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**ABSTRACT:** Considerable knowledge has been accumulated on the biochemical processes occurring during ripening of Cheddar cheese, which in turn has major consequences on flavor and texture development. The present review outlines major metabolic pathways and agents involved in the modification of milk constituents in Cheddar cheese ripening. Mechanisms of volatile flavor and off-flavor production and recent developments in the analysis, both sensory and instrumental, of Cheddar flavor and flavor compounds are also detailed here.

## Introduction

Cheesemaking originated as a crude form of food preservation. The preservation of cheese is a result of the combined action of:

- Dehydration. The stability of foods is inversely related to moisture content. Cheese is a medium-moisture food, containing about 30 to 50% moisture. The water activity ( $a_w$ ) of cheese varies from 0.98 to 0.87, and these values are highly correlated with the total nitrogen and ash content (mainly NaCl). Biochemical reactions that occur during the ripening of cheese contribute to the depression of  $a_w$  by increasing the number of dissolved low-molecular weight compounds and ions
- Acid. During the manufacture and ripening of cheese, starter bacteria ferment lactose to lactic acid. The pH of Cheddar cheese is about 5.0 to 5.2
- Production of antimicrobial factors
- Anaerobic condition
- Addition of NaCl

Cheese is the generic name for a group of fermented milk-based food products. More than 500 varieties of cheeses are listed by the International Dairy Federation (IDF 1982), and numerous minor and/or local varieties also exist (Fox 1987). The flavor profiles of cheeses are complex and variety- and type-specific. This was realized back in the 1950s, when Mulder (1952) and Kosikowski and Mocquot (1958) proposed the "component balance" theory. According to this theory, cheese flavor is the result of the correct balance and concentration of a wide variety of volatile flavor compounds.

The volatile flavor compounds in cheese originate from degradation of the major milk constituents; namely lactose, citrate, milk lipids, and milk proteins (collectively called caseins) during ripening which, depending on the variety, can be a few weeks to more than 2 years long. The physicochemical parameters—pH, water

activity, and salt concentration—necessary to direct biochemical reactions in the right direction are set during manufacturing of cheese curd, which on its own is bland. In case of deviation of any of these 3 parameters, cheeses could potentially develop texture and/or flavor inconsistencies.

Extensive knowledge of the primary degradation pathways of milk constituents in cheese curd, glycolysis (lactose and citrate), lipolysis (milk lipids), and proteolysis (caseins), has been accumulated. Primary degradation of milk constituents leads to the formation of a whole range of precursors of flavor compounds. Only some of the compounds formed by glycolysis, lipolysis, and proteolysis directly contribute to cheese flavor; for example, short-chain fatty acids, acetaldehyde diacetyl, peptide, and amino acids. Primary degradation of major caseins; for example,  $\alpha_{s1}$ -caseins, has major consequences for cheese texture. These changes are followed and/or overlapped by a concerted series of secondary catabolic reactions which are responsible for the unique aroma profile of a particular variety or type of cheese.

A number of groups in the past have worked on identification of volatile flavor compounds from Cheddar cheese. The list of volatile flavor compounds identified in Cheddar is quite extensive and includes a wide variety of compounds; namely acids, alcohols, esters, aldehydes, ketones, sulfur-containing compounds, phenolics, and so on. Only limited information is available on the characterization of the flavor of most cheese varieties, and none is characterized sufficiently to permit duplication of its complete flavor by mixtures of pure compounds. The body of knowledge in the area of cheese and Cheddar cheese flavor is rapidly expanding. Previous reviews have addressed cheese ripening and volatiles found in cheese, but have not addressed generation and identification of volatiles, the sensory impact of specific volatiles, and methods to measure and identify Cheddar cheese flavor from an

instrumental and sensory perspective. The present review outlines the biochemical changes involved during manufacture and ripening of cheese in general and Cheddar cheese in particular. Literature on Cheddar cheese flavor and off-flavor, including recent developments in chemical/instrumental and sensory methodologies, are also reviewed.

### Manufacture of Cheddar cheese

The manufacture of rennet-coagulated cheeses, such as Cheddar, can be divided into two more or less distinct phases: (1) conversion of milk to curd, which is essentially complete within 24 h; and (2) ripening of the curd (Figure 1). Cheese manufacture is essentially a dehydration process in which the fat and casein in milk are concentrated between 6- and 12-fold, depending on the variety.

Cheddar curd manufacture commences with the selection and pretreatment of milk of high microbiological and chemical quality. Most Cheddar cheese milk is now pasteurized just before use, but raw milk is still used in both commercial and farmstead cheesemaking. In general, cheese made from raw milk develops the characteristic Cheddar flavor more rapidly, reaching its best flavor at 3 to 6 months (Price and Call 1969). 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 others (1993a) 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 atypical—its flavor was much more intense and developed much faster than that of the other cheeses. Peptide profiles, by urea-polyacrylamide gel electrophoresis (PAGE), of the 3 cheeses were indistinguishable throughout ripening, but the rate of formation of soluble nitrogen was faster in the raw milk cheese. The number of lactobacilli was about 10-fold higher in the raw milk cheese than in the other 2, and the species of lactobacilli also differed. It was concluded from the above results that lactobacilli were responsible for differences in proteolysis in the cheese made from raw milk, particularly in regard to the formation of short peptides and free amino acids. The species of lactobacilli involved in faster ripening and development of flavor intensity in raw milk cheeses appear to have been killed by pasteurization. In a further study, aseptic cheeses with selected species of lactobacilli added as adjunct starters were evaluated by Lynch and others (1994). Adjunct lactobacilli-added cheeses showed slightly higher levels of free amino acids, but no significant differences in flavor intensity and texture were found between control and experimental cheeses. From the above studies, it appears that pasteurization of milk prior to cheese manufacture

influences both the extent and characteristics of proteolysis during Cheddar cheese ripening. 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 and others 1991).

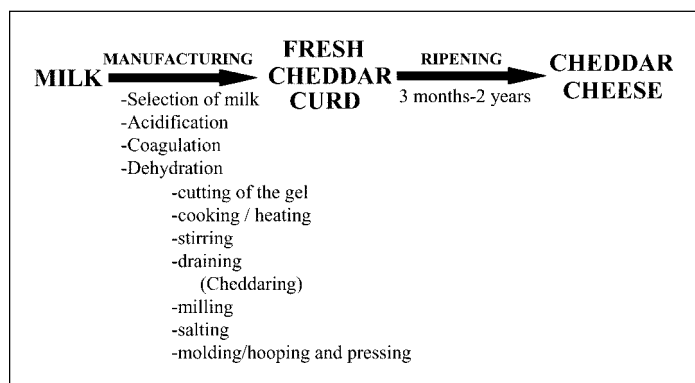
Acidification during cheese manufacture is one of the primary events in the manufacture of most, if not all, cheese varieties and involves the fermentation of lactose to lactic acid by selected lactic acid bacteria (*Lactococcus lactis* ssp *cremoris* and ssp *lactis* for Cheddar) or, in traditional cheesemaking, by the indigenous microflora. 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/hooping, while in most other varieties, it occurs mainly after molding. Acid production affects almost all facets of cheese manufacture and, hence, 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.

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, below about pH 5.5, a progressive dissociation of the sub-micelles into smaller aggregates occurs (Lawrence and others 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 2-step process. The 1st step involves the enzymatic hydrolysis of  $\kappa$ -casein, and the 2nd involves the coagulation of casein by  $\text{Ca}^{2+}$  at temperature  $> 20^\circ\text{C}$ . Chymosin in rennet specifically cleaves  $\kappa$ -casein at Phe<sup>105</sup>-Met<sup>106</sup>, which leads to the release of the hydrophilic caseinomacropptide [ $\kappa$ -CN (f106-169)] part of  $\kappa$ -casein, located at the surface of the casein micelles. When intact, the micelles are kept colloiddally dispersed in milk by steric and electrostatic repulsion involving the negatively charged caseinomacropptide part of  $\kappa$ -casein (Dalgleish 1993). The casein micelles become unstable following the removal of these hydrophilic peptides; then, at an appropriate temperature (for example,  $30^\circ\text{C}$ ), the milk coagulates under the influence of  $\text{Ca}^{2+}$  in the medium (Dalgleish 1993).

A rennet milk gel is quite stable if maintained under quiescent conditions, but if it is cut or broken, syneresis occurs rapidly, expelling whey (Fox 1993). During practical cheesemaking, cutting the curd into small pieces gives faster (initial) syneresis which is proportional to the area of the surface exhibiting syneresis (Walstra and others 1987). The rate and extent of syneresis are influenced by milk composition, especially  $\text{Ca}^{2+}$  level, casein concentration, pH of the whey, cooking temperature, rate of stirring of the curd-whey mixture, and time. In Cheddar manufacture, after cooking and whey drainage, the curd is allowed to rest for a considerable time to develop sufficient acidity (often while allowing the curd to flow: cheddaring), 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. This leads to higher moisture content (1 to 2% more water) compared to curd kept for the same time but which is prevented from spreading. The main cause of



**Figure 1—General description of Cheddar cheese manufacture**

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, since this is readily under the control of the cheesemaker, it is here that the differentiation of individual cheese varieties really begins (Fox 1993).

The last manufacturing operation is salting. Salting is performed in Cheddar by mixing dry salt with broken or milled curd at the end of manufacture. Salt exercises 1 or more of the following functions:

- Directly modifies flavor: unsalted cheese is insipid, which is overcome by 0.8% salt
- Promotes curd syneresis, and thus regulates the moisture content of cheese
- Reduces  $a_w$
- Influences the activity of rennet, starter, and nonstarter lactic acid bacteria (NSLAB) and of their enzymes, and indigenous milk enzymes
- Suppresses the growth of undesirable nonstarter microorganisms
- By its influence on post-cheddaring starter activity, salt in Cheddar-type cheeses controls the metabolism of lactose and thus the pH of the fresh cheese, which in turn affects the rate of maturation and cheese quality (Fox 1987).

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 hence the flavor, aroma, and texture of mature cheese, are largely predetermined by the manufacturing process; that is, by composition, especially moisture, salt, and pH; by the type of starter; and in many cases by secondary inocula added to, or gaining access to, the cheese milk or curd (Fox 1993). Cheeses are ripened under controlled temperature conditions (for Cheddar 8 °C for 6 to 9 months or even longer), and possibly under controlled humidity. The ripening time is generally inversely related to the moisture content of the cheese.

#### Biochemical reactions during manufacture and ripening of Cheddar cheese

Cheese ripening is a slow process, involving a concerted series of microbiological, biochemical, and chemical reactions. 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 manufacturing process: (a) composition, especially moisture, pH, and salt, and (b) microflora, starter, and especially nonstarter microflora and adjunct starter (that is, microorganisms added to cheese milk for purposes other than acidification).

Considerable knowledge on the principal changes and pathways involved in Cheddar cheese ripening has been accumulated over the last several decades, but it is still not possible to predict or guarantee premium quality. Based on the analysis of young (14 d) experimental and commercial Cheddar cheeses, the standards prescribed in New Zealand for premium grade are: pH: 4.95 to 5.1; salt-in-moisture (S/M): 4 to 6%; moisture in solid-not-fat (MSNF): 52 to 56%; fat in dry matter (FDM): 52 to 55%. The corresponding values for 1st grade cheeses are: pH 4.85 to 5.20; 2.5 to 6%; 50 to 57% and 50 to 56%; young cheeses with a composition outside these ranges are considered unlikely to yield good quality matured cheese (Gilles and Lawrence 1973).

The ripening of cheese involves 3 primary biochemical pro-

**Table 1—Flavor compounds generated from the 3 principal milk constituents during ripening of cheese (adapted from Fox and others 1995a)**

Casein	Milk fat	Lactose & Citrate
Peptides	Fatty acids	Lactate
Amino Acids	Keto acids	Pyruvate
Acetic acid	Methyl ketones	CO <sub>2</sub>
Ammonia	Lactones	Diacetyl
Pyruvate		Acetoin
Aldehydes		2,3-butandiol
Alcohols		Acetaldehyde
Carboxylic acid		Acetic acid
Sulfur compounds		Ethanol

cesses—glycolysis, lipolysis, and proteolysis—the relative importance of which depends on the variety (Fox and others 1994). These primary changes are followed and overlapped by a host of secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids,  $\beta$ -oxidation of fatty acids, and even some synthetic changes; that is, esterification (Fox 1993). The above-mentioned primary reactions are mainly responsible for the basic textural changes that occur in cheese curd during ripening, and are also largely responsible for the basic flavor of cheese. However, the secondary transformations are mainly responsible for the finer aspects of cheese flavor and modify cheese texture.

The compounds listed in Table 1 are present in most, if not all, cheese varieties. The concentration and proportions of these compounds are characteristic of the variety and are responsible for individuality. These complex biochemical changes occur through the catalytic action of the following agents:

- Coagulant
- Indigenous milk enzymes, especially proteinase, lipase, and phosphatases
- Starter bacteria and their enzymes
- Secondary microflora and their enzymes

The biochemistry of the primary events in cheese ripening is now fairly well characterized, but the secondary events are understood only in general terms. In the next few sections, glycolysis, lipolysis, proteolysis, and other related reactions are discussed with corresponding relevance to Cheddar cheese flavor and texture.

#### Contribution of glycolysis and related reactions to Cheddar cheese flavor

During Cheddar cheese manufacture, mesophilic starter bacteria ferment lactose to (mainly L+) lactic acid (Figure 2). In the case of Cheddar-type cheeses, most of the lactic acid is produced in the vat before salting and molding. During manufacture or shortly thereafter, curd pH reaches ~5.0, but the rate is characteristic of variety (6 to 24 h). Even after losing ~98% of the total milk lactose in the whey as lactose or lactate, the cheese curd still contains 0.8 to 1.5% lactose at the end of manufacture (Huffman and Kristoffer- sen 1984). The pH decreases after salting, presumably due to the action of starter, at S/M levels < 5.0%, but at high values of S/M, starter activity decreases abruptly (Fox and others 1990) and the pH remains high. The quality grade assigned to the cheese also decreases sharply at S/M levels > 5.0% (Lawrence and Gilles 1982).

Commercial lactic cultures are stimulated by low levels of NaCl, but are very strongly inhibited at > 2.5% NaCl. Thus, the activity of the starter and its ability to ferment residual lactose is strongly dependent on the S/M level in the curd. *Lc. lactis* ssp. *cremoris* is more salt-sensitive than *Lc. lactis* ssp. *lactis* which, in turn, is more sensitive than nonstarter lactic acid bacteria (Turner and Thomas

1980). Therefore, the % S/M also determines the products of post-manufacture lactose fermentation.

If starter activity is inhibited after manufacture, residual lactose will be metabolized by nonstarter lactic acid bacteria (NSLAB), mainly pediococci and mesophilic lactobacilli, which are more salt-tolerant than starter bacteria and metabolize lactose to DL-lactate and racemize L-lactate (see Figure 3). 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  $g^{-1}$  (Fox and others 1990).

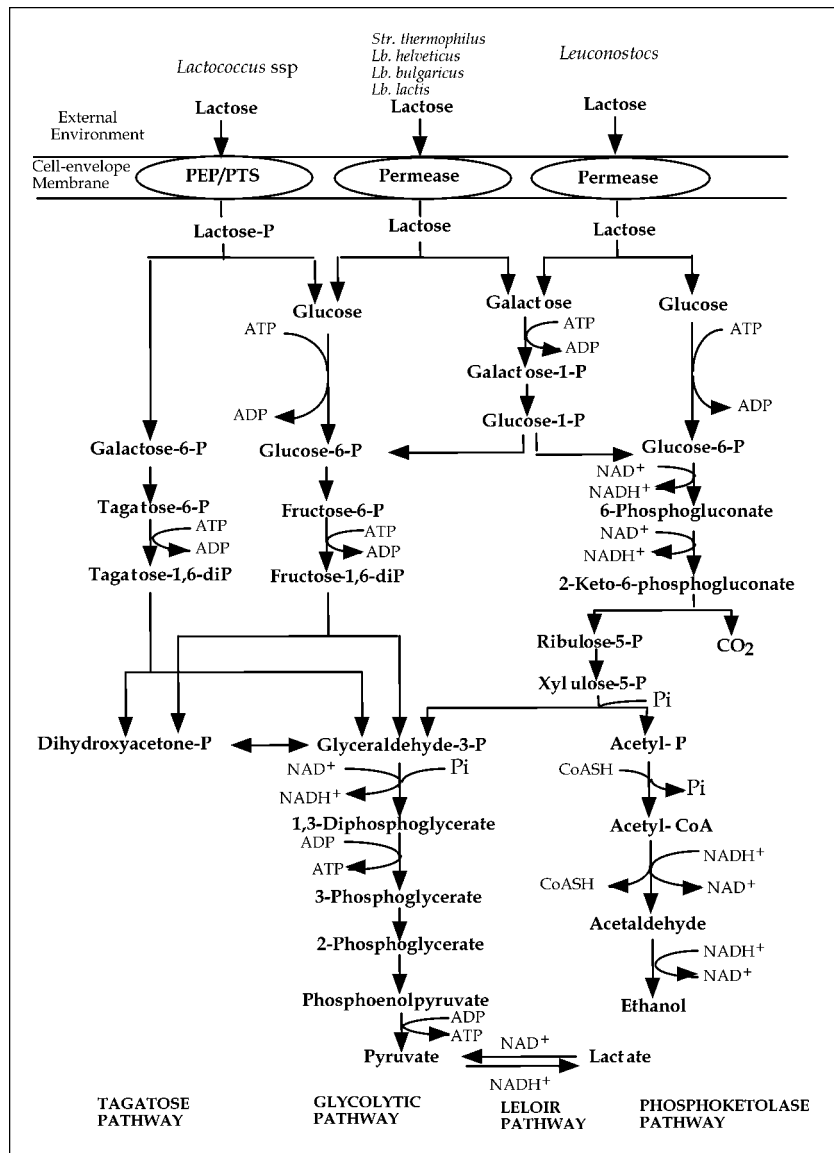
The pH at whey drainage largely determines the mineral content of a cheese. The loss of  $Ca^{2+}$  and phosphate from casein micelles determines the extent to which they are disrupted, and this largely determines the basic structure and texture of a cheese (Lawrence and others 1983). In general, curds with a low pH have a crumbly texture, that is, Cheshire, while high pH curds tend to be more elastic, that is, Emmental.

**Metabolism of lactose and lactic acid**

Experimental and commercial Cheddar cheeses contain con-

siderable amounts of D-lactate, which could be formed by fermentation of residual lactose by lactobacilli or by racemization of L-lactate (Fox and others 1990). Racemization of L-lactate by both pediococci and lactobacilli is pH-dependent (optimum pH 4 to 5), and is retarded by NaCl concentrations > 2% or > 6% 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, especially on cut surfaces (Dybing and others 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 off-flavor (Aston and Dulley 1982).

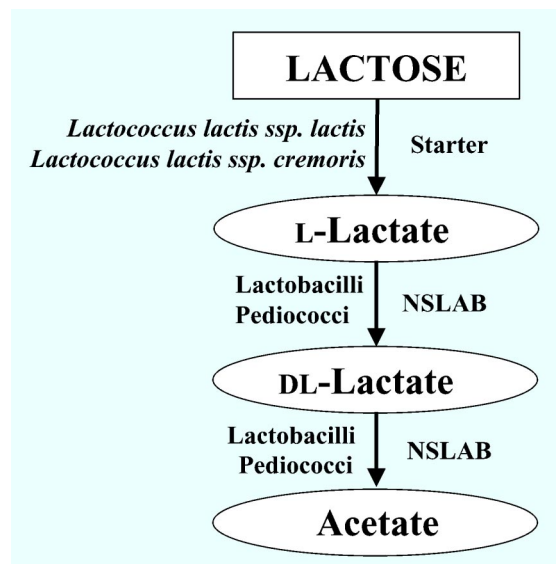


**Figure 2—Probable pathways for the metabolism of lactose by mesophilic and thermophilic lactic acid bacteria (adapted from Fox and others, 1990).**

**Citrate metabolism in Cheddar cheese**

Bovine milk contains relatively low levels of citrate (~8 mM). 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 ~3 times that in whey (Fryer and others 1970), presumably reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2 to 0.5% (w/w) citrate which is not metabolized by *Lc. lactis* ssp. *lactis* or ssp. *cremoris*, but is metabolized by *Lc. lactis* biovar *diacetylactis* and *Leuconostoc* spp, with the production of diacetyl and  $CO_2$  (Figure 4). Due to  $CO_2$  production, citrate metabolism is responsible for the characteristic eyes in Dutch-type cheeses. Diacetyl and acetate produced from citrate contribute to the flavor of Dutch-type and Cheddar cheeses (Aston and Dulley 1982; Manning 1979a, 1979b).

Several species of mesophilic lactobacilli metabo-



**Figure 3—Pathway for the metabolism of lactose in Cheddar cheese**

lize citrate with the production of diacetyl and formate (Fryer 1970); the presence of lactose influences the amount of formate formed. Thomas (1987) showed that the concentration of citrate in Cheddar cheese decreases slowly to almost zero at 6 mo, presumably as a result of metabolism by lactobacilli, which become the major component of the nonstarter microflora. Inoculation of cheese milk with *Lb. plantarum* accelerated the depletion of citrate (Thomas 1987).

The principal flavor compounds produced from metabolism of citrate are acetate, diacetyl, acetoin, and 2,3-butandiol (Cogan 1995). Diacetyl is usually produced in small amounts, but acetoin is generally produced in much higher concentration (10 to 50 fold higher than diacetyl concentration). Acetate is produced from citrate in equimolar concentrations.

#### Contribution of lipolysis and related reactions to Cheddar flavor

Cheese is a high-fat food; fresh Cheddar cheeses contain 30.5% or more fat (wet weight) (Renner 1993). The fat fraction of cheese is important for the development of typical flavor and texture. It is well known that a higher fat content leads to a less firm and more elastic body, while low-fat products tend to be harder, more crumbly, and less smooth than characteristic (Emmons and others 1980). In low-fat products, there is increased crosslinking within the curd, which is carried through into the cheese. Increasing the moisture content in an attempt to overcome these defects leads to weak body and encourages an undesirable flora and atypical flavor. Cheddar cheese made from nonfat milk does not develop full aroma, even after 12 mo (Ohern and Tuckey 1969). Substituting vegetable or even mineral oil for milkfat seems to favor a certain aroma development in Cheddar (Foda and others 1974). This indicates that one important function of fat is to dissolve and hold the flavor components. Foda and others (1974) also suggested that the fatty acid composition and natural emulsion of milkfat are important for flavor development. In recent years there has been an increased interest in low-fat cheeses. Cheeses with reasonably good flavor and texture were successfully made by substituting fat with whey proteins (de Boer and Nooy 1980; McGregor and White 1990a, 1990b).

Like all types of food with a high fat content, lipolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) changes are likely to occur in cheese. 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 free fatty acids (FFA), di- and monoglycerides and possibly glycerol. FFA contribute to the aroma of cheese. Individual FFA, particularly acids between  $C_{4:0}$  and  $C_{12:0}$ , have specific flavors (rancid, sharp, goaty, soapy, coconut-like). The flavor intensity of FFA depends not only on the concentration, but also

on the distribution between aqueous and fat phases, the pH of the medium, the presence of certain cations (that is,  $Na^+$ ,  $Ca^{2+}$ ) and protein degradation products (Adda and others 1982). The pH of cheese has major influence on the flavor impact of FFA. At the pH of Cheddar (pH ~5.2), a considerable portion of FFA are present as salts, which are nonvolatile, thus reducing their flavor impact. 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 free fatty acids to be rancid. However, extensive lipolysis is desirable as part of overall flavor development in certain cheeses, such as hard Italian cheeses (Romano, Provolone), Blue, and Feta.

Lipases and esterases in Cheddar cheese originate from milk, starter, and nonstarter bacteria. A number of psychrotrophic organisms, which can dominate the microflora of refrigerated milk, produce heat-stable lipases. These lipases adsorb onto the fat globules, are incorporated into the cheese curd, and may cause rancidity in cheese over a long ripening time (Fox 1989). Milk contains a well-characterized indigenous lipoprotein lipase (LPL) (Olivecrona and others 1992), as well as a number of esterases (Deeth and FitzGerald 1983). Milk lipase is reported to be more active than starter lipases in Cheddar (Reiter and Sharpe 1971). Bovine LPL is rather nonspecific and readily liberates fatty acids from the sn-1 and sn-3 positions of mono-, di- and triglycerides and the sn-1 position of glycerophospholipids. However, lipolysis in milk preferentially releases short and medium-chain fatty acids, because in milk triglycerides, short-chain fatty acids are esterified predominantly at the sn-3 position. This specificity probably explains the disproportionate concentration of free butyric acid in cheese. Milk lipase appears to hydrolyze the fat selectively and is able to act on triglycerides, while lactococcal lipases seem to be active mainly on mono- and diglycerides (Stadhouders and Veringa 1973). Cheddar cheeses of different flavor intensity showed only small differences between the concentrations of individual FFA (Bills and Day 1964). The relative proportions of FFAs,  $C_{6:0}$  to  $C_{18:3}$ , were similar to those in milkfat, indicating that these FFAs were released nonspecifically. However, free butyric acid was found at higher concentrations than could be explained by its proportion in milkfat, suggesting that it was selectively hydrolyzed or synthesized by the cheese microflora. The lipolytic activity of lactic acid bacteria produce low levels of FFA that can contribute to the background flavor of Cheddar cheese (Olson 1990). Model cheeses, manufactured using gluconic acid  $\delta$ -lactone instead of starter, contained low levels of FFA which did not increase during ripening (Reiter and others 1967). 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 (Kamaly and Marth 1989; Singh and others 1973) and lactobacilli (El-Soda and others 1986) lipases.

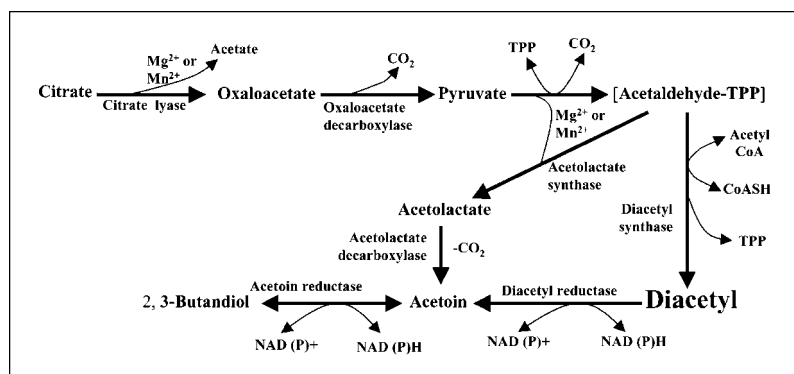


Figure 4—Metabolism of citrate by *Lactococcus* & *Leuconostoc* (Fox and others 1995).

#### Metabolism of fatty acids

The FFA are involved in several types of reactions which vary in importance with the type of cheese involved (Figure 5). Methyl ketones are produced from fatty acids by oxidative degradation. The production of methyl ketones involves oxidation of fatty acids to  $\beta$ -ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less, mainly from  $C_{6:0}$  to  $C_{12:0}$  fatty acids (Hawke 1966). Methyl ketones are responsible for the characteristic aroma of blue-veined cheeses (Gripone and others 1991). However, they do play a limited role in Cheddar cheese flavor. Ultimately, methyl ketones can be reduced to secondary alcohols, which do not contribute to cheese aroma.

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 and others 1982).

Aliphatic and aromatic esters play an important part in the flavor and, sometimes, the off-flavor of Cheddar cheese. This synthesis mainly concerns the above-mentioned short- or medium-chain fatty acids, and the alcohols involved may be aliphatic (ethanol), aromatic (phenylethanol), or thiols (methanethiol). Esters can be produced enzymatically by lactic acid bacteria (Hosono and others 1974; Harper and others 1980), but can also easily result from a purely chemical reaction. Amides have been identified in cheese (Wirotna and Ney 1973); that is, Cheddar, Emmental, Manchego, but no mechanism has been proposed for their formation.

$\gamma$ - and  $\delta$ -Lactones have been identified in cheeses, particularly in Cheddar, where they have been considered as important for flavor (Wong and others 1973). Lactones are cyclic esters resulting from the intramolecular esterification of hydroxy acids through the loss of water to form a ring structure. Lactones possess a strong aroma which, although not specifically cheese-like, may be important in the overall cheese flavor impact. The accepted mechanism of formation of lactones in cheese presumes the release of hydroxy fatty acids, which are normal constituents of milk fat, followed by lactonization.

**Contribution of proteolysis and related reactions to Cheddar flavor**

During the manufacture and ripening of Cheddar cheese, a gradual decomposition of caseins occurs due to the combined action of various proteolytic enzymes. These generally include enzymes from the coagulant, milk, starter and nonstarter lactic acid bacteria, and secondary starter.

- Coagulant
  - (a) Chymosin (genetically engineered)
  - (b) Chymosin/pepsin (from calf stomach)
- Indigenous milk enzymes
  - (c) Plasmin
  - (d) Cathepsin
- Starter and nonstarter bacterial enzymes

- (e) Cell envelope-associated proteinases (CEP)
- (f) Peptidases
  - i. Endopeptidases
  - ii. Aminopeptidases
  - iii. Di- and tripeptidases
  - iv. Proline specific peptidases

Enzymes from the first 4 sources are active in most ripened cheeses. The secondary starter (that is, microorganisms added to cheese milk or curd for purposes other than acidification) exerts considerable influence on the maturation of cheese varieties in which they are used. Exogenous enzymes used to accelerate ripening could be added to the above list and, when present, can be very influential.

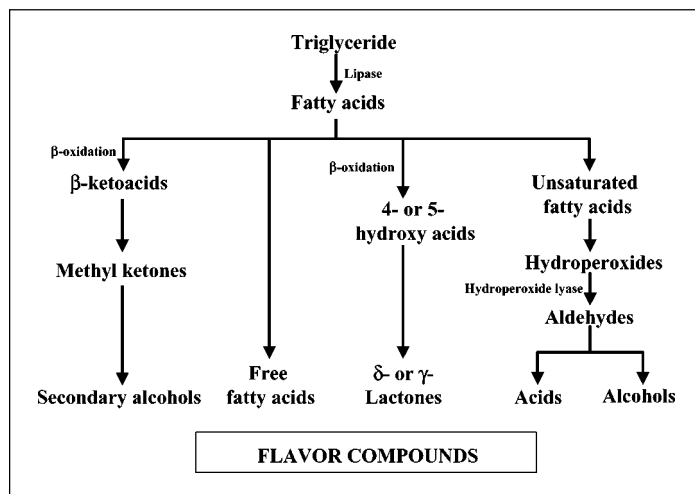
The correct pattern of proteolysis is generally considered to be a prerequisite for the development of the correct flavor of Cheddar cheese. Products of proteolysis per se (that is, peptides and free amino acids) probably are significant in cheese taste, at least to "background" flavor and some off-flavors, for example, bitterness, but are unlikely to contribute much to aroma. 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 and others 1995b). In at least some instances, these parameters correlate with flavor and body development, but they provide little information about the mechanism of cheese ripening. The ultimate description of proteolysis requires identification of the peptide bonds cleaved, which requires isolation of proteolytic products and determination of their structures.

The contribution of the above enzymes, individually or in various combinations, has been assessed using 3 complimentary approaches:

- model cheese systems from which the nonstarter microflora have been eliminated by aseptic techniques, in which acidification is accomplished by an acidogen (usually gluconic acid- $\delta$ -lactone) rather than starter, and in which coagulant and indigenous milk enzymes may be inactivated or inhibited
- activity and specificity of the principal proteinases and peptidases on caseins or casein-derived peptides in solution
- isolation of peptides from cheese and, based on the known specificity of the proteinases/peptidases on the caseins in solution, identification of the agent(s) responsible for their formation in cheese (Fox and others 1994)

In Cheddar cheese, the coagulant is responsible for the initial hydrolysis of caseins (Fox and others 1994, 1995a). Coagulant activity is restricted largely to  $\alpha_{s1}$ -casein, with limited hydrolysis of  $\beta$ -casein and probably not of  $\alpha_{s2}$ -casein. Indigenous milk and starter proteinases are less important at the initial stages of proteolysis, but the production of small peptides and amino acids is due primarily to the activity of starter bacteria enzymes. The principal indigenous milk proteinase, plasmin, appears to be mainly responsible for the relatively limited proteolysis of  $\beta$ -casein in Cheddar and Dutch-type cheese, but is more significant in high-cooked cheeses (for example, Swiss types), in which chymosin is extensively or completely inactivated (Visser 1993).

The starter lactic acid bacteria (*Lactococcus lactis* ssp) possess a very comprehensive proteolytic system. Previously, it was believed that these enzymes contribute little to primary proteolysis in cheese but are principally responsible for the production of small peptides and free amino acids (O'Keefe and others 1978). Aseptic starter-free cheeses, containing normal amounts of rennet, show the development of high levels of soluble N (peptides and free amino soluble in various aqueous extracts), indicating the importance of rennet for soluble N production. However, the production of soluble N in these cheeses is less than in normal aseptic cheeses, indicating that the starter bacteria also contribute to the production of soluble N. Significant amounts of soluble N



**Figure 5—General pathways for the metabolism of milk triglycerides and fatty acids.**



were produced in aseptic rennet-free cheeses, suggesting that starter bacteria are capable of attacking paracasein in cheese and converting it to soluble products, independently of rennet action (Visser 1977a,b,c).

NSLAB, predominantly mesophilic lactobacilli, usually dominate the microflora of Cheddar-type cheese during much of its ripening. NSLAB possess a wide range of proteolytic enzymes (Atlan and others 1993) and may contribute toward the formation of short peptides and free amino acids in Cheddar.

The final pH, 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 since 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 and others 1983). The level of chymosin incorporated in the cheese curd is dependent on the initial level of chymosin and the pH at whey drainage (more rennet is retained in the curd at lower pH) (Holmes and others 1977; Lawrence and others 1983, Creamer and others 1985). It has been suggested that the casein in low-pH cheese is hydrolyzed more rapidly than in normal pH cheese because depletion of colloidal calcium phosphate from the curd causes micelle dissociation and renders the caseins more susceptible to proteolysis (O’Keeffe and others 1975). Being an acid proteinase, chymosin is optimally active at low pH and this is considered to be mainly responsible for the increased proteolysis in low-pH cheese (Creamer and others 1985). The S/M is a primary factor controlling the enzymatic activities of rennet, plasmin, and bacterial proteinases. Proteolysis, and thus the incidence of bitterness, decreases with an increase in salt concentration (Thomas and Pearce 1981; Kelly 1993). At S/M levels > 5.0, bitter flavors are rarely encountered (Lawrence and others 1983), while below this level there is more or less a linear relationship between S/M and the intensity of bitterness.

**Proteolysis of milk proteins in Cheddar cheese**

For the development of an acceptable Cheddar cheese flavor, a well-balanced breakdown of the curd protein (that is, casein) into small peptides and amino acids is necessary (Thomas and Pritchard 1987; Visser 1993). These products of proteolysis themselves are known to contribute to flavor (McGugan and others 1979; Aston and others 1983; Aston and Creamer 1986; Cliffe and others 1993; Engels and Visser 1994) or act as precursors of flavor components during the actual formation of cheese flavor.

The residual chymosin rapidly hydrolyses  $\alpha_{s1}$ -casein at the bond Phe<sup>23</sup>-Phe<sup>24</sup>, and possibly Phe<sup>24</sup>-Val<sup>25</sup> also, during the initial stages of ripening (Creamer and Richardson 1974; Hill and

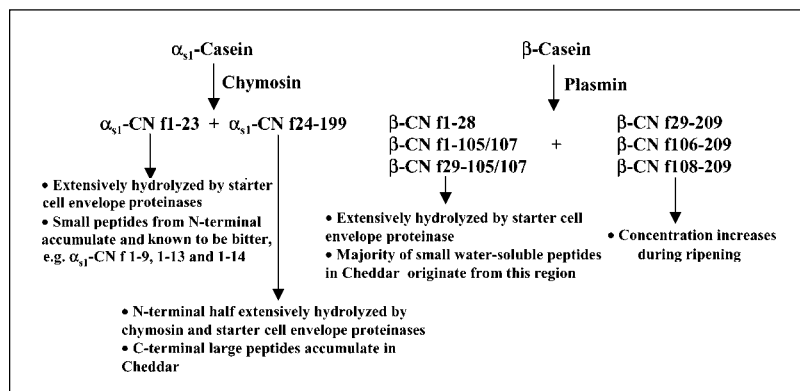
others 1974) (Figure 6). The hydrolysis of bond Phe<sup>23</sup>-Phe<sup>24</sup> results in the formation of a large  $\alpha_{s1}$ -CN f24-199 [called  $\alpha_{s1}$ -I casein], and small  $\alpha_{s1}$ -CN f1-23 peptides. Hydrolysis of this single bond of  $\alpha_{s1}$ -casein causes a rapid change in the rubbery texture of young Cheddar curd into a smoother, more homogeneous product (Lawrence and others 1987). Increasing S/M does not influence the initial hydrolysis of  $\alpha_{s1}$ -casein, but inhibits the subsequent hydrolysis of  $\alpha_{s1}$ -CN f24-199 (Exterkate and Alting 1995).

The peptide  $\alpha_{s1}$ -CN f1-23, produced by chymosin action on the bond Phe<sup>23</sup>-Phe<sup>24</sup> of  $\alpha_{s1}$ -casein, is further hydrolyzed in Cheddar cheese (Singh and others 1994) by proteinase from *L. lactis* ssp. *cremoris*, resulting in the production and accumulation of peptides  $\alpha_{s1}$ -CN f1-9, f1-13 and f1-14. The small peptides produced from  $\alpha_{s1}$ -CN f1-23 by proteinase from starter representing N-terminal ( $\alpha_{s1}$ -CN f1-7, 1-9, 1-13 and 1-14) and C-terminal ( $\alpha_{s1}$ -CN f14-17, 17-21) sequences were identified in Cheddar and found to be bitter in taste (Lee and others 1996; Richardson and Creamer 1973). According to Exterkate and Alting (1995), the action of chymosin is the limiting factor in the initial production of amino acid-N. In the absence of CEP,  $\alpha_{s1}$ -CN f1-23 accumulates in cheese and the production of amino acid-N decreases. In such a cheese, only a slow conversion of  $\alpha_{s1}$ -CN f1-23, probably catalyzed by an intracellular endopeptidase, could be detected. In the presence of CEP, the early appearance in cheese of products of the action of this endopeptidase indicates significant cell lysis (Exterkate and Alting 1995). CEPs with clearly different specificities may direct gross proteolysis to the extent that, ultimately, distinct perceptible effects on flavor development occur (Exterkate and Alting 1995).

Chymosin-produced large peptide  $\alpha_{s1}$ -CN f24-199 is further hydrolyzed by chymosin and CEP (for details, see Singh and others 1995, 1997). The concentration of  $\alpha_{s1}$ -CN f24-199 increases initially, but it is further hydrolyzed by chymosin with the formation of  $\alpha_{s1}$ -CN f102-? (‘?’ means C-terminal end of the peptide undetermined),  $\alpha_{s1}$ -CN f24-? and  $\alpha_{s1}$ -CN f33-?, which are present in water-insoluble fraction of Cheddar cheese (McSweeney and others 1994), and correspond to chymosin cleavage sites (McSweeney and others 1993b). It was noted that only the N-terminal half of this large  $\alpha_{s1}$ -CN peptide is extensively hydrolyzed and the corresponding C-terminal part was represented by a number of large peptides (McSweeney and others 1994, Singh and others 1995, 1997).

Chymosin has limited action on  $\beta$ -casein in Cheddar, although some activity is indicated by the presence of the peptide  $\beta$ -CN f1-192 [also called  $\beta$ -I casein] in the water-insoluble fraction of Cheddar (McSweeney and others 1994). Hydrolysis of the bond Leu<sup>192</sup>-Tyr<sup>193</sup> of  $\beta$ -casein by chymosin releases a small corresponding C-terminal fragment,  $\beta$ -CN f193-209, which is extremely bitter (Visser and others 1983a,b).  $\beta$ -CN f193-209 was identified in Cheddar cheese (Kelly 1993).

In the cheese environment, with a high ionic strength and a low  $a_w$ , rennet-induced breakdown of  $\alpha_{s1}$ -casein proceeds much faster than that of  $\beta$ -casein ( $\alpha_{s2}$ - and  $\kappa$ -caseins are quite resistant to hydrolysis by the rennet) (Visser 1993). Nearly half of the  $\beta$ -casein in Cheddar cheese is hydrolyzed during the ripening. Plasmin, an indigenous milk proteinase, is mainly responsible for the initial proteolysis of this protein. According to Visser and de Groot-Mostert (1977), proteolysis in aseptic starter- and rennet-free cheese is due exclusively to indigenous milk proteinases. Plasmin hydrolysis of  $\beta$ -casein results in the formation of 3  $\gamma$ -caseins [ $\gamma_1$ - ( $\beta$ -CN f29-209),  $\gamma_2$ - ( $\beta$ -CN f106-209), and  $\gamma_3$ - ( $\beta$ -CN f108-209) caseins], representing C-terminal region, and 5 proteose-peptones [ $\beta$ -CN f1-28,  $\beta$ -CN 1-105/107, and  $\beta$ -CN f29-105/107] representing the corresponding N-termi-



**Figure 6—Pathway for the degradation of major caseins in Cheddar cheese during ripening (compiled from the information in Fernandez and others 1998 and Singh and others 1994, 1995, 1997).**

**Table 2—Amino acid catabolites formed by lactic acid bacteria isolated from Cheddar cheese**

Catabolic products	Precursor	Aroma note
2-Methyl propanoic acid	Valine	rancid butter, sweaty, sweet, apple-like
2-Methyl-1-propanol	Valine	penetrating, alcohol, wine-like
2-Methyl propanal	Valine	malt
3-Methyl butanoic acid	Leucine	cheesy, sweaty, old socks, rancid, faecal, rotten fruit
3-Methyl-1-butanol	Leucine	fruity, alcohol, solvent-like, grainy
3-Methyl butanal	Leucine	dark chocolate, malt
2-Methyl butanoic acid	Isoleucine	fruity, waxy, sweaty-fatty acid
2-Methyl-1-butanol	Isoleucine	—
2-Methyl butanal	Isoleucine	dark chocolate, malt
3-(Methylthio) propanal	Methionine	cooked/boiled potato
3-(Methylthio) propanol	Methionine	cooked/boiled potato
Methanethiol	Methionine/cysteine	cabbage, boiled cabbage, sulfurous
Methyl sulfide	S-containing	cabbage, sulfurous
Dimethyldisulfide	S-containing	onion
Dimethyltrisulfide	S-containing	garlic
Dimethyltetrasulfide	S-containing	cabbage
Acetophenone	Phenylalanine	almond, musty, glue
Benzaldehyde	Phenylalanine	almond, bitter almond
Phenyl acetaldehyde	Phenylalanine	rosy, violet-like
Phenylethyl alcohol	Phenylalanine	unclean, rose, violet-like, honey
Phenyl acetic acid	Phenylalanine	flowery, rosy, plastic
Phenol	Tyrosine	medicinal
p-OH-phenyl aldehyde	Tyrosine	—
p-OH-phenyl lactate	Tyrosine	—
p-OH-phenyl acetate	Tyrosine	—
p-Cresol	Tyrosine	unclean, medicinal
Indole	Tryptophan	unclean, mothball
Skatole	Tryptophan	unclean, mothball
Benzaldehyde	Tryptophan	almond

nal region (Figure 6). Formation of  $\gamma$ -caseins and proteose-peptones has been demonstrated in Cheddar cheese (McSweeney and others 1994; Singh and others 1995, 1997). The  $\gamma$ -caseins seem to accumulate in Cheddar over the ripening period. The proteose-peptones are extensively hydrolyzed by the starter bacterial CEP and peptidases to produce small peptides and free amino acids. A majority of the  $\beta$ -casein peptides identified in Cheddar originated from the proteose-peptones (Singh and others 1995, 1997).

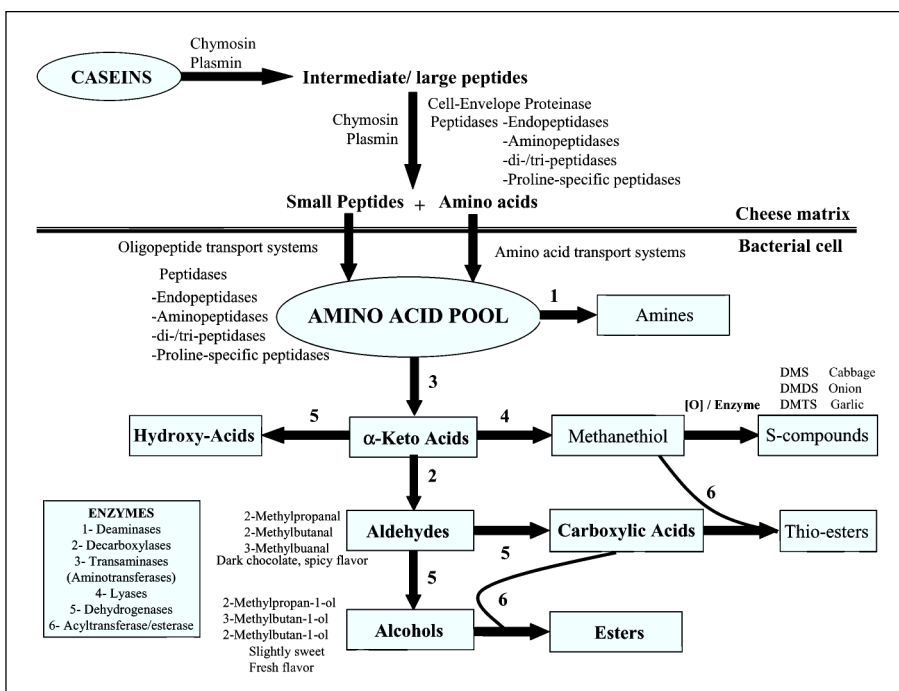
A hydrophobic peptide ( $\beta$ -CN f58-72) identified in Cheddar was found to inhibit intracellular endopeptidase (Stepaniak and others 1995); these results demonstrate that cheese ripening may be influenced by the formation of such inhibitory peptides originating from  $\beta$ -casein.

Proteolysis in cheese seems to be a sequential process involving rennet, milk proteinase (particularly plasmin), the starter culture, secondary microorganisms, and NSLAB (Fox 1989). The hydrolysis of casein to high molecular weight peptides is thought to be primarily the result of chymosin and plasmin (Olson 1990; Fox and others 1994, 1995a). The subsequent hydrolysis of high molecular weight peptides is primarily the result of proteolytic enzymes from lactic acid bacteria.

**Metabolism of amino acids**

In lactococci, the 1st step in the degradation of amino acids is transamination (see Figure 7; Gao and others 1997), leading to formation of  $\alpha$ -keto acids ( $\alpha$ -KA). Aromatic aminotransferase enzymes have been previ-

ously characterized from *Lactococcus lactis* subsp *cremoris* (Yvon and others 1997; Rijnen and others 1999a) and *Lactococcus lactis* subsp *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 (see Table 2 for various catabolites and aroma notes). Inactivation of aminotrans-



**Figure 7— Generation of flavor compounds from milk protein degradation. DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS dimethyl trisulfide. Modified from Kranenburg and others 2002.**



ferase enzymes involved in the breakdown of amino acids by lactococci has been shown to reduce aroma formation during cheese ripening (Rijnen and others 1999b).

Ney (1981) reported  $\alpha$ -keto acids corresponding to almost every amino acid in Cheddar cheese.  $\alpha$ -keto-3-methyl butyric acid and  $\alpha$ -keto-3-methyl valeric acid (Ney and Wirotma 1978) were shown to have an intense cheese-like odor. It was also shown that phenyl pyruvic acid formed from Phe by transamination was further degraded to the flavor compounds phenyl lactate and phenyl acetate by lactococcal cells in vitro (Yvon and others 1997). This degradation of phenyl pyruvic acid to phenyl lactate, phenyl acetate, and also to benzaldehyde in semihard cheese was confirmed by Yvon and others (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, no sugar) Phe, Tyr, and Trp transamination and dehydrogenation pathways were active in both species and, interestingly, these reactions were found to be reversible. Major products of Phe catabolism were phenyl lactic acid, phenyl acetic acid, and benzoic acid, while Tyr degradation resulted in the formation of p-hydroxy phenyl lactic acid and p-hydroxy phenyl acetic acid (Gummalla and Broadbent 2001). Production of p-cresol was not detected for any of the lactobacilli tested. The authors also showed that some of these products were likely to be formed by nonenzymatic processes, since spontaneous chemical degradation of Tyr intermediate p-hydroxy phenyl pyruvic acid produced p-hydroxy phenyl acetic acid, p-hydroxy propionic acid, and p-hydroxy benzaldehyde, while chemical degradation of Phe intermediate phenyl pyruvic acid resulted in production of phenyl acetic acid, benzoic acid, phenyl ethanol, phenyl propionic acid, and benzaldehyde. Trp degradation by both lactobacilli was assessed under carbohydrate starvation (pH 6.5, 30/37 °C, no sugar) and near Cheddar cheese ripening conditions (Gummalla and Broadbent 1999). Cell-free extract of both species of lactobacilli catabolized Trp to indole-3-lactic acid. Intact cells of *Lactobacillus casei* metabolized Trp in both conditions, and also the reaction was found to be reversible. In contrast, Trp catabolism by strains of *Lactobacillus helveticus* showed varied behavior: (i) detected Trp catabolism in near cheese ripening conditions, and (ii) did not catabolize Trp under both conditions, but did convert indole-3-pyruvic acid to Trp in carbohydrate starvation medium and to Trp and indole-3-lactic acid under near cheese-ripening condition.

The Cheddar cheese starter *Lactococcus lactis* initiated Trp catabolism via transaminase under some of the conditions found in cheese, but did not convert indole-3-pyruvic acid to indole-3-lactic acid (Gao and others 1997). Instead, indole-3-pyruvic acid formed by starter underwent enzymatic or spontaneous degradation to indole-3-aldehyde and indole-3-acetic acid. These secondary reactions may be important because some lactobacilli can convert indole-3-acetic acid to skatole, which is responsible for unclean flavor in cheese (Yokoyama and Carlson 1989).

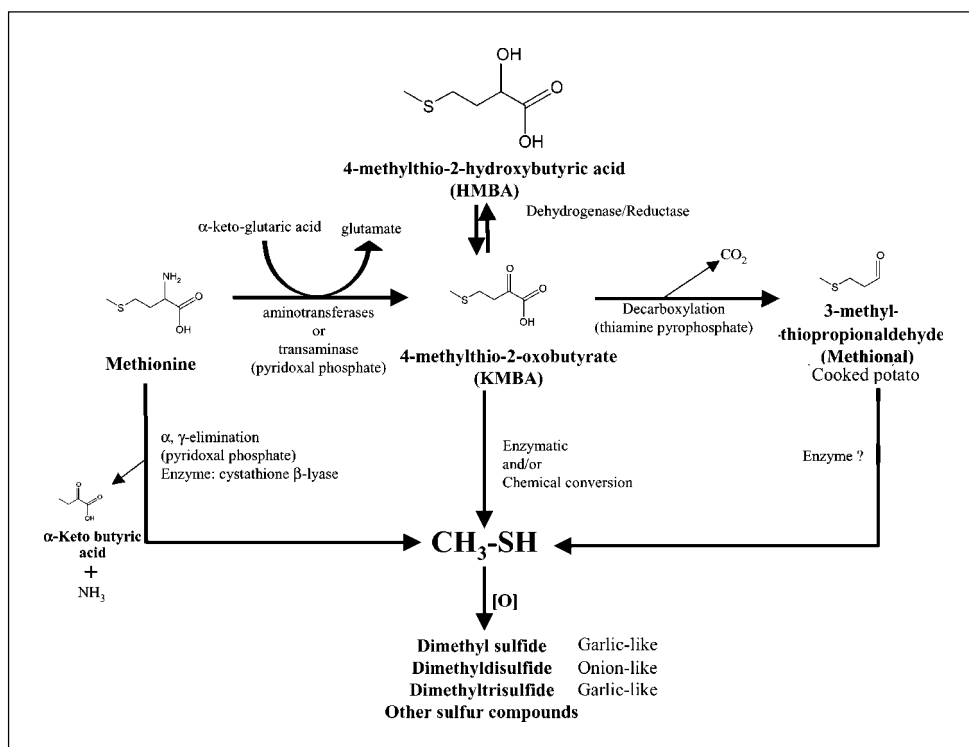
Starter lactococci are present in very

high cell numbers in cheese during the early stages of ripening, and nonviable cells may also contribute to amino acid catabolism (Gao and others 1997). Starter 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 and others 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 (Manning and Price 1977; Manning and More 1979; Price and Manning 1983). However, alone or in excess, methanethiol does not produce typical Cheddar cheese flavor (Weimer and others 1999).

Two enzymatic pathways potentially leading to the formation of methanethiol from Met has been postulated to exist in lactococci (Figure 8). A pathway for Met catabolism via  $\alpha, \gamma$  elimination was proposed by Alting and others (1995). In this pathway, a lyase catalyzes deamination and demethylthiolation of Met simultaneously, resulting in the formation of methanethiol and  $\alpha$ -keto butyric acid. Both a cystathionine  $\beta$ -lyase and a cystathionine  $\gamma$ -lyase have been purified from *Lactococcus lactis* and characterized (Alting and others 1995; Bruinenberg and others 1997). However, both of these enzymes have relatively low activities on Met. The other potential pathway is initiated by transamination of Met to 4-methylthio-2-oxobutyric acid (KMBA). The characterized aromatic aminotransferases from lactococci exhibit substantial activity with Met (Yvon and others 1997; Gao and Steele 1998).

Gao and others (1998) utilized  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$  NMR) and gas chromatography (GC) to demonstrate that Met catabolism, leading to formation volatile sulfur compounds, by lactococci is initiated mainly by an aminotransferase. The cells of



**Figure 8—Methionine degradation pathways in cheese ripening microorganisms**

4 of the 5 *Lactococcus lactis* strains examined completely converted Met to 4-methylthio-2-hydroxybutyric acid (HMBA) in the presence of  $\alpha$ -KA. Whole cells of *Lactococcus lactis* HP were not capable of converting Met to KMBA or HMBA in the presence of  $\alpha$ -KA, but this conversion was achieved with the permeabilized HP cells. These results suggested that cells of *Lactococcus lactis* HP lacked the ability to transport free Met under these conditions. However, this probably does not affect Met catabolism by HP in cheese as peptides are believed to be the primary sources of Met in the cheese matrix (Juillard and others 1995, Kunji and others 1996).

Under cheese-like conditions (pH 5.2, 5.1% NaCl), results of  $^{13}\text{C}$  NMR studies indicate Met catabolism occurs predominately via the transamination pathway (Gao and others 1998). Production of methanethiol was not detected in  $^{13}\text{C}$  NMR experiments. Headspace GC analysis indicated that methanethiol formation from Met occurred via an aminotransferase pathway which converts Met to KMBA, followed by either enzymatic conversion or chemical decomposition of KMBA to methanethiol (Figure 8).

Methanethiol is readily oxidized to dimethyl disulfide and dimethyl trisulfide (Parliament and others 1982; Chin & Lindsay 1994) (Figure 8). Occurrence of these compounds is a direct result of methanethiol content and is modulated by the low redox potential present in cheese. Methanethiol can potentially oxidize during analysis to form these compounds, and this may account for some reports of dimethyl disulfide and dimethyl trisulfide in cheese. Dimethyl sulfide (Milo and Reineccius 1997) and dimethyl trisulfide were recently noted as important odorant (Milo and Reineccius 1997; Suriyaphan and others 2001b; Zehentbauer and Reineccius 2002). Further work is needed to define the mechanism and cheese conditions needed for production.

Intact cells and autolyzed cells are capable of producing methanethiol, but the two types of cells utilize different pathways. In whole cells, KMBA (Figure 8), is primarily enzymatically converted to methanethiol. The release of aminotransferases from lactococci by autolysis could result in accumulation of KMBA from Met. The KMBA could then decompose to form methanethiol directly or could be converted to methanethiol enzymatically by whole cells (Gao and others 1998). Lactococcal cell autolysis is thought to play a role in flavor development in Cheddar cheese, and the balance of autolyzed and intact cells is believed to be important for the desired cheese-ripening events (Wilkinson and others 1994; Crow and others 1995).

The discussion shows that amino acid degradation plays a vital role in flavor development in Cheddar cheese. 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 and others 1994, Christensen and others 1995). But increased amino acid content in Cheddar did not affect the flavor development, which led Yvon and others (1998) to hypothesize that the rate limiting factor in flavor biogenesis was not the release of amino acids, but their subsequent conversion to aroma compounds. Yvon and others (1998) identified transaminase acceptor  $\alpha$ -ketoglutarate as the first limiting factor in degradation of amino acid. Addition of  $\alpha$ -ketoglutarate to Cheddar curd resulted in increased volatile components originating from branched-chain and aromatic amino acids (Banks and others 2001).

#### Characterization of Cheddar cheese flavor

Early work on Cheddar cheese flavor was based on the hypothesis that there was 1 compound or 1 class of compounds that provided the characteristic Cheddar flavor. Since no such compound could be found, Mulder (1952) and Kosikowski and Mocquot (1958) suggested the "Component Balance Theory." This theory

suggested that Cheddar cheese flavor was produced by a correct balance and concentration of a wide range of sapid and aromatic compounds. If a proper balance of components was not achieved, then undesirable or defective flavor occurred. During the intervening 50 years, there has been extensive research on the flavor of Cheddar cheese and other cheese varieties, but despite this effort, only limited information is available on the chemistry of flavor of most varieties and the flavor of none is characterized sufficiently to permit its reproduction by mixtures of pure compounds in a cheese model (Fox and others 1995a; McGorin 2001; Parliament and McGorin 2000).

It is generally accepted that the flavor quality of Cheddar cheese in the marketplace today differs considerably from that manufactured before the wide use of pasteurization, microbial rennets, and other modern manufacturing practices (Mabbit 1961; 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. Additionally, 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, which are as follows: (1) determination of the factors/agents which influence/control the development of flavor, and (2) isolation and identification of components which contribute to the flavor.

Reiter and Sharp (1971) performed experiments using cheese model systems, where 1 or more of the ripening agents were eliminated. This involved making (1) aseptic starter-free cheeses and (2) 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  $\delta$ -gluconic acid lactone as the acidulant. This cheese was completely devoid of Cheddar flavor, demonstrating 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  $\delta$ -gluconic acid lactone, developed mild but characteristic Cheddar flavor after 6 mo of ripening, and at 12 mo the flavor was fairly strong. These results clearly showed the important role played by the starter culture in the flavor development.

With the aseptic model system, Reiter and Sharp (1971) were also able to demonstrate that different single-strain starter cultures produced different flavors. Results of this study confirmed that the organisms isolated from commercial cheese or milk, added along with the single-strain starter culture, increased the flavor intensity, and that the contamination from the atmosphere, nonstarter lactic acid bacteria, can significantly increase the flavor development.

A vast amount of work has been done on various aspects of cheese composition, glycolysis, lipolysis, and proteolysis, but very few studies have characterized effects on the composition of volatile flavor compounds. Cheeses in many previous studies were simply analyzed for flavor by cheese graders. Such qualitative sensory data has limited use. More defined and analytical information using descriptive sensory and instrumental analysis is required. In the last couple of decades, a number of published works attempted to characterize the mechanism/enzymology of various reactions involved in the generation of volatiles in cheese. Only recent work in the last decade has attempted to study cheese flavor in detail.

Suzuki and others (1910, cited from Dacre 1955) worked on a neutral "flavor solution" which possessed an aroma similar to that of steam distillate of an American Cheddar cheese. Chemical analysis of the solution showed that it contained alcohols and esters (made up of ethanol and acetic acid components). In the pre-

**Table 3—Aroma compounds identified in Cheddar cheese in various studies. Compounds are listed in the order of their importance in odor.**

Mild Cheddar <sup>a</sup>	Mild Cheddar <sup>b</sup>	Mild Cheddar <sup>c</sup>	Sharp Cheddar (British Farmhouse Cheddar) <sup>d</sup>
Homofuraneol	Butyric acid	Furaneol	2-Isopropyl-3-methoxy-pyrazine
Butyric acid	Homofuraneol	(E)-2-Nonenal	3-(Methylthio) propanal
Furaneol	3-(Methylthio) propanal	2,3-Butanedione	p-Cresol
δ-Decalactone	δ-Decalactone	(Z)-4-Heptenal	δ-Dodecalactone
Skatole	6-(Z)-Dodeceny-γ-lactone	3-(Methylthio) propanal	Butanoic acid
6-(Z)-Dodeceny-γ-lactone	Furaneol	1-Octen-3-one	Isovaleric acid
3-(Methylthio) propanal	2,3-Butanedione	2-Acetyl-2-thiazoline	2-Phenylethanol
(E)-β-Damascenone	Skatole	Dimethyl trisulfide	Ethyl octanoate
2,3-Butanedione	Acetic acid	(Z)-1,5-Octadien-3-one	Acetic acid
Nonanal	Hexanoic acid	(Z)-2-Nonenal	β-Damascenone
<i>trans</i> -4,5-Epoxy-2-(E)-decenal	2/3-Methyl butanal	Ethyl butanoate	Octanoic acid
Acetic acid	1-Octen-3-one	Hexanal	4,5-Dimethyl-3-hydroxy-2-(5H)-furanone (sotolon)
(E)-2-Nonenal	(Z)-2-Nonenal	2-Isobutyl-3-methoxy-pyrazine	Phenyl acetic acid
δ-Dodecalactone	(E)-2-Nonenal	<i>trans</i> -4,5-Epoxy-2-(E)-decenal	Ethyl butanoate
1-Octen-3-one	Hexanal	2-Nonanone	Ethyl hexanoate
(Z)-2-Nonenal	Octanal	2-Isopropyl-3-methoxy-pyrazine	Dimethyl trisulfide
2-Acetylthiazoline	2-Acetyl-2-thiazoline	Decanal	Phenyl acetaldehyde
	β-Damascenone	2/3-Methyl butanal	Pentanoic acid
	2-Acetyl-1-pyrroline	Ethyl octanoate	Guaiacol
	Nonanal	1-Hexen-3-one	γ-Decalactone
	(E,Z)-2,6-Nonadienal	Methyl propanal	δ-Decalactone
	(E,E)-2,4-Nonadienal	Ethyl hexanoate	1-Octen-3-one
		Homofuraneol	2-Acetylpyrazine
		Butyric acid	2-Isobutyl-3-methoxy-pyrazine
			Linalool
			(E,Z)-2,6-Nonadienal
			Geosmin
			Furaneol

<sup>a</sup>Diethyl ether extract of mild Cheddar cheese analyzed by GCO/AEDA/GC-MS (Milo and Reineccius, 1997).

<sup>b</sup>Diethyl ether extract and dynamic headspace

<sup>c</sup>of mild Cheddar cheese analyzed by GCO/AEDA/GC-MS and GCO-H/DHDA/GC-MS (Zehentbauer and Reineccius, 2002).

<sup>d</sup>Diethyl ether extract of British farmhouse Cheddar cheese analyzed by GCO/AEDA/GC-MS (Suriyaphan and others, 2001b).

vious study, the authors could not identify any component/components definitely responsible for the characteristic cheese flavor. Dacre (1955) extracted volatiles from Cheddar cheese by steam distillation. Besides volatile fatty acids (acetic, butyric, and hexanoic acids), the distillate was found to contain ethanol, butanal, ethyl acetate, and ethyl butyrate. 2,3-Butanedione does not appear to be involved in mature cheese flavor, although it is present in young cheese; it decreases in concentration as the cheese matures. None of the aroma compounds identified, singly or in combination, gave the odor or taste of Cheddar, but the authors also commented on the possibility of some other organic compounds, sensitive to oxidation, present in trace amounts being involved in the cheese flavor.

Methyl ketones were identified in New Zealand Cheddar cheese and were found to play a role in cheese flavor (Walker and Harvey 1959). A range of carbonyl compounds were identified, which included compounds such as acetoin, 2,3-butanedione, acetone, 2-butanone, 2-pentanone, 2-heptanone, 2-nonanone, and 2-undecanone. In a further study, Walker (1959) showed that hydrogen sulfide (H<sub>2</sub>S) played an important role in Cheddar flavor. The removal of carbonyls and H<sub>2</sub>S from steam distillate completely destroyed the cheesy aroma.

In an interesting study, Manning and Robinson (1973) characterized volatile flavor compounds in the headspace over the distillate, prepared by cryotrapping of volatiles of Cheddar cheese using low-temperature vacuum distillation. The aroma emanating from the trap as it thawed, at temperatures between -100 and -70 °C, was found to have a distinct Cheddar aroma and mainly contained volatiles such as H<sub>2</sub>S, methanethiol, and dimethyl sul-

fide. The compounds with low vapor pressure/high boiling points in the distillate, such as 2,3-butanedione, methyl ketones, and volatile fatty acids were also considered to play an important role in Cheddar flavor. Analysis of Cheddar headspace volatiles also reconfirmed the important role played by H<sub>2</sub>S, methanethiol, and dimethyl sulfide in flavor (Manning and Price 1977; Manning and More 1979; Price and Manning 1983).

The availability of improved sensory and instrumentation methodologies for analysis of volatiles in the last decade or so have been immensely helpful in understanding and characterization of cheese flavor. Sensory methods are discussed later in this review. 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 3-y-old Cheddar cheese were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, α-pinene, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, methional, propionic acid, butyric acid, valeric acid, caproic acid, capric acid, and lauric acid. The authors pointed out that the technique did not allow the determination of the most volatile odor fraction, which included hydrogen sulfide, acetaldehyde, and methanethiol. Descriptive sensory analysis was not conducted on the cheese used in the study, which limited conclusions about the role of individual compounds on specific cheese flavors. Based on these results, a subsequent sensory study using a concept matching technique was conducted. Dacremont and Vickers (1994) found that a recognizable Cheddar aroma was produced by a mixture of 2,3-butanedione, methional, and butyric acid. However, the authors also indicated a possible contribution of

other aroma compounds that were not commercially available at that time.

Milo and Reineccius (1997) applied both traditional high-vacuum isolation/aroma extract dilution analysis (AEDA) and static headspace-olfactometry (GCOH) to further study the aroma of a regular and a low-fat Cheddar cheese (see Table 3). 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 above-mentioned 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 (Preininger 1995, cited from Milo and Reineccius 1997). While 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. Also, 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 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 and others 1996). Addition of methanethiol to bland slurry of reduced-fat Cheddar produced a strong Cheddar aroma (Urbach 1997b).

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 to GCO-H and AEDA (Zehentbauer and Reineccius 2002) (Table 3). Results of DHDA showed that, in addition to the odorants previously identified by AEDA and GCO-H, (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.

The volatile aroma components of 2 sharp Cheddar cheeses of British Farmhouse origin, made using raw milk and ripened for at least 1 y, were analyzed by AEDA (Suriyaphan and others 2001b) (Table 3). Descriptive sensory analysis of these cheeses was also conducted. Key flavors in the sharp Farmhouse Cheddar cheeses were "barnyard" and "earthy." Following instrumental analysis, model system addition was used to confirm compounds responsible for specific flavor notes. p-Cresol was mainly responsible for a "cowy-barny" note, whereas an intense "soil-like" note was due to 2-isopropyl-3-methoxypyrazine. At much lower odor intensity, 2-isobutyl-3-methoxypyrazine contributed a "bell pepper-like" note. Direct addition of p-cresol (> 100 ppb) or 2-isopropyl-3-methoxypyrazine (> 3 ppb) in a mild domestic Cheddar cheese resulted in increases in intensities of cowy/phenolic and earthy/bell pepper aroma notes. Additionally, within the same wedge of cheese, the concentrations of p-cresol and 2-isopropyl-3-methoxypyrazine were lower at the center than at the rind.

It is important to note that, in each of the studies mentioned

previously, different Cheddar cheeses of different ages, microflora, and biochemistry were studied. Cheddar cheese encompasses a wide category and there are numerous potential flavor profiles (see Table 4 for summary of volatile flavor compounds identified in Cheddar). Thus, to elucidate Cheddar cheese flavor is a large task and descriptive sensory analysis should be conducted in conjunction with any instrumental study to provide clarification.

### Sensory studies of Cheddar cheese

The dairy industry has long recognized that sensory quality is a crucial aspect of sales and marketing. In fact, the dairy industry developed and used traditional tools for evaluating sensory quality before modern-day sensory analysis techniques were developed. These traditional tools include grading and dairy products judging. Grading was established by the Federal government with the founding of the Office of Markets (currently known as the Agricultural Marketing Service) in 1913 ([www.ams.usda.gov](http://www.ams.usda.gov)). Dairy products judging developed as a way to stimulate student interest and education, and the first contest was held in 1916 (Bodyfelt and others 1988). Both grading and judging operate on the same premise. Products are scored for overall flavor or texture quality based on an idealized concept and a predetermined list of defects (Table 5). The "defects" were common problems encountered in cheesemaking. Describing or defining the defects and their intensities can be subjective, as many of the defects can constitute an "ideal" flavor and only become defects if they are out-of-balance in the cheese. These tools can function well in a setting where a large number of samples need to be rapidly assessed for basic quality. Grading is still used in the cheese industry today for this purpose. However, these fundamental sensory tools were not designed to be specific, analytical tools that are often required for interpretations of flavor research and the nuances of consumer marketing preferences. Two cheeses may receive identical AA grades, indicating that they are both good quality (based on the presence or absence of predefined defects); however, specific differences may exist in descriptive flavor profiles. These differences can significantly impact research interpretations and consumer preferences. Further, what represents "good" and "bad" quality to an expert grader are not necessarily synonymous to the cheese consumer. Consumer concepts and preferences for cheese flavor profiles are diverse. Thus, cheese grade results cannot be used in research to superimpose consumer preferences. For these reasons, grading and judging are not ideal tools for cheese flavor research.

The most powerful sensory tool in cheese flavor research is descriptive sensory analysis. In this technique, a panel of 6 to 12 individuals is trained to identify and quantify sensory aspects of a food, which may include appearance, aroma, flavor, texture, or any single aspect (Meilgaard and others 1999). The panel acts as a highly trained instrument. A trained descriptive panel can be a powerful and versatile tool with applications to instrumental analysis. Such panels require training and regular maintenance. Descriptive analysis of flavor is 1 of the most complex modalities to train, and a good cheese flavor panel may require as many as 75 to 100 h of training. For a review of descriptive sensory analysis of flavor, see Drake and Cville (2003). Affective tests involve the use of consumers to assess acceptability or preference. These tools are certainly useful to determine consumer perception, but cannot give analytical data on the presence and intensity of specific flavors or flavor profiles.

Cheddar cheese flavor research using descriptive sensory analysis has been conducted primarily within the past 10 years. Descriptive analysis can be used to differentiate cheeses, or it can be used to interpret research parameters such as cheese make-procedure modifications. Muir and others (1995a) described 9 aroma terms for characterization of aroma profiles of hard and semi-hard cheeses, including Cheddar cheese. In a subsequent study, a

set of key attributes for texture and flavor of hard cheeses were defined (Muir and others 1995b). Piggot and Mowat (1991) determined 23 descriptive flavor and aroma terms in a study of maturation of Cheddar cheese. Muir and Hunter (1992), Muir and others

(1996b), and Roberts and Vickers (1994) also developed descriptive flavor and aroma terms to study flavor during Cheddar cheese aging. Banks and others (1993) used descriptive analysis to determine sensory properties of low-fat Cheddar cheese while factory

**Table 4—Volatile compounds identified in Cheddar cheese**

Chemicals	Aroma note*	Chemicals	Aroma note*
Acetaldehyde	sweet, pungent	Indole	mothball
Acetic acid	vinegar	Isobutanol	—
Acetone	—	Isohexanal	—
Acetophenone	almond, musty, glue	Limonene	citrus
$\beta$ -Angelicalactone	—	Linalool	sweet, floral, honey
Benzaldehyde	almond	Methanethiol	cabbage, boiled cabbage, sulfurous
Butanal	pungent	Methyl acetate	—
n-Butanol	floral, fragrant, fruity, sweet	Methyl propionate	—
2-Butanol	alcoholic	Methyl hexanoate	pineapple
2,3-Butanediol	fruity	2-Methyl butanal	dark chocolate, malt
2-Butanone	etheric	3-Methyl butanal	dark chocolate, malt
2,3-Butanedione (Diacetyl)	buttery	2-Methyl-1-butanol	wine
n-Butyl acetate	pear	3-Methyl-1-butanol	fruity, alcohol, solvent-like, grainy
n-Butanoic acid	sweaty, cheesy, fecal	3-Methyl-2-butanone	camphor
2-Butyl acetate	—	3-Methylbutanoic acid (isovaleric acid)	Swiss cheese, waxy, sweaty, old socks, fecal
n-Butyl butyrate	—	3-Methyl-2-pentanone	—
p-Cresol	unclean, medicinal, cowy, barny	2-Methyl propanal	malt
(E)- $\beta$ -Damascenone	apple sauce	3-(Methylthio) propanal (Methional)	baked/boiled potato
Decanal	soapy, flowery	Nonanal	green
n-Decanoic acid	rancid	(E)-2-Nonenal	green, fatty
(E,E)-2,4-Decadienal	mayonnaise, bread, fatty, tallow, fruity	(Z)-2-Nonenal	green
(E,Z)-2,4-Decadienal	mayonnaise, bread, fatty, tallow, fruity	(E,Z)-2,6-Nonadienal	melon, cucumber
Dimethyl sulfide	cabbage, sulfurous	(E,E)-2,4-Nonadienal	soapy
Dimethyl disulfide	onion, sulfurous	2-Nonanone	green, earthy, blue cheese, fatty, fruity, musty, varnish
Dimethyl trisulfide	cabbage, garlic, sulfurous	(Z)-1,5-Octadien-3-one	green, metallic
Dimethyl tetrasulfide	putrid, cabbage, sulfurous	$\delta$ -Octalactone	fruity, peach, sweet
$\gamma$ -Decalactone	coconut	n-Octanoic acid	body odor, sweaty
$\delta$ -Decalactone	peachy, coconut	Octanal	green, fatty, soapy, fruity, orange peel
$\delta$ -Dodecalactone	cheesy, coconut	2-Octanol	mushroom, coconut, oil, rancid
6-(Z)-Dodecenyl- $\gamma$ -lactone	soapy	2-Octanone	floral, fruity, soapy, ketone, musty
trans-4,5-Epoxy-2-(E)-decenal	metallic	1-Octen-3-one	mushroom
Ethanol	alcohol	2,4-Pentanediol	—
2-Ethyl butanol	—	n-Pentanoic acid	Swiss cheese
Ethyl acetate	fruity, solvent, sweet	Pentanal	pungent, almond-like
Ethyl propionate	fruity	2-Pentanol	sweet, alcoholic, fruity, nutty
Ethyl butyrate	bubble gum, fruity	Pentan-2-one	acetone, sweet, fruity, ketone
Ethyl hexanoate	fruity	Phenyl acetaldehyde	rosy
Ethyl octanoate	fruity	Phenyl acetic acid	flowery
Furanone, 4,5-dimethyl-3-hydroxy-2(5H)-(Sotolon)	curry, seasoning	2-Phenyl ethanol	rosy
Furanone, 2,5-dimethyl-4-hydroxy-3(2H)-(Furaneol)	sweet, caramel, burnt sugar	$\alpha$ -Pinene	pine
Furanone, 2-Ethyl-4-hydroxy-5-methyl-3(2H)-(Homofuraneol or Ethyl Furaneol)	caramel	Propionic acid	pungent
Geosmin	earthy, moistened soil	n-Propanol	pungent
Guaiacol	smoky, spicy	Propanal	solvent-like
Heptanal	fatty, oily, green	Propyl acetate	—
2-Heptanone	blue cheese, fruity, musty, soapy	n-Propyl butyrate	pineapple
(Z)-4-Heptenal	creamy, biscuit	Propenal	—
n-Hexanal	green	Pyrazine, 2-acetyl	popcorn
n-Hexanol	fatty, floral, green	Pyrazine, 2-isobutyl-3-methoxy-	bell pepper-like, green
n-Hexanoic acid	goat-like	Pyrazine, 2-isopropyl-3-methoxy-	earthy, soil, green, beany
2-Hexanone	fruity, ketone	Pyrroline, 2-acetyl-1-	roasted
Hexanethiol	sulfur	Skatole	unclean, mothball, fecal
2-Hexenal	almond bitter, green, fatty	Thiazoline, 2-Acetyl-2-	roasted
1-Hexen-3-one	cooked vegetable, plastic	Thiophen-2-aldehyde	—
Hydrogen sulfide (H <sub>2</sub> S)	rotten eggs, cabbage	2-Tridecanone	—
3-Hydroxy-2-butanone (Acetoin)	buttery	2-Undecanone	floral, fruity, green, musty, tallow

\*Additional information on aroma notes were obtained from (1) "Flavornet-Gas chromatography-olfactometry (GCO) of natural products" at [www.nysaes.cornell.edu/flavornet/index.html](http://www.nysaes.cornell.edu/flavornet/index.html) (maintained by Dr. T. Acree and Dr. H. Arn, 1997, Dept of Food Science, Cornell Univ.), (2) "The LRI and Odour Database" at [www.odour.org.uk](http://www.odour.org.uk) (maintained by Dr. R. Mottram, Flavor Research Group, School of Food Biosciences, Univ. of Reading), and (3) Rychlik and others (1998). Retention indices and threshold values of flavor compounds can also be found in the above mentioned databases.

and farmhouse Cheddars have also been compared (Muir and others 1997). Muir and others (1996a), Drake and others (1996, 1997), and Lynch and others (1999) used descriptive sensory panels to determine the effect of starter culture and adjunct cultures on Cheddar cheese flavor. Descriptive analysis was used to determine the impact of emulsifying agents on processed Cheddar cheese flavor (Drake and others 1999). Recently, studies have used descriptive sensory analysis to address the role of specific starter culture enzyme systems on Cheddar cheese flavor (Broadbent and others 2002; Banks and others 2001).

Research has also focused on standardizing descriptive sensory languages used to describe Cheddar cheese flavor. Precise definitions and references (food or chemical) for each term are necessary to be able to calibrate panels over time or panels at multiple locations (Drake and Civille 2003). Descriptive sensory languages used in many studies do not have standardized definitions and references. A standard list of descriptors and references was identified for a descriptive sensory language used with aged natural

cheeses (Heisserer and Chambers 1993). Descriptive sensory languages specifically for Cheddar cheese flavor with definitions and references were more recently developed independently in Ireland and the United States (Murray and Delahunty 2000a; Drake and others 2001) (Table 6). In a later study, Drake and others (2002a) demonstrated that a defined and anchored descriptive language for Cheddar cheese flavor could be used by different panels at different sites to obtain similar results. Current work is focused on international cross validation of Cheddar cheese flavor languages.

Relating instrumental analysis to the actual sensory perception of particular flavors is another area where descriptive sensory analysis is crucial. Descriptive analysis can be done simultaneously with instrumental analysis and the results analyzed for statistical relationships by univariate and multivariate analysis of variance. Alternatively, descriptive sensory results and GCO data can be linked using threshold testing and model systems. Several studies have been conducted in this area and are addressed in

**Table 5—List of cheese flavor defects used with judging cheese flavor**

Flavor defect	Definition
High acid	Excessive acid or sour taste
Bitter	Bitter taste resembling caffeine or quinine
Fruity/fermented	Aroma of fermenting or overripe fruit
Flat	Devoid of flavor
Garlic/onion	Flavor resembling garlic, onion, or leeks
Heated	Not the clean cooked flavor of pasteurized milk but a flavor resembling the odor of old or spoiled milk
Malty	Flavor similar to Grape Nuts cereal
Metallic	A flat metal-like taste and a lingering puckery mouthfeel
Moldy	Musty, reminiscent of a damp cellar
Rancid	Also called lipase, caused by short-chain fatty acids, flavor described as bitter, soapy, disagreeable
Sulfide	Also called skunky. Similar to water with high sulfur content.
Unclean	Dirty aftertaste that fails to clean-up after the cheese is expectorated
Whey taint	Also called sour whey. The dirty sweet acidic taste and odor characteristic of fermented whey
Yeasty	Sour, bread-dough, earthy aroma characteristic of yeast.

Adapted from Bodyfelt and others (1988)

**Table 6—The basic Cheddar cheese flavor lexicon**

Descriptor	Definition	Reference
Cooked/milky	Aromatics associated with cooked milk	Skim milk heated to 85 °C for 30 min
Whey	Aromatics associated with fresh Cheddar cheese whey	Fresh Cheddar whey
Diacetyl	Aromatic associated with diacetyl	Diacetyl, 20 ppm
Milk fat/lactone	Aromatics associated with milk fat	Fresh coconut meat, heavy cream, $\delta$ dodecalactone, 40 ppm
Fruity	Sweet aromatics associated with different fruits, primarily pineapple	Fresh pineapple Ethyl hexanoate, 20 ppm
Sulfur	Aromatics associated with sulfurous compounds	Boiled mashed egg $H_2S$ bubbled through water
Brothy	Aromatics associated with boiled meat or vegetable soup stock	Canned potatoes Low sodium beef broth cubes Methional, 20 ppm
Free fatty acid	Aromatics associated with short-chain free fatty acids	Butyric acid, 20 ppm
Nutty	Nutty aromatic associated with various nuts	Roasted peanut oil extract Lightly toasted unsalted nuts
Catty	Aroma associated with tomcat urine	2-mercapto-2-methyl-pentan-4-one, 20 ppm
Cow/barny	Aromas associated with barns and stock trailers, indicative of animal sweat and waste	A mixture of p-cresol (160 ppm) + isovaleric acid (320 ppm)
Sweet	Fundamental taste sensation elicited by caffeine	Sucrose (5 % in water)
Sour	Fundamental taste sensation	Citric acid (0.08 % in water)
Salty	Fundamental taste sensation	Sodium chloride (0.5 % in water)
Bitter	Fundamental taste sensation	Caffeine (0.08 % in water)
Umami	Chemical feeling factor elicited by certain peptides and nucleotides	Mono sodium glutamate (1 % in water)

Chemical references prepared in 95% ethanol, then blotted onto filter paper into jars for sniffing. Adapted from Drake and others (2001).



other sections of this review. Affective sensory tests involve consumers and can be used to probe not only consumer likes and dislikes, but can be linked with descriptive sensory analysis of the same sample set to identify specific flavor profiles that drive consumer acceptability. The concept of Cheddar cheese flavor is a consumer concept and likely varies widely among consumers, as does Cheddar cheese flavor itself. The analytical descriptive sensory properties that describe exactly what attributes are perceived, and at what levels, are related to consumer preferences using combinations of multivariate techniques. The process is called preference mapping. Preference mapping has been applied to a wide number of commodities and products, and there are a few applications to cheese. Consumer preferences for specialty cheeses were clarified and preference mapping was used to compare farmhouse and factory-produced cheeses (Lawlor and Delahunty 2000; Murray and Delahunty 2000b). Murray and Delahunty (2000c) used preference mapping to explore packaging preferences for Cheddar cheeses. Recently, Young and others (2003) conducted preference mapping on Cheddar cheeses. Consumers did distinguish young and aged Cheddar cheeses but, as expected, the concept of Cheddar flavor varied widely. Six distinct clusters of consumers with specific preferences for flavor profiles of Cheddar cheeses were identified.

#### **Bitterness and other off-flavors in Cheddar cheese**

In addition to the characteristic desirable flavors, cheese frequently suffers from specific flavor defects. While desirable flavor has been difficult to define in chemical and sensory terms, since consumers vary in preference and definition of Cheddar flavor, the specific cause(s) of many of these specific flavor or off-flavor notes have been established more or less definitively. This section presents an overview on bitterness and chemical anchors for the specific flavor/off-flavor notes in Cheddar cheese.

**Bitterness in Cheddar cheese.** Bitterness is a problem in many cheese varieties, especially those made with mesophilic cultures, and have been associated with the production (by rennet and starter bacteria) of bitter peptides, which predominantly contain hydrophobic amino acid residues. Certain sequences in the caseins are particularly hydrophobic and, when excised by proteinases, can lead to bitterness. Aged Cheddar cheese often develops bitterness due to the accumulation of hydrophobic peptides, consisting of 2 to 23 amino acid residues, or in the molecular weight range of 500 to 3000 Da (Sullivan and Jago 1972; Lee and others 1996). Bitterness is detected when the concentration of bitter peptide exceeds the threshold. Although the bitter taste is considered a normal component of cheese taste, excessive bitterness may limit consumer acceptance of the cheese. The problem has been the subject of considerable research which has been reviewed by Lemieux and Simard (1991, 1992).

Bitter peptides found in normal aseptic, aseptic starter-free, aseptic rennet-free, and aseptic starter- and rennet-free Gouda cheeses have been shown to have a molecular weight of < 1400 Da (Visser 1977b,c). Chymosin has been implicated in the formation of bitter peptides in cheese, and thus factors that affect the retention and activity of rennet in the curd may influence the development of bitterness. The concentration of NaCl has a major effect on the hydrolysis of  $\beta$ -casein by chymosin in solution and in cheese, and thus may also be a factor in the control of bitterness. Kelly (1993) found that the formation of  $\beta$ -CN f193-209 (a primary product of chymosin action on  $\beta$ -casein and potentially bitter) is inhibited by increasing NaCl concentrations. In Cheddar, large amounts of this peptide were produced in an unsalted cheese, which was extremely bitter, but in cheeses made with increasing salt content, the production of this peptide was inhibited (Kelly 1993). The rate of chymosin-induced hydrolysis of  $\beta$ -casein is largely dependent on the state of aggregation of the substrate,

which affects the accessibility of the susceptible peptide bonds. A nonuniform distribution of dry salt in the milled curd during manufacture can lead to production of  $\beta$ -CN f193-209 in Cheddar during ripening. This particular bitter peptide is not further hydrolyzed by chymosin or starter proteinases (Lemieux and Simard 1992). Visser and others (1983b,c) concluded that the relatively slow degradation of  $\beta$ -casein by rennet and starter proteinases inevitably leads to the gradual appearance of the bitter peptide,  $\beta$ -CN f193-209, as the first degradation product.

It is interesting to note that Lee and others (1996) identified several peptides in Cheddar cheese as bitter originating from N-terminal of  $\alpha_{s1}$ -casein, identified as  $\alpha_{s1}$ -CN f1-7,  $\alpha_{s1}$ -CN f1-9,  $\alpha_{s1}$ -CN f1-13 and  $\alpha_{s1}$ -CN f1-14. These peptides, especially  $\alpha_{s1}$ -CN f1-9 and  $\alpha_{s1}$ -CN f1-13, were found to accumulate in Cheddar (Singh and others 1994) and Gouda (Kaminogawa and others 1986) during ripening. These peptides are produced from  $\alpha_{s1}$ -CN f1-23, formed by chymosin action on the bond Phe<sup>23</sup>-Phe<sup>24</sup> of  $\alpha_{s1}$ -casein, followed by the action of lactococcal CEP on  $\alpha_{s1}$ -CN f1-23. The corresponding C-terminal fragment of  $\alpha_{s1}$ -CN f1-23 was also found to be bitter, and  $\alpha_{s1}$ -CN f14-17 and f17-21 are also produced in Cheddar cheese made using strain HP as the starter (Richardson and Creamer 1973). The above observation indicates that the role of rennet in the development of bitterness may be the production of long peptides which are subsequently degraded to small, bitter peptides by starter proteinases.

Some authors suggest that bitterness is simply related to starter cell numbers; for example, fast acid-producing, heat-tolerant strains, but others maintain that there are inherent differences between bitter and nonbitter starter strains with respect to proteinase and peptidase profiles (see Lemieux and Simard 1991). It has been shown earlier that growth conditions of the culture significantly affect the debittering activity. From experiments on the ability of different cultures to hydrolyze  $\beta$ -CN f193-209 under different growth conditions, Smit and others (1996) concluded that, in general, cells grown under pH-controlled conditions have a stronger debittering ability than cells grown in acidifying conditions. Similar observations were made in cheesemaking trials using cultures grown under different growth conditions. These differences were the result of increased sensitivity to lysis of cultures grown under pH-controlled conditions than to cells grown under acidifying conditions. This clearly shows that bitterness in cheese can be controlled by adaptation of the lactic acid bacterial culture strains to growth conditions. The debittering assay (based on the ability to hydrolyze  $\beta$ -CN f193-209) allowed rapid screening for and prediction of debittering activity of lactic acid bacteria. Aminopeptidase activity (Pep N) appears to be important in reducing the intensity of bitterness. The result of Baankreis (1992) demonstrated increasing bitterness in cheese made with PepN negative mutants, whereas cheese made with PepX negative mutants exhibited no increase in bitterness. Since lactococcal peptidases are intracellular, the rate of lysis of starter cells may be important both with respect to the rate of peptidolysis in general and to the control of bitterness in particular. Soeryapranata and others (2002b) reported higher bitterness score and concentration of  $\beta$ -CN f193-209 in Cheddar made using starter culture with low susceptibility to lysis. The primary action of plasmin on  $\beta$ -casein probably does not produce bitter peptides. The fate of  $\alpha_{s2}$ -casein in cheese is unclear, but plasmin can release potentially bitter peptides from this protein in solution (Fox and others 1994).

Bitterness appears to be a particular problem in low-fat cheeses (Banks and others 1992; Drake and Swanson 1995). In normal-fat cheese, bitter peptides, being hydrophobic, probably partition into the fat phase where they are less likely to be perceived as being bitter. In reduced-fat cheeses, bitterness may be more pronounced.

The majority of studies on the identification of bitter peptides

**Table 7—Bitter peptides identified in Cheddar cheese**

Peptide	Hydrophobicity	Sequence	Reference
$\alpha_{s1}$ -CN f1-7	1771.0	H.Arg.Pro.Lys.His.Pro.Ile.Lys.OH	Lee & others (1996)
$\alpha_{s1}$ -CN f1-13	1363.0	H.Arg.Pro.Lys.His.Pro.Ile.Lys.His.Gln.Gly.Leu.Pro.Gln.OH	Lee & others (1996)
$\alpha_{s1}$ -CN f11-14	1367.0	H.Leu.Pro.Gln.Glu.OH	Lee & others (1996)
$\alpha_{s1}$ -CN f14-17	1162.5	H.Glu.Val.Leu.Asn.OH	Hodges & others (1972), Richardson & Creamer (1973), Hamilton & others (1974)
$\alpha_{s1}$ -CN f17-21	1074.0	H.Asn.Glu.Asn.Leu.Leu.OH	Hodges & others (1972), Richardson & Creamer (1973), Hamilton & others (1974)
$\alpha_{s1}$ -CN f26-32	1930.0	H.Ala.Pro.Phe.Pro.Glu.Val.Phe.OH	Richardson & Creamer (1973)
$\alpha_{s1}$ -CN f26-33	1688.8	H.Ala.Pro.Phe.Pro.Glu.Val.Phe.Gly.OH	Hodges & others (1972), Hamilton & others (1974)
$\alpha_{s2}$ -CN f191-197	2010.0	H.Lys.Pro.Trp.Ile.Gln.Pro.Lys.OH	Lee & others (1996)
$\beta$ -CN f8-16	1390.0	H.Val.Pro.Gly.Glu.Ile.Val.Glu.Ser(P).Leu.OH	Lee & others (1996)
$\beta$ -CN f 46-67	1580.5	H.Gln.Asp.Lys.Ile.His.Pro.Phe.Ala.Gln.Thr.Gln.Ser.Leu.Val.Tyr.Pro.Phe.Pro.Gly.Pro.Ile.(Pro/His).OH	Richardson & Creamer (1973), Hamilton & others (1974)
$\beta$ -CN f46-84	1508.5	H.Gln.Asp.Lys.Ile.His.Pro.Phe.Ala.Gln.Thr.Gln.Ser.Leu.Val.Tyr.Pro.Phe.Pro.Gly.Pro.Ile.(Pro/His).Asn.Ser.Leu.Pro.Gln.Asn.Ile.Pro.Pro.Leu.Thr.Gln.Thr.Pro.Val.Val.Val.OH	Hamilton & others (1974)
$\beta$ -CN f193-209	1762.4	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly.Pro.Val.Arg.Gly.Pro.Phe.Pro.Ile.Ile.Val.OH	Kelly (1993), Broadbent & others (1998), Soeryapranata and others (2002a, b)

were conducted on model systems consisting of isolated casein incubated with various enzymes. Bitter peptides have been identified in hydrolyzates of  $\alpha_{s1}$ -,  $\alpha_{s2}$ - and  $\beta$ -caseins; para- $\kappa$ -casein is also a potential source of bitter peptides. Bitter peptides originating from  $\alpha_{s1}$ -casein in solution include f22-43 and f45-50, f23-34, f91-100 and f145-151. Three short peptides produced from the C-terminal region of  $\alpha_{s2}$ -casein by plasmin (f198-207, f182-207 and f189-207) may be bitter. Bitter peptides found in hydrolysates of  $\beta$ -casein include f203-208, f195-209, f201-209, and f53-79. A number of bitter peptides isolated from cheese are summarized in Table 7.

Various methodologies have been adopted for the analysis of bitter peptides in cheeses. Isolation of bitter peptides from cheese invariably involved water and/or solvent extraction of cheese followed by the analysis using 1 or more steps of liquid chromatography (Lee and Warthesen 1996; Kelly 1993). Bitter peptides from Cheddar have been studied by capillary electrophoresis and reversed phase-high performance liquid chromatography (Kelly 1993; Lee and Warthesen 1996; Smit and others 1996; Broadbent and others 1998). Most of these techniques required extensive separation, which is labor-intensive and time-consuming. In a recent work, Soeryapranata and others (2002a) reported a sensitive and rapid quantitative method, using an internal standard, for analysis of synthetic  $\beta$ -CN f193-209 by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The concentration ratio of synthetic  $\beta$ -CN f193-209 to an internal standard was linearly correlated to the peak-height ratio obtained from MALDI-TOF analysis. The internal standards developed in this study were: (1) Asn substitution for Glu at residue 195, and (2) penta-deuterated Gln at residues 194 and 195 in  $\beta$ -CN f193-209, which are separated by mass of -14 and +10 from  $\beta$ -CN f193-209 (m/z 1880), respectively. The method was used for monitoring the decrease of the m/z 1880 peak as a function of incubation time, accompanied by the decrease in bitterness intensity of a water-soluble extract of bitter cheese after incubation with cell-free extract of *Lb. helveticus* (a debittering culture). This clearly shows that peptide  $\beta$ -CN f193-209 is a useful marker to follow the development of bitterness in cheese. The method was used to monitor concentration of  $\beta$ -CN f193-209 in Cheddar cheese extract during ripening (Soeryapranata and others 2002b). The relationship of  $\beta$ -CN f193-209 concentration and bitterness intensity

was found to be weaker as the aging time progressed, probably due to formation of other bitter peptides more responsible for bitterness at longer aging time and/or the presence of compounds produced during ripening that mask bitterness perceived by sensory panelists. The decreased correlation coefficient between [ $\beta$ -CN f193-209] in cheese extract and bitterness intensity of cheese suggests that  $\beta$ -CN f193-209 is a better marker for bitterness development at the initial stages of Cheddar cheese ripening. This peptide could also be used for categorizing cheese starter culture as bitter/nonbitter. In addition to peptides, a number of other compounds can contribute to bitterness in cheese, including amino acids, amines, amides, substituted amides, long-chain ketones, and some monoglycerides (Adda and others 1982).

**Chemical anchors for specific flavors or off-flavors in Cheddar cheese.** In the grading and judging of dairy products, the term “unclean” has been used for describing a range of unpleasant flavors that vary both in flavor character and intensity. Unclean-types of flavors in Cheddar may vary from vague, dulling suppression of flavors to several distinct unpleasant flavors such as rose, violet-like, solvent-like, medicinal, phenolic, barny, unclean-utensil-like (Dunn and Lindsay 1985). In many cases of cheeses exhibiting distinct unclean-types of flavors, bitterness was also present. It is important to note that the terms “unpleasant” and “unclean” and “off-flavor” are subjective in nature. Many of these flavors may represent acceptable or desired flavors in Cheddar cheese to certain consumers.

Cheddar cheeses with unclean-type flavors that were described as subtle “floral” or “rose-like” aftertastes were mostly aged. This flavor note is attributed to compounds like phenyl ethanol and phenyl acetaldehyde, which originate from the hydrophobic amino acid, phenylalanine (Phe), through enzyme-mediated transamination, decarboxylation, and reduction reactions (Dunn and Lindsay 1985). Addition of phenyl acetaldehyde (50 to 500 ppb) to clean-flavored mild Cheddar cheese imparted distinct intensities of unclean-rosy taint (Dunn and Lindsay 1985). An astringent, bitter, and stinging sensation was noted at higher concentrations of phenyl acetaldehyde. Unclean-utensil-like off flavor in Cheddar cheese was attributed to increased concentration of p-cresol, 100 to 1320 ppb. The concentration of p-cresol in clean-flavored Cheddar was below 100 ppb (Dunn and Lindsay 1985). The authors also noticed a synergistic enhancement of unclean utensil-

like note by short-chain fatty acids. Additionally, the branched-chain fatty acids, such as isobutyric and isovaleric acids, could also contribute to unclean flavor (Nakae and Elliot 1965a, 1965b). These studies did not utilize defined descriptive sensory analysis to pinpoint specific relationships between compounds. Suriyaphan and others (2001b) used descriptive sensory analysis with instrumental flavor analysis to determine that p-cresol was responsible for "cowy/barny" flavors in aged Cheddar cheese. Cowy/barny flavors would be classified as an unclean-type of flavor in grading terminology.

The presence of branched-chain Strecker-type aldehydes, 2/3-methyl butanal, and 2-methyl propanal, were also hypothesized for unclean flavors (dull harsh) in Cheddar cheese, but only when their concentration in cheese exceeded 200 ppb. Addition of these compounds, below 200 ppb, to mild clean-flavored Cheddar did not cause any flavor defect (Dunn and Lindsay 1985). The lack of defined descriptive sensory analysis in these studies precludes the exact determination of sensory flavors and causant chemicals.

Production of 2/3-methyl butanal by lactic acid bacteria in milk was shown to cause malty flavor, which was considered to be a defect (Miller and others 1974; Morgan 1970a,b; Sheldon and others 1971). Addition of 0.34 ppm of 3-methyl butanal to good quality homogenized milk was enough to develop a malty defect (Morgan 1970a,b). Cheeses made with co-encapsulated cell-free extracts of *Gluconobacter oxydans* (which produces acetic acid from ethanol) and *Streptococcus lactis* subsp. *maltigenes* (which converts leucine to 3-methyl butanal and 3-methyl-1-butanol) in a milk-fat coat exhibited a stronger malty flavor than cheese made with broken capsule or incomplete cell-free extract mixture (Braun and Olson 1986). Distinct malty aroma significance of 3-methyl butanal has already been characterized in cereal products (Grosch and Schieberle 1997) and buckwheat honey (Zhou and others 2002). In recent work, Drake and others (2002b) found high concentrations of 2/3-methyl butanal and 2-methyl propanal in aged Cheddar cheeses with high intensities of nutty flavors. The addition of 2/3-methyl butanal and/or 2-methyl propanal to mild or aged Cheddar cheese resulted in the sensory perception of nutty flavors in these cheeses.

Esters are formed via the esterification of alcohols and free fatty acids. These precursors are present in cheese at various concentrations (Urbach 1997a) and ethyl acetate, ethyl butanoate, and ethyl hexanoate are important volatile components in the cheese flavor spectrum. At low concentrations, the esters contribute to cheese flavor attributes, but high concentrations may cause fruity flavor defects in Cheddar cheese (Bill and others 1965; Morgan 1970a,b; Liu and others 1998). In cheese where high levels of esters gave rise to fruity flavor defects, it was suggested that excessive production of ethanol by starter lactic acid bacteria was responsible (Bill and others 1965; Morgan 1976). Liu and others (1998) suggested that the normal cheese pH (~5.0) and ripening temperature are not critical factors in the ester formation, but NaCl concentration and water activity level are pivotal in determining ester formation.

Mayonnaise/bread-like off-flavor was noted in 33% reduced-fat Cheddar cheese containing soy lecithin (Suriyaphan and others 1999). This off-flavor note was attributed to the chemical agents (E,E)- and (E,Z)-2,4-decadienal. The same authors also noted the formation of off-flavor due to (E,E)- and (E,Z)-2,4-decadienal in a model system composed of pasteurized skim milk fortified with lecithin, fermented by inoculation of *Lactococcus lactis* ssp. *lactis* (Suriyaphan and others 2001a). Pure (E,E)-2,4-decadienal and (E,Z)-2,4-decadienal provide fatty fried and corn chip/stale/hay-like aromas, respectively, but in cheese these compounds give mayonnaise/bread-like off-flavor notes. It has been well documented that the above-mentioned compounds are the decompo-

sition products through oxidation of linoleic acid (Hwang and others 1994, Ho and Chen 1994).

Commercial low-fat Cheddar cheeses were reported to possess a meaty-brothy odor. This has been hypothesized to be due to high concentration of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol™), homofuraneol, and methional (Milo and Reineccius 1997). Sensory studies have not confirmed this observation. Furaneol has been found in cultures of *Lactobacillus helveticus* (Kowalewska and others 1985).

Catty flavors in cheese were caused by 2-mercapto-2-methyl-pentan-4-one (Badings 1967). The development of the odor proceeded through the ripening period and could not be detected before the cheese was about 2 wk old. Thus, a reaction between mesityl oxide and a sulfide-containing group liberated during cheese ripening was hypothesized to be the cause of the odor. Mesityl oxide can be introduced in cheese by varnish used on the racks or it could also be produced from acetone, used in many types of plastic materials (for example, floor coating), in the presence of strong acid or base under heated condition (Steinsholt and Svensen 1979). This flavor was usually absent in normal cheese, but occasionally occurred in cheese (Badings 1967). Spencer (1969a,b) discussed in detail sources of mesityl oxide contamination and prevention of catty taint in foods. Catty flavor and other strong sulfur notes can be prevalent in aged Cheddar cheese (Drake and others 2001, 2002), so most likely pathways other than mesityl oxide contamination are sources of these flavors.

#### Acceleration of Cheddar cheese ripening

Cheese ripening is an expensive and time-consuming process, depending on the variety of cheese (for example, Cheddar cheese typically ripens for 6 to 9 mo, while Parmesan usually is ripened for 2 y. Owing to the cost of cheese ripening, there are obvious economic advantages to be gained by accelerating the process.

A vast amount of literature is available on studies related to acceleration of the cheese-ripening process. From these studies, it is quite obvious that acceleration of cheese ripening involves acceleration of 1 or more of the primary biochemical events occurring during conventional ripening process; namely, glycolysis, lipolysis, and proteolysis. A better understanding of the agents and factors affecting the biochemical reactions will help in better control of an accelerated ripening process, and greater control of ripening may also be gained by manipulating the process whereby end product quality may be predicted with greater certainty. Acceleration of cheese ripening is, therefore, of benefit to the producer from both economic and technological points of view—provided, of course, that the final product has the same flavor profile and rheological attributes as conventional cheese.

Acceleration of glycolysis, which occurs rapidly, is considered to be of no benefit in most, if not all, cheese varieties. Acceleration of lipolysis may be of benefit in Blue- or Italian-type cheeses where lipolysis plays a major role in generating characteristic flavor. The contribution of lipolysis to the flavor of Cheddar or Dutch cheeses is unclear, and acceleration of lipolysis in these types is not usually undertaken as a means of enhancing flavor development. But many of the commercially available enzyme-modified cheeses which are sold as Cheddar cheese flavors and flavor enhancers contain lipases. Proteolysis occurs in all varieties and is considered to be a prerequisite for good flavor development.

The main methods used for accelerating cheese ripening may be summarized as:

- Elevated ripening temperature
- Modified starters
- Cheese slurries
- Adjunct nonstarter lactic acid bacteria
- Exogenous enzymes

In most, if not all, of the studies published on the acceleration

of Cheddar cheese ripening, the workers did not study in detail the effect on volatile composition. The aroma of these cheeses was only characterized by sensory analysis by a trained panel of cheese graders. Details on various acceleration mechanisms employed were reviewed exhaustively by Fox (1988/1989); Fox and others (1996a,b); El-Soda (1997); Klein and Lortal (1999); Wilkinson (1999); and Law (2001).

#### Instrumental analysis of Cheddar cheese flavor

Because of excellent separation efficiency and versatility, gas chromatographic methods have found growing acceptance and application in food science and technology for the separation of a wide variety of compounds. A product cannot be generally injected directly onto a GC system without some sample preparation. Separating volatile compounds from the matrix is required to avoid degradation and formation of artifacts due to the high temperatures in the injection port, and also to permit concentration of the components until a desirable detectable limit is reached. Therefore, sample preparation or volatile isolation is the first problem to solve in a flavor research project.

The sample preparation for flavor analysis is complicated and pushes the flavor researcher to deal with certain factors to produce meaningful and reproducible results. The concentration level of volatile compounds is as low as  $10^{-8}$  to  $10^{-14}\%$  in the food, thus it is necessary to isolate and concentrate the components. The aroma composition is generally very complex, covering the range of polarities, solubilities, pHs, functional groups, vapor pressures, and volatilities. Instability to certain conditions such as oxygen, light, heat, and/or pH, and the complexity of the food matrices with which the aroma compounds interact, are other important factors one must consider in preparation of flavor extracts. Weurman (1969) emphasized that more than one procedure may be required for optimum recovery of flavor compounds.

A number of sample preparation and novel analytical methods for the analysis of flavors and off-flavors were recently reviewed by Wilkes and others (2000) and Stephan and others (2000). The most common sample preparation methods used for cheese volatiles are reviewed below.

**Solvent extraction and distillation.** Solvent extraction typically involves the use of an organic solvent; for example, acetonitrile (Wong and Parks 1968; Vandeweghe and Reineccius 1990), pentane (Benkler and Reineccius 1980); dichloromethane (Zehentbauer and Reineccius 2002), or diethyl ether (Milo and Reineccius 1997; Suriyaphan and others 2001b; Zehentbauer and Reineccius 2002). This limits the method to the isolation of fat-free foods, or an additional procedure must be employed to separate the extracted fat; for example, dialysis (Benkler and Reineccius 1979, 1980; Vandeweghe and Reineccius 1990) or low-temperature high-vacuum distillation (Suriyaphan and others 2001b). The main solvent extraction techniques are direct solvent extraction of cheese samples (Benkler and Reineccius 1980; Milo and Reineccius 1997; Suriyaphan and others 2001b; Zehentbauer and Reineccius 2002) or the solvent extraction of aqueous distillate prepared by low-temperature high-vacuum distillation (Suriyaphan and others 1999, 2001a). Solvent extraction has the disadvantage that one is usually faced with the problem of evaporating relatively large quantities of solvent while retaining the volatile flavor components (Libbey and others 1963).

Solvent extraction of Cheddar followed by dialysis against pure solvent using a perfluorosulfonic acid membrane was used in the isolation of volatile aroma compounds (Benkler and Reineccius 1979). Chromatograms of cheese aroma extracts prepared by dialysis were compared with those prepared by steam distillation-extraction. The dialyzed sample showed good recovery of a wide range of compounds, while the distilled sample showed greater recovery of more volatile compounds. Besides, dialysis is a slow

process; some other disadvantages of the method are adsorption of basic components on the membrane surface, catalysis of acetone condensation due to membrane acidity, and differential diffusion rates of compounds across the membrane (Benkler and Reineccius 1980).

A low-temperature high-vacuum distillation technique utilizing a molecular still was described by Libbey and others (1963). The technique was used for isolation of volatile aroma compounds from Cheddar cheese fat, obtained by centrifugation (at 30000 g at 43 °C) and possessing typical Cheddar aroma. Day and Libbey (1964) separated the aroma fraction from the fat of raw-milk Cheddar into 130 components by gas chromatographic separation on capillary column; major components included aldehydes, methyl ketones, primary and secondary alcohols, esters of primary and secondary alcohols and fatty acids, and  $\delta$ -lactones.

Steam distillation followed by solvent extraction is another method that can be employed (Dacre 1955). Among those, the most popular and valuable technique is simultaneous steam distillation extraction (SDE), which employs an apparatus that condenses simultaneously the steam distillate and an immiscible organic solvent (Chaintreau 2001). This method provides near quantitative recoveries of volatiles. But, since this method is performed at elevated temperatures, it can lead to formation of thermal artifacts; for example, methyl ketones during isolation of cheese flavor volatiles. Parliment (1998) described a detailed procedure for isolation and fractionation of volatile aroma compounds from cheese. Aqueous slurry of Swiss cheese was atmospherically steam distilled using a Likens-Nickerson extractor with diethyl ether as solvent. Volatile components in Cheddar, Gouda, Edam, Swiss, and Parmesan cheeses were concentrated by SDE procedure (using modified Likens-Nickerson apparatus) and analyzed by capillary GC (Aishima and Nakai 1987). Stepwise discriminant analysis was applied to the peaks in the resulting chromatogram to objectively classify cheese varieties. Most of the cheese samples were successfully assigned to the correct varieties. Clusters corresponding to young, mild, and old cheeses were observed in a canonical plot based on the GC data of Cheddar samples.

Engel and others (1999) developed a new distillation unit called "solvent assisted flavor evaporation (SAFE)," which is described as versatile and fast for the direct isolation of volatile compounds from complex aqueous food matrices, such as fruit juices, beer, and milk. SAFE is a modification of the traditional high-vacuum technique. For SAFE extraction, the distillation vessel and "transfer tubes" are thermostated at low temperatures (20 to 30 °C) to avoid condensation of compounds with high boiling points, and the sample is added by dropping aliquots from the funnel into the vessel to reduce the time of extraction. The authors reported that this new method allows the use of solvents other than diethyl ether and dichloromethane, and it could be useful for extracts containing high concentrations of saturated fat. It was also demonstrated that the SAFE method resulted in better recovery and yield than the traditional "high vacuum transfer" system for a solution of several selected food aroma compounds dissolved in diethyl ether and containing 50% fat (Engel and others 1999). Distillation methods often produce discrimination effects; that is, highly volatile substances tend to be lost while those with medium- to low-volatility are enriched in the final aroma isolate.

**Headspace methods (HS).** These techniques are frequently divided into static and dynamic headspace. In static headspace, a small sample of the atmosphere around the food is injected directly onto the GC column. It is assumed that equilibrium of the volatile compounds in the headspace and food sample is reached in the vial prior to sampling. This method is satisfactory when rapid analysis is required for some major components (Reineccius 1998).

Manning and Moore (1979) analyzed the headspace sample of a bore hole in a Cheddar cheese block. A new device for head-

space sampling was developed by Price and Manning (1983). This headspace sampling device involved cutting out a plug from a block of cheese which was subsequently extruded in a nitrogen-flushed chamber and, upon equilibration, a headspace sample was drawn for GC analysis. Manning and Robinson (1973) studied the highly volatile aroma compounds in the headspace over a cryotrapped low-temperature-vacuum distillate of Cheddar cheese as it thawed. The above-mentioned 3 methodologies were useful in the analysis of highly volatile sulfur compounds, such as H<sub>2</sub>S, methanethiol, and dimethyl sulfide, which play an important role in Cheddar cheese flavor. A method involving analysis of a headspace gas sample above a steam distillate of Cheddar slurry by capillary GC system was developed by Lin and Jeon (1985).

Dynamic headspace takes larger amounts of headspace sample by sweeping the sample with a flow of carrier gas and concentrating it prior to injection in a cryogenic trap or an adsorbent trap (Reineccius 1998). Dynamic headspace concentration (also known as purge and trap) on Tenax (poly-2,6-diphenyl-p-phenylene oxide) is frequently used for flavor volatile concentration to compare flavor differences among dairy products because it is moderately sensitive, can be performed rapidly, and is not prone to thermally-derived artifacts.

Volatile compounds from a progressively heated Cheddar sample were isolated onto a Tenax trap by a dynamic headspace technique, desorbed by heating, concentrated by cryofocusing, and thermally desorbed onto a GC column and analyzed by multidimensional GC sniff/FID/MS system (Arora and others 1995). Simultaneous sensory analysis and mass spectrometry detection permitted the assignment of aroma descriptors to odor-active components in the complex mixture of volatiles. In total, 5 aldehydes, 6 ketones, 8 alcohols, 3 esters, 11 hydrocarbons, 3 halides, and 3 sulfur compounds were positively identified. However, certain variables in headspace analysis techniques need to be standardized to make the system quantitative. Yang and Min (1994) followed the formation of volatile compounds in Cheddar and Swiss cheeses during ripening for 9 wk. Aroma compounds were adsorbed onto Tenax trap by dynamic headspace method, followed by thermal desorption and analysis by GC.

In an interesting methodology, flavor released from various cheeses in headspace of an artificial mouth model system were analyzed. In the artificial mouth model, cheese and artificial saliva were crushed at 37 °C, resulting in the release of volatile aroma compounds which were purged using nitrogen onto a Tenax trap. Lawlor and others (2002) compared the composition of volatiles from 8 hard-type varieties by using this technique. Flavor attributes were correlated with volatile compounds, free amino acids, free fatty acids, and gross compositional constituents. Volatiles adsorbed on Tenax could also be desorbed by solvent extraction for direct GC analysis. One such method was developed by Olafsdottir and others (1985) which involves simple diethyl ether washing of the Tenax trap for the elution of volatile compounds from cheese.

**Solid-phase micro-extraction (SPME).** SPME is a relatively new solventless isolation method that can be used to extract and concentrate a wide range of volatile compounds from various matrices in a single step (Kataoka and others 2000). This technique was initially developed for sampling organic contaminants in water, but it has also been applied with success to the analysis of volatile flavor compounds in foods and beverages (Kataoka and others 2000). SPME has the potential to reduce the time required for sampling, works well in combination with many separation and detection systems, has relatively low cost, and only requires a small amount of sample (Kataoka and others 2000).

Unlike some of the commonly used methods such as solvent extraction, SDE, conventional solid phase extraction, and purge and trap sampling, quantitative adsorption with SPME is often

very difficult, if not impossible. SPME headspace and liquid sampling were tested for 25 common flavor components in dilute aqueous solution (Yang and Peppard 1994). The addition of salt generally enhances SPME adsorption by salting-out hydrophobic compounds from aqueous solution. Large-sample volume and a smaller headspace volume over liquid samples were shown to increase the sensitivity of SPME analysis. A small-dia GC injector liner (1 mm internal dia) improves resolution and eliminates the need for cryogenic focusing. In one of the 1st applications of SPME to food flavor analysis, the technique was applied to study the volatiles of samples such as ground coffee, fruit juice beverage, and a butter flavor in vegetable oil. SPME headspace sampling was shown to be effective in detecting characteristic components of butter flavor such as diacetyl,  $\delta$ -decalactone, and  $\delta$ -dodecalactone (Yang and Peppard 1994).

Cheddar and Swiss cheese headspace volatile compounds were extracted with SPME fibers, coated with nonpolar polydimethylsiloxane (PDMS) and polar polyacrylate (PA), and thermally desorbed in the injector port for GC analyses (Chin and others 1996). Results with PA-coated fibers were better than those with PDMS-coated fibers. Major cheese volatile components such as fatty acids and  $\delta$ -lactones were readily extracted by both SPME fibers, but minor volatile components such as volatile sulfur compounds were not observed. SPME-GC patterns were distinctly different among the cheese varieties, and characteristic volatile aroma compounds were identified using multivariate techniques. Dufour and others (2001) studied Cheddar cheese flavor by isolation of volatiles in the headspace by SPME combined with GCO and GC-MS. The authors also compared the extraction efficiency of Cheddar cheese volatiles with different SPME fiber coatings; namely PDMS, polydimethylsiloxane/divinylbenzene (PDMS-DVB), PA, carboxen/polydimethylsiloxane (CAR-PDMS), and carbowax/divinylbenzene (CW-DVB). The bipolar coating PDMS-DVB, and to a lesser extent CAR-PDMS, showed the highest selectivity (highest number of peaks adsorbed). In this study, the authors also emphasized the importance of sample temperature and exposure time of fiber. In a similar study, Lecanu and others (2002) compared different SPME fibers for the extraction of headspace volatiles from surface-ripened cheeses.

A new technique using SPME, mass spectrometry, and multivariate analysis (SPME-MS-MVA) was developed for the study of off-flavors in milk (Marsili 1999). In this technique, the analytical GC column of a GC/MS system was replaced with a 1 m deactivated fused-silica column, which served as a transfer line to deliver volatiles isolated from milk samples with carboxen-SPME fiber to the mass spectrometer. Mass fragmentation data resulting from unresolved milk volatile components were subjected to MVA. Principal component analysis based on SPME-MS-MVA provided differentiation of control reduced-fat milk samples from reduced-fat milk samples with various off-flavor notes. This technique could possibly also be employed in rapid analysis of cheese varieties, cheese flavor, or off-flavor notes.

Quantification of trace volatile compounds is more problematic with SPME. Since dairy flavors are present at low concentrations, prolonged heating of samples during isolation to increase flavor volatility and recovery can potentially alter the composition of labile components.

**Analysis of flavor extracts.** Sensory-directed analytical flavor techniques for evaluation of key aroma compounds in cheese and dairy products were exhaustively reviewed by Parliment and McGorin (2000) and McGorin (2001). Gas chromatography-olfactometry (GCO) is a widely used method that works with the separation capacity of the gas chromatography techniques and the sensitivity of the human nose. The past 2 decades have experienced tremendous growth in the development and application of GCO in flavor research (Acree 1993, 1997; Grosch 1993). Nu-

merous GCO techniques are currently in use, with the most popular being the so-called “dilution analysis” methods, which include aroma extract dilution analysis (AEDA) (Grosch 1993), CharmAnalysis™ (Acree 1997), and a recent variation—aroma extract concentration analysis (AECA) (Kerschler and Grosch 1997). In these methodologies, a dilution (or concentration) series of an aroma extract is evaluated by GCO and attempts to rank the key odorants in order of potency. The highest dilution at which a substance is smelled is defined as its flavor dilution factor, which is proportional to the odor activity value of the compound. In recent work on Cheddar cheese flavor characterization, various studies performed AEDA (Christensen and Reineccius 1995; Milo and Reineccius 1997; Suriyaphan and others 2001b; Zehentbauer and Reineccius 2002).

A major limitation of such dilution techniques is that they do not account for highly volatile compounds that are lost during concentration of aroma extracts by distilling off the solvent. Consequently, the odor potency of highly volatile compounds can be underestimated by GCO in comparison with the odor activity of these compounds. Another disadvantage of the dilution methods is the great duration necessary to complete the required sniffing.

AECA could be a milder alternative to AEDA (Kerschler and Grosch 1997; Grosch and others 2001). This procedure starts with GCO of original extract from which the nonvolatile components have been removed. Then the extract is concentrated stepwise by distilling off the solvent and, after each step, an aliquot is analyzed by GCO. Headspace GCO techniques, such as GCO of decreasing headspace volumes (GCO-H) (Milo and Grosch 1995) and dynamic headspace volumes (GCO-DHS) (Cadwallader and Baek 1998) have been used to augment the data from AEDA to provide a more complete evaluation of food aroma composition.

Both AEDA and Charm methodologies originally proposed that the larger the flavor dilution value, the greater the potential contribution of that compound to the overall aroma. With time, data interpretation has changed. Researchers now consider AEDA, OAV, and Charm methodologies to be screening in nature. These methodologies are used to determine which aroma compounds will “most likely” make a contribution to the odor of a food, recognizing that sensory work (for example, recombination studies) needs to be done to determine which aroma compounds are truly contributory (Audouin and others 2001).

Odor properties detected during GCO can also be used to characterize aroma components along with their chromatographic properties. It is important to consider that aroma-active compounds usually do not correspond to the major volatile components because of the variability of their odor thresholds. In general, the aroma quality of a volatile component combined with more than one RI value (on different GC stationary phases) is considered as equivalent to identification by GC-MS. This is very useful for the identification of odorants with very low thresholds at very low concentrations (Rychlik and others 1998).

One of the best methods of qualitative/quantitative analysis is the combination of gas chromatography with mass spectrometry. A chromatographic parameter that can be used for qualitative analysis is the retention time. However, the retention index (Kovats) (RI) has become the standard method for reporting GC data. To identify unknowns, it is necessary to combine chromatography and mass spectrometry (Rychlik and others 1998).

## Conclusions

The biogenesis of Cheddar cheese flavor is a complex and slow process. General biochemical pathways; for example, glycolysis, lipolysis, and proteolysis involved in the degradation of milk constituents during cheese ripening, are now fairly well characterized.

Recent works on the enzymology and genetic manipulation of the starter and nonstarter lactic acid bacteria have helped in the better understanding of further catabolic modification of the products of primary degradation pathways. So far, a large number of volatile compounds have been characterized from Cheddar, but still it is not possible to duplicate Cheddar flavor by pure chemicals in cheese model systems. There is now good understanding of the causes of bitterness and specific flavors/off-flavors in Cheddar cheese. Developments in sensory and instrumental methodologies in flavor analysis have been of immense help in the understanding of Cheddar cheese flavor chemistry. Further work on the characterization of flavor compounds, volatile flavor compounds-matrix interaction mechanisms, and flavor release mechanisms are needed to fully elucidate Cheddar flavor. The better understanding of Cheddar cheese flavor chemistry will be useful in the development of new technology/mechanisms for the effective control and acceleration of the ripening process.

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