (Murtaza et al., 2012). It suppresses the growth of undesirable non-starter microorganisms and, by its influence on post-cheddaring, starter activity. Salt in Cheddar cheese controls the metabolism of lactose and the pH of the fresh cheese, which in turn affects the rate of maturation and cheese quality (Fox, 1987). In addition, it modifies cheese proteins, which influence the texture and protein solubility (Fox et al., 2000).

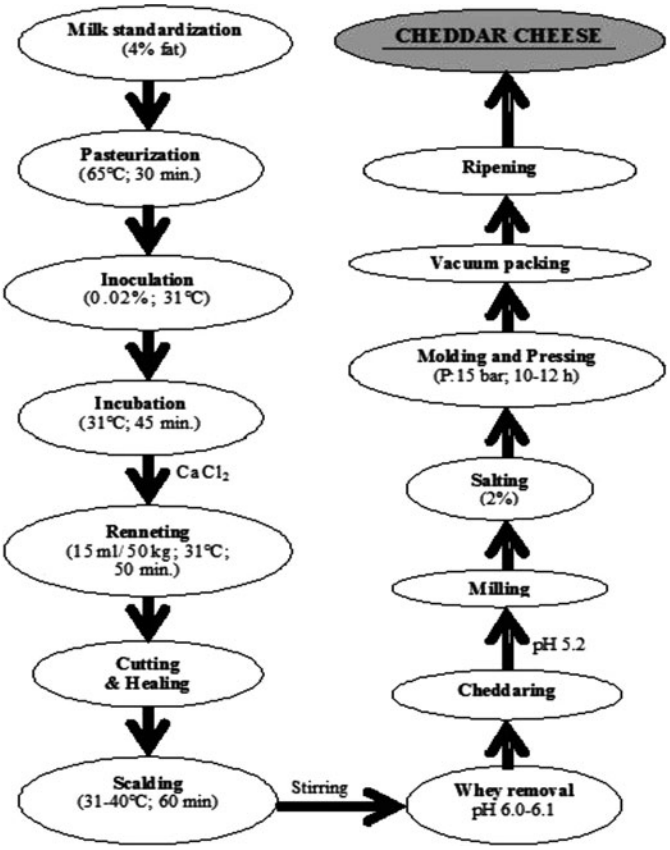
The last step in Cheddar manufacture is molding and pressing. Molding is a process of forming the salted curd into shape by the use of metal, plastic, or wooden moulds (Scott, 1986). During molding, the curds are allowed to form a continuous mass (Fox et al., 2000). Matting of high-moisture curds occurs readily under their own weight, but pressing is required for low-moisture cheese. The functions of pressing are to assist final whey expulsion, leading to a closed-texture, well-shaped cheese with a rind after long ripening periods (Bylund, 1995). Pressing should be gradual at first because initial high pressure compresses the surface layer and entraps moisture in the body of cheese. Stainless steel moulds are lined with muslin cloth soaked in warm and slightly salted water. The use of perforated liners is necessary to allow the remaining whey to escape. After 18 hours of pressing, the cheese blocks are removed from the moulds, wrapped in plastic film, and thermally sealed (Kosikowski and Mistry, 1997b).

At industrial scale, Cheddar cheese manufacture results in the production of rectangular blocks of cheese ranging in weight from approximately 12 to 290 kg. Within these blocks, localized variations exist in parameters such as salt, pH, moisture, starter populations, and proteolytic enzyme activities that can influence the flavor and texture of Cheddar cheese (Sheehan et al., 2009).

Curds for different cheese varieties are recognizably different at the end of manufacture, mainly as a result of compositional and textural differences arising from differences in milk composition and processing factors. The unique characteristics of the individual cheeses develop during ripening, although in most cases the biochemical changes that occur during ripening and, therefore, the flavor, aroma, and texture of mature cheese,

are largely predetermined by the manufacturing process (Fox, 1993).

PROCESS LINE FOR MANUFACTURING OF CHEDDAR CHEESE



Cheddar Cheese Ripening

Cheese ripening is an outcome of several microbiologic, biochemical, and metabolic processes usually referred to as glycolysis, lipolysis, and proteolysis (Farkye, 2004; Singh et al., 2003). These are responsible for the basic flavor and textural changes (Collins et al., 2003a; Lucey and Singh, 2003; Smit et al., 2005). The relative importance of each of the processes depends on the variety of cheese (Farkye, 2004).

Although considerable differences in curd are apparent, as mentioned earlier, the characteristic flavor, aroma, texture, and appearance of individual cheese varieties develop during ripening. These changes are predetermined by the composition, particularly moisture, pH, and salt, and microflora, starter, and, especially, nonstarter microflora and adjunct starter (Singh et al., 2003).

The primary changes – proteolysis, lipolysis, and glycolysis – are followed and overlapped by a host of secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids, oxidation of fatty acids, as well as some synthetic changes—that is, esterification (Fox, 1993). The primary reactions are mainly responsible for the basic textural changes and flavor production of cheese whereas the secondary transformations are mainly responsible for the finer aspects of cheese flavor and modify cheese texture (Singh et al., 2003).

GLYCOLYSIS

As cheese is a fermented dairy product, a key feature of its manufacture is the metabolism of milk sugar (lactose) to lactate by selected cultures of lactic acid bacteria (McSweeney, 2004). Lactose degradation influences cheese flavor and a number of flavor compounds, including diacetyl, acetic acid, and propionic acid, are produced from this phenomenon (Forde and Fitz-Gerald, 2000). The main isomer produced during this change is L-lactate (Fox and Law, 1991). Lactose metabolism is decreased by addition of salt during Cheddar cheese manufacture. In this case, most of the lactic acid is produced in the cheese before salting and molding. The rate of lactose fermentation depends on the percentage salt-in-moisture content of the curd. At low salt-in-moisture concentrations and low populations of NSLAB, residual lactose is converted mainly to L (+)-lactate by the starter bacteria (Choisy et al., 2000).

The salt-in-moisture concentrations also determine the products of post-manufacture lactose fermentation. If starter activity is inhibited after manufacture, residual lactose will be metabolized by NSLAB, mainly *Pediococci* and mesophilic *Lactobacilli*, which are more salt-tolerant than starter bacteria and metabolize lactose to D and L-lactate. NSLAB grow in all cheeses, but their growth is markedly dependent on temperature. They have little influence on lactose or lactate concentration until their numbers exceed 10^6 to 10^7 cfu/g (Fox et al., 1990).

Demineralization of the casein micelles has a major effect on cheese texture and increases cheese proteolysis (Le Graet and Gaucheron, 1999; Pastorino et al., 2003). The pH at whey drainage largely determines the mineral content of a cheese. The loss of calcium and phosphate from casein micelles determines the extent to which they are disrupted, and this largely determines the basic structure and texture of a cheese (Lawrence et al., 1983). In general, curds with a low pH have a crumbly texture, while high-pH curds tend to be more elastic.

CATABOLISM OF LACTATE AND CITRATE

Lactate produced from lactose by the growth of the starter is an important substrate for a range of reactions that occur in cheese during ripening (McSweeney, 2004). The level of lactate in Cheddar is about 1.5%. Matured Cheddar contains a considerable concentration of D (−) lactate, which may be formed by fermentation of residual lactose by *Lactobacilli* or by racemization of L (+) lactate (Fox et al., 1990). Racemization of L-lactate

by both *Pediococci* and *Lactobacilli* is pH-dependent (optimum pH four to five), and is retarded by NaCl concentrations more than two percent or more than six percent for *Pediococci* and *Lactobacilli*, respectively. The racemization of L-lactate is probably not significant from a flavor viewpoint, but D-lactate may have undesirable nutritional consequences in infants. Calcium D-lactate is believed to be less soluble than calcium L-lactate and may crystallize in cheese, particularly on cut surfaces (Dybing et al., 1988). The crystals may be mistaken by consumers as spoilage, and crystal formation is generally considered negative. Oxidation of lactate can also occur in cheese. During this process, lactate is converted to acetate and CO_2. This oxidative activity is dependent on NSLAB population and on the availability of O_2, which is determined by the size of the blocks and the oxygen permeability of the packaging material (Thomas, 1987). Acetate is present at fairly high concentrations in Cheddar and is considered to contribute to cheese flavor, although a high concentration may cause an off-flavor (Aston and Dulley, 1982).

Cheddar cheese curd typically contains 0.2–0.5% (w/w) citrate (McSweeney and Fox, 2004). Citrate is an important precursor for flavor compounds in certain varieties (McSweeney and Fox, 2004; Parente and Cogan, 2004). It is not metabolized by *Lactococcus lactis* ssp. *lactis* or *Lactococcus lactis* ssp. *cremoris*, but is metabolized by *Lactococcus lactis* ssp. *biovar diacetylactis* and *Leuconostoc* spp., which leads to production of flavor compounds, such as diacetyl, acetoin, and 2, 3-butyleneglycol, whereas, in some cheeses, citrate is metabolized rapidly in the presence of fermentable carbohydrate with the production of acetic acid, diacetyl, and CO_2 (Aston and Dulley, 1982; Fox and Law, 1991; Cogan, 1995). However, this citrate metabolism is responsible for undesirable openness and floating curd in Cheddar cheese (Cogan and Hill, 1993).

Approximately 90% of the citrate in milk is soluble and most is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is three times that in whey (Fryer et al., 1970), presumably reflecting the concentration of colloidal citrate.

Lipolysis

Lipolysis is usually understood as the accumulation of FFA during ripening, with most of the free fatty acids (FFA) being released from triglycerides. Total FFA concentration and short/long-chain FFA ratio have been related to the type and the amount of lipase used during cheese ripening (Hernandez et al., 2009).

The fat fraction of cheese is important for the development of typical flavor and texture (Emmons et al., 1980). Fresh Cheddar cheeses contain 30% or more fat (Renner, 1993).

Lipids in foods may undergo lipolytic or oxidative degradation. However, in cheese, oxidative changes are very limited due to the low oxidation/reduction potential (McSweeney and Sousa, 2000; Collins et al., 2003b), while lipolytic (enzymatic hydrolysis by lipases and esterases) changes are likely to occur (Adda et al., 1982; Walstra et al., 1999). Lipases in cheese originate from milk lipase, chymosin paste, starter, adjunct starter, non-starter bacteria, and exogenous lipases (McSweeney and Sousa, 2000; Perotti et al., 2005). Lipases catalyze the hydrolysis of triglycerides, diglycerides, monoglycerides, fatty acids, and glycerol (Thomson et al., 1999; McSweeney and Sousa, 2000). The hydrolysis of triglycerides, which constitute more than 98% of cheese fat, is the principal biochemical transformation of fat during ripening, which leads to the production of FFA, diglycerides, monoglycerides, and possibly glycerol. It has an important effect on flavor and aroma development in cheese during ripening (Adda et al., 1982). The relative proportions of FFAs C6:0 to C18:3 are similar to those in milk fat, indicating that these FFAs are released nonspecifically. However, free butyric acid is found at higher concentrations than can be explained by its proportion in milk fat. The lipolytic activity of lactic acid bacteria produces low levels of FFA that can contribute to the background flavor of Cheddar cheese (Olson, 1990).

Lipase and esterase activities have been detected in cell-free extracts of numerous *Lactococcus* and *Lactobacillus* species (Kamaly and Marth, 1989). A preference for short-chain fatty acids has been observed for *Lactococcal* and *Lactobacilli* lipases (El Soda et al., 1986; Kamaly and Marth, 1989).

The degree of lipolysis depends on the variety of cheese. In most cheese varieties, relatively little lipolysis occurs during ripening and too much is considered undesirable; most consumers would consider Cheddar, Dutch, and Swiss-type cheeses containing even moderate levels of FFAs to be rancid. However, extensive lipolysis is essential and desirable as part of overall flavor development in certain cheeses, such as hard Italian cheeses, Blue and Feta (Forde and Fitz-Gerald, 2000; Alewijn et al., 2005; Perotti et al., 2005).

CATABOLISM OF FATTY ACIDS

Fatty acid composition, lipolytic enzymes, moisture, temperature, storage time, oxygen, and surface area all affect lipolysis. Lipolytic degradation of triglycerides of milk fat leads to the formation of FFAs, which are catabolized to volatile compounds, such as methyl ketones, thioesters, and lactones (Walstra et al., 1999; Forde and Fitz–Gerald, 2000; Collins et al., 2003a; Collins et al., 2003b).

FFAs play a major role in the flavor of Cheddar cheese. Large quantities of short-chain fatty acids, such as butyric acid, produce rancid off flavors (Deeth and Touch, 2000). The free caproic acid to linolenic acid ratio of Cheddar cheese is similar to that of milk fat. However, free butanoic acid occurs at a greater concentration in cheese than in milk fat (Collins et al., 2003a). In Cheddar cheese, LAB esterolytic or lipolytic enzymes hydrolyze esters of FFAs, mono-, di-, and triglycerides (Liu at al., 2001). The lipolytic activity of LAB, especially of *Lactobacillus* and *Lactococcus* spp., is weaker than that of species such as *Pseudomonas, Acinetobacter*, and *Flavobacterium*. However,

because of their high numbers at the extended ripening period, they are responsible for the liberation of significant levels of FFA (Collins et al., 2003a). The FFA act as precursors to produce flavor and aroma compounds in catabolic reactions leading to the formation of methyl ketones, lactones, esters, alkanes, and secondary alcohols (Alewijn et al., 2005; Collins et al., 2003a)

The ketones are produced from fatty acids by oxidative degradation. The production of methyl ketones involves oxidation of fatty acids to ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less, mainly from C6:0 to C12:0 fatty acids. These are responsible for the characteristic aroma of blue-veined cheeses. However, they do play a limited role in Cheddar cheese flavor. Ultimately, the ketones can be reduced to secondary alcohols, which do not contribute to cheese aroma (Gripon et al., 1991).

Another reaction in which polyunsaturated and, perhaps, monounsaturated, fatty acids can be involved, is oxidation. The extent of oxidation in cheese is, however, rather limited, possibly due to a low redox potential together with the presence of natural antioxidants, which could prevent the initiation of oxidation mechanisms or create conditions in which the primary oxidation products are reduced (Adda et al., 1982).

Proteolysis

Proteolysis is the most complex and, in most varieties, the most important primary event which occurs during cheese ripening (McSweeney and Sousa, 2000; Sousa et al., 2001; Upadhyay et al., 2004) and plays a vital role in the development of texture and flavor (Forde and Fitz-Gerald, 2000; Sousa et al., 2001; Smit et al., 2005).

Coagulating proteases, plasmin, and microbial proteases (from starter and non-starter bacteria) influence proteolysis during ripening. These proteolytic enzymes provide a mixture of small peptides and amino acids which directly change the taste of cheese (Law, 2001).

The principal indigenous milk proteinase, plasmin, appears to be mainly responsible for the relatively limited proteolysis of α-casein in Cheddar type cheese (Visser, 1993). *Lactococcus lactis* possess a very comprehensive proteolytic system that is believed to contribute little to primary proteolysis in cheese, but are principally responsible for the production of small peptides and free amino acids (O'Keeffe et al., 1978).

The final pH, moisture, salt in moisture (S/M), temperature, and duration of ripening, to a large extent, control the proteolysis in cheese. The point in the manufacturing process at which the whey is drained is the key stage in the manufacture of Cheddar because drainage of whey influences the cheese mineral content, the proportion of residual chymosin in the cheese, the final pH, and moisture-to-casein ratio (Lawrence et al., 1983). The level of chymosin incorporated in the cheese curd is dependent on the initial level of chymosin and the pH at whey drainage (Holmes et al., 1977; Lawrence et al., 1983; Creamer et al., 1985).

The primary proteolysis results from the actions of chymosin and plasmin (Forde and Fitz-Gerald, 2000). Hydrolysis of caseins leads to the formation of large- and intermediate-sized peptides, which in turn are degraded to smaller peptides by coagulants and enzymes derived from starter and non-starter bacteria. However, intracellular bacterial peptidases that are released after cellular lysis are responsible for degradation of small peptides to free amino acids. The amino acid catabolism is accomplished essentially by LAB and is responsible for aroma formation in Cheddar cheese (Sousa et al., 2001).

Catabolism of Amino Acids

Amino acids are precursors of volatile aroma compounds (Smit et al., 2005), which are degraded into flavoring compounds such as amines, aldehydes, alcohols, and ammonia (Fox et al., 1996; Law, 2001).

The compounds arising from the catabolism of free amino acids contribute directly to cheese taste and aroma. The total amount and composition of the amino acid mixture in cheese has long been used as an index of cheese ripening (Fox et al., 1995). A number of works in the past attempted to enhance free amino acid content in Cheddar cheese by direct addition of amino acids (Wallace and Fox, 1997) and genetic modification of *Lactococci* with increased aminopeptidase N activities (McGarry et al., 1994; Christensen et al., 1995). However, increased amino acid content in Cheddar does not affect the flavor development, which leads to the hypothesis that the rate-limiting factor in flavor biogenesis is not the release of amino acids, but their subsequent conversion to aroma compounds (Yvon et al., 1998).

Moreover, a transamination reaction that is the main pathway for degradation leads to formation of α-keto acids, which in turn are degraded to various aroma compounds (Yvon and Rijnen, 2001; Kieronczyk et al., 2003). LAB and NSLAB cooperate in aroma formation in Cheddar cheese: the conversion of amino acids to keto- and hydroxyl acids is initiated by *Lactobacilli*, while *Lactococcus* strains further convert these products to carboxylic acids. This cooperation between LAB and NSLAB leads to an enhanced cheese flavor.

In case of *Lactococci*, the first step in the degradation of amino acids is transamination (Gao et al., 1997), leading to formation of α-keto acids (α-KA). Aromatic aminotransferase enzymes have been previously characterized from *Lactococcus lactis* ssp. *cremoris* (Yvon et al., 1997; Rijnen et al., 1999a) and *Lactococcus lactis* ssp. *lactis* (Gao and Steele, 1998). These enzymes initiated the degradation of Val, Leu, Ile, Phe, Tyr, Trp, and Met, all of which are known precursors of cheese flavor compounds. Inactivation of aminotransferase enzymes involved in the breakdown of amino acids by *Lactococci* has been shown to reduce aroma formation during cheese ripening (Rijnen et al., 1999b).

Ney (1981) reported α-keto acids corresponding to almost every amino acid in Cheddar cheese. Phenyl pyruvic acid formed

from Phe by transamination is further degraded to the flavor compounds phenyl lactate and phenyl acetate by *Lactococcal* cells in vitro (Yvon et al., 1997). This degradation of phenyl pyruvic acid to phenyl lactate, phenyl acetate, and also to benzaldehyde in semi-hard cheese is confirmed by Yvon and colleagues (1998).

Gummalla and Broadbent (1999; 2001) studied the catabolism of Phe, Tyr, and Trp by *Lactobacillus helveticus* and *Lactobacillus casei*, which are widely used as starter or flavor adjuncts. Under near-Cheddar cheese-ripening conditions (pH 5.2, 4% NaCl, 15°C, and no sugar) pathways of Phe, Tyr, and Trp transamination and dehydrogenation are found active in both species and, interestingly, these reactions are found to be reversible. Major products of Phe catabolism are phenyl lactic acid, phenyl acetic acid, and benzoic acid, while Tyr degradation results in the formation of phydroxy phenyl lactic acid and *p*-hydroxy phenyl acetic acid (Gummalla and Broadbent, 2001).

Starter *Lactococci* are present in very high cell numbers in cheese during the early stages of ripening, and nonviable cells may also contribute to amino acid catabolism (Gao et al., 1997). These bacteria are likely to have a greater role in the initial conversion of Trp to indole-3-pyruvic acid in the cheese matrix. Gummalla and Broadbent (1999) suggested that nonstarter and adjunct lactobacilli may have an important role in secondary reactions involving indole-3-pyruvic acid and other starter-derived aromatic metabolites.

The volatile fraction of cheese has several sulfur-containing compounds such as methanethiol, methional, dimethyl sulfide, dimethyldisulfide, dimethyltrisulfide, dimethyltetrasulfide, carbonyl sulfide, and hydrogen sulfide (Lindsay and Rippe, 1986; Urbach, 1995; Weimer et al., 1999), and they contribute to the aroma of cheese (Milo and Reineccius, 1997). Methanethiol has been associated with desirable Cheddar-type sulfur notes in good-quality Cheddar cheese (Price and Manning, 1983). However, alone or in excess, methanethiol does not produce typical Cheddar cheese flavor (Weimer et al., 1999).

The breakdown of the protein network during proteolytic activity leads to textural changes in the cheese matrix. Carboxyl and amine groups that are liberated during proteolysis cause a decrease in water activity by binding water molecules (Sousa et al., 2001).

The biochemistry of cheese ripening is summarized in the figure:

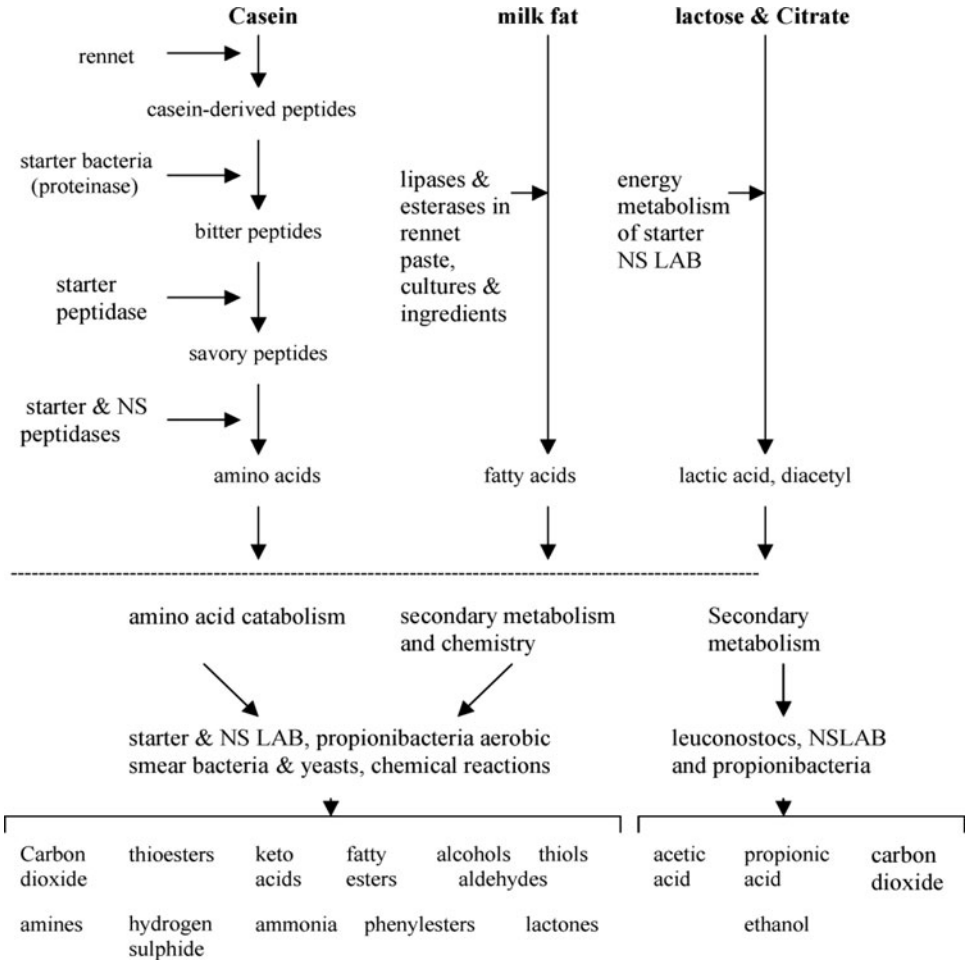


Fig. Biochemistry of cheese ripening (Law, 2001; Azarnia et al., 2006).

ACCELERATING CHEDDAR CHEESE RIPENING

Ripening is a slow and consequently an expensive process that is not fully predictable or controllable (Sihufe et al., 2010). Both traditional and modern methods used to accelerate the ripening process fall into the following main categories:

(1) Exogenous enzymes
(2) Modified starters
(3) Use of cheese slurries
(4) Adjunct cultures
(5) Attenuated starters
(6) Elevated temperatures

(Wilkinson, 1993; Azarnia et al., 2006).

Each method has associated advantages and disadvantages. Exogenous enzymes are relatively cheap, have specific action, and give a choice of flavor options, but the choice of useful enzymes is rather limited. There is a risk of over-ripening, difficulties with uniform incorporation, and possible legal barriers. Modified starters are easy to incorporate and the natural enzyme balance can be retained, but modification of starters, either by physical or genetic approaches, is technically complex. Enzyme-modified and high-moisture cheeses have been used successfully as food ingredients, but in general, do not develop flavor or texture characteristic of the corresponding natural cheese. Adjunct cultures and NSLAB may have potential to accelerate ripening, but to date, their use has been limited by the availability of suitable strains. Ripening at elevated temperature poses a risk in terms of the growth of unwanted microbial contaminants; however, it may be safely used when cheese is made with pasteurized milk under good manufacturing conditions (Law, 2001; Azarnia et al., 2006). Undoubtedly, elevated ripening temperatures offer the most effective, and certainly the simplest and cheapest, method for accelerating ripening (Sihufe et al., 2010).

CHARACTERIZATION OF CHEDDAR CHEESE FLAVOR

Cheese flavor is one of the most important criteria determining consumer choice and acceptance. It is generally accepted that the volatile profile reflects the image of the odor and aroma of cheeses. Therefore, the unique flavor of a cheese variety is the result of a complex balance among volatile and non-volatile chemical compounds (Delgado et al., 2010).

Despite of extensive research on flavor of Cheddar and other cheese varieties, only limited information is available on the chemistry of flavor and the flavor of none is characterized sufficiently to consent its reproduction by mixtures of pure compounds in a cheese model (Parliment and McGorrin, 2000; McGorrin, 2001).

The flavor of aged Cheddar cheese is attributed to a complex mixture of chemical compounds and is influenced by the cheese microflora (Ong and Shah, 2009). Flavor development in cheese is the result of a complex series of microbiologic, biochemical, and chemical processes that occur during ripening (Fox and Wallace, 1997). Flavor compounds are formed by various processes acting in a concerted and/or sequential manner, that is, conversion of lactose and citrate (glycolysis and pyruvate metabolism), fat (lipolysis), and caseins (proteolysis; ref. McSweeney and Sousa, 2000). However, proteolysis and lipolysis are major sources of cheese flavor and odor compounds. These enzymatic processes must occur in a coordinated way to give each cheese type its unique and appreciated sensory characteristics (Hernandez et al., 2009).

LAB, milk, and rennet provide the enzymes involved in the biochemical conversions that give rise to volatile and nonvolatile compounds which contribute to cheese flavor. However, the ripening of some cheeses can take a considerable length of time and a better understanding of the processes involved could enable reduction in the ripening time required to produce mature flavors (Hannon et al., 2007).

The flavor quality of Cheddar cheese in the market today differs considerably from that manufactured before the wide use of pasteurization, microbial rennets, and other modern manufacturing practices (Dunn and Lindsay, 1985). Much of the differences between traditional and contemporary Cheddar flavors probably should be attributed to current marketing of bland-flavored young cheeses. However, even longer-aged cheeses are frequently criticized for a lack of adequate Cheddar-type flavor. In addition, the development of stronger flavors in aged Cheddar, often, is accompanied by the occurrence of distinct off-flavors, especially bitterness.

Different approaches have been attempted to biochemically characterize Cheddar cheese flavor such as the determination of the factors or agents which influence or control the development of flavor and isolation and identification of components which contribute to the flavor.

Reiter and Sharpe (1971) conducted experiments using cheese model systems, where one or more of the ripening agents were eliminated. This involved the making of aseptic starter-free cheeses and aseptic cheeses. With the aseptic starter-free cheeses, the authors were able to eliminate the effects of both starter culture and nonstarter lactic bacteria. Cheese was made in aseptic vats using α-gluconic acid lactone as the acidulant. This cheese was completely devoid of Cheddar flavor, showing that indigenous milk enzymes, which survive pasteurization and coagulant rennet by themselves, do not produce Cheddar flavor. The aseptic cheese, which involved starter culture in place of acidulant α-gluconic acid lactone, developed mild but characteristic Cheddar flavor after six months of ripening and, at 12 months, the flavor was fairly strong. These results clearly showed the important role played by the starter culture in the flavor development.

In order to evaluate important odorants, aroma extract dilution assay (AEDA) was first applied to Cheddar cheese by

Christensen and Reineccius (1995). The components found to have the highest potency (dilution factor) in three-year-old Cheddar cheese were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, methional, propionic acid, butyric acid, valeric acid, caproic acid, capric acid, and lauric acid.

Dacremont and Vickers (1994) found that a recognizable Cheddar aroma was produced by a mixture of 2,3-butanedione, methional, and butyric acid. Methional is considered to have a boiled-potato-like aroma, while methanethiol, dimethyldisulphide, and dimethyltrisulphide are considered to add garlic notes to the flavor of matured cheddar cheese. 3-methyl-butanol is associated with a green malty odor which, at high concentration, impacts flavor. 3-methylbutanoic acid derived from leucine has a rancid, cheesy, and sweaty odor which may contribute to mature Cheddar aroma. Butyric acid derived from lipolysis has a cheesy and sweaty odor, which is considered as an important component of cheddar flavor (Urbach, 1995).

Milo and Reineccius (1997) applied both traditional high-vacuum isolation or AEDA and static headspace-olfactometry (GCOH) to study the aroma of a regular and a low-fat Cheddar cheese. After the quantification and calculation of respective odor activity values, based on sensory thresholds in oil and water, they suggested acetic acid, butyric acid, methional, 2,3-butanedione, and homofuraneol as the primary odorants responsible for the pleasant mild aroma of Cheddar cheese. In addition to the abovementioned compounds, the contribution of highly volatile sulfur compounds such as methanethiol and dimethyl sulfide to nasal perception of Cheddar cheese was quite obvious on the basis of GCO analysis of static headspace samples. The authors further hypothesized that the meaty–brothy odor characteristic of low-fat Cheddar was caused by high concentrations of methional, furaneol, and especially homofuraneol. The furaneol-type odorants are known to be produced by certain strains of lactobacilli (Milo and Reineccius, 1997). Although the mixture of these volatile organic compounds in a model cheese base had Cheddar aroma, attribute profiling described it as lacking in sour, moldy, and sulfurous notes relative to the real cheese. In addition, the overall odor was described as weak. This discrepancy in sensory character between the aromatized model and real cheese was partially caused by aroma–matrix interactions which resulted in quantitative errors (Wang and Reineccius, 1998).

A comparison of the volatile compositions of full- and reduced-fat Cheddar cheese showed that the level of methanethiol in the cheese is highly correlated with the flavor grade. This observation indicates that the lack of aroma in reduced fat Cheddar is likely to be mainly due to lack of methanethiol, but a combination of methanethiol and decanoic acid or butanoic acid in all cheeses gave a better correlation with Cheddar flavor than methanethiol alone (Dimos et al., 1996). Further, addition of methanethiol to bland slurry of reduced-fat Cheddar produced a strong Cheddar aroma (Urbach, 1997a).

The use of dynamic headspace dilution analysis (DHDA) methodology, previously described by Cadwallader and Baek (1998), has suggested additional volatiles as being important to Cheddar cheese aroma as compared with GCO-H and AEDA (Zehentbauer and Reineccius, 2002). Results of DHDA showed that, in addition to the odorants previously identified by AEDA and GCOH, (Z)-4-heptenal, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, (Z)-1,5-octadiene-3-one, and (E)/(Z)-2-nonenal, which have been underestimated or not even perceived during AEDA, may also contribute to the overall aroma of Cheddar cheese.

Analysis of cheese aroma compounds by traditional methods typically involves the use of concentration–extraction equipment such as vacuum distillation, liquid–liquid extraction, and, more recently, purge and trap techniques (Engels et al., 1997; Thierry et al., 1999). Vacuum distillation, while effective, involves delicate equipment, use of organic solvents, and can be prohibitively time consuming for general application. Microscale liquid–liquid extraction apparatus offers a less costly alternative, but suffers the drawback of requiring elevated temperatures, leading to the generation of chemical artifacts and loss of highly volatile components. A number of purge and trap approaches have been successfully applied to cheese aroma analysis; however, these require specific equipment. In addition, these techniques are not easily automated and precluding their wide-scale use. In contrast, solid-phase micro extraction (SPME) flavor analyses can be conducted at low cost with relatively simple equipment (Frank et al., 2004).

Volatile compounds are generally analyzed by gas chromatography (GC) coupled with mass spectrometry (MS), with an earlier step involving the extraction and pre-concentration of the volatile fraction. The technique of SPME requires only a small amount of sample and permits the isolation of volatile from matrices both in the solid and liquid states in a short time, in a simple way. For this reason, nowadays, SPME is commonly used for the extraction of flavor compounds in cheese (Lecanu et al., 2002; Lee et al., 2003; Guillen et al., 2004; Coda et al., 2006).

SPME can effectively extract and concentrate aroma compounds and also provides high sensitivity with minimum artifact formation. With the use of SPME fibers, there is no requirement for organic solvents, and sample preparation can be completed in minimal time (Burbank and Qian, 2005). In addition, SPME equipment can be automated. Although there are a growing number of available fiber coatings, the carboxen-polydimethylsiloxane (CAR-PDMS) fiber has repeatedly shown its exceptional ability to extract sulfur compounds including methanethiol and dimethyl sulfide from food (Roberts et al., 2000; Hill and Smith, 2002; Lecanu et al., 2002; Pinho et al., 2003; Pinho et al., 2004; Frank et al., 2004). The process of concentration with CAR-PDMS fiber involves adsorption of small molecules into micro-pores by the Carboxen phase in addition to absorption by the PDMS coating (Frank et al., 2004), lending to its greater capacity for extracting highly volatile, low-molecular-weight molecules, which includes most volatile sulfur compounds.

CONCLUSION

During Cheddar cheese ripening, microbiologic and biochemical changes occur that result in the development of flavor and texture characteristics of the variety. These changes are grouped into primary (glycolysis, proteolysis, and lipolysis) or secondary (catabolism of fatty acids and of amino acids) events. Secondary reactions lead to the production of volatile flavor compounds from fatty acids and amino acids. Ripening is, relatively, an expensive process for the cheese industry and reducing maturation time without destroying the quality of the ripened cheese has economic and technologic benefits. Because the ripening temperature influences the rate of biochemical processes, elevated temperature is the simplest method for accelerating the ripening of Cheddar cheese.

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