

# Flavour Compounds in Cheese

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We take for granted the familiar flavours of our favourite cheeses because we do not need to concern ourselves with their origin or composition. However, the flavourist or food technologist seeking to control or simulate cheese flavours is only too aware that their development is extremely complex in most varieties. It depends on interactions between metabolic pathways, individual enzyme reactions and non-enzymic reactions, all taking place in the presence of a myriad of substrates, and often in the presence of an ever-changing population of microorganisms. The microbiology of cheese manufacture, and the general mechanisms of flavour development in cheese

have been reviewed recently,<sup>1-3</sup> but a summary of the factors which determine the overall properties of different cheese varieties is included here for those unfamiliar with the subject.

The vast range of cheese varieties can be grouped according to their moisture content and the complexity of their microfloras (Table I.) For example, soft cheeses have high (50-80%) water content and may be classed either as unripened (e.g., cottage cheese), with a simple mesophilic lactic acid bacterial flora (the acid-producing starter bacteria used to make the product), or as ripened, with a similar basic flora but with a surface mould growth which contributes the flavour compounds characteristic of the mature

Table I. Major Flavour Compounds Formed in Cheeses

	<u>From fermentation</u>	<u>From maturation</u>
<u>Soft Cheeses</u>		
Cottage (unripened)	lactic acid diacetyl	
Camembert, Brie (surface-ripened)	lactic acid, diacetyl	amino acids, ammonia, fatty acids, alcohols, ketones, phenols, aromatic hydrocarbons, methanethiol, bis (methylthio) methane, oct-1-en-3-ol, phenylethanol
<u>Semi-Hard Cheeses</u>		
Caerphilly Limburg (surface ripened)	lactic acid, diacetyl lactic acid	? amino acids, volatile fatty acids, ammonia, methanethiol, acetyl methyl disulphide
<u>Hard Cheeses</u>		
Cheddar	lactic acid, diacetyl, acetic acid	amino acids, amines, volatile fatty acids, pentanone, hydrogen sulphide, methanethiol
Emmental, Gruyere	lactic acid, acetic acid, propionic acid	amino acids (esp. proline), peptides, butyric acid, methanethiol, thio-esters, dimethyl sulphide, alkyl pyrazines
Blue-vein		volatile fatty acids, ketones, amino acids, lactones, aromatic hydrocarbons, oct-1-en-3-ol
Italian		volatile fatty acids, amino acids, alcohols, ketones

cheese (e.g., Camembert after 6-8 weeks' storage).

Semi-hard varieties are made with similar mesophilic starters but the manufacturing process is more complex and includes a short curd cooking stage to reduce its moisture content to approximately 45% and render it firmer. Some varieties which are salted internally develop little flavour beyond that of fresh curd, though texture softens due to protein breakdown (e.g., Caerphilly). Others are salted by immersion of the lightly pressed curd block in brine and subsequently by rubbing of the surface with salt or a cloth soaked in brine. This treatment results in the development of a characteristic surface flora of yeasts and bacteria (Limburg is a classical example of this type).

The hard cheeses (approximately 40% moisture or less) fall into three major categories. Those with relatively simple microfloras resemble Cheddar and are made with the same mesophilic starters used in soft and semi-hard cheese. Subsequent bacterial growth in the cheese is limited by the conditions of acidity, salt concentration and redox potential so that full, mature flavour may take up to twelve months to develop.

The second group differs from the first by being inoculated with mould spores which germinate when air is admitted to the cheese by "spiking." The metabolism of the growing mycelium, and later of the spores, generates the flavour and aroma compounds characteristic of the cheese; Stilton, Danish Blue, Roquefort and Gorgonzola are examples of this type.

Emmental and Gruyère form a third group. They are distinguished both by the thermophilic starters used to make them, and by the subsequent growth in the cheese of propionic acid bacteria which not only contribute to flavour development but also produce the gas-filled "eyes" characteristic of this cheese. Gruyère "mountain cheese" varieties also have a surface flora of yeasts and bacteria which add to their basic flavour and aroma.

Italian hard cheeses form a special group in which added mammalian lipases are used to develop strong fatty acid (rancid) flavours. Davis has reviewed the history of cheesemaking and the modern manufacturing methods used for different types.<sup>4</sup>

This article considers recent advances in our understanding of the nature of flavour compounds themselves, and how they may contribute to the organoleptic properties of natural cheese and artificial cheese flavour formula-

tions. A consideration of the literature from the last twenty-five years shows that sophisticated analytical techniques (chiefly GC and MS) have revealed the presence of a wide range of potentially flavourful compounds in cheeses. These include the following categories: volatile and nonvolatile fatty acids, alcohols, esters, lactones, ketones, aldehydes, hydrocarbons, pyrazines, peptides, amino acids, amines and sulphur compounds.

Although "cheesy" aromas and flavours can be imparted to fresh curds or other foods using formulations of such compounds, the faithful reproduction of the characteristic flavours of individual varieties is extremely difficult and, in some cases, impossible. This situation can be explained by invoking the Component Balance Theory which states that cheese flavour is composed of a number of different compounds which must be present in the correct proportions to give a balanced flavour.<sup>5,6</sup> Unfortunately the theory is too all-embracing to be practically useful in designing flavour mixtures which could lead to its experimental verification.

### Unripened Cheese

Cottage cheese, a typical unripened cheese, is made using *Streptococcus cremoris*, *lactis* or *diacetylactis* in the acidifying starter culture. The metabolism of these organisms not only produces the lactic acid which gives the product its sharp flavour, but it also generates diacetyl from milk citrate which imparts the characteristic fresh "buttery" flavour to the cheese. The enzymic reactions leading to diacetyl are now well known (fig. 1) but other less desirable products also result from citrate metabolism. For example, acetaldehyde can be formed in excess by some starters and causes a harsh, "green" flavour. Ratios of diacetyl to acetaldehyde should be between 5:1 and 3:1 for balanced cottage cheese flavour.<sup>7</sup> This can be achieved even with high acetaldehyde producers by including an acetaldehyde-utilizing strain of *Leuconostoc* in the starter culture.<sup>8</sup>

Carbon dioxide is another undesirable but inevitable by-product of diacetyl synthesis which can cause floating curds and excessive fragmentation of the curd particles. Most manufacturers now use non-citrate fermenters (*Str. lactis* or *cremoris*) to form the curd, then give it flavour by applying a dressing of cream previously cultured with *Str. diacetylactis* or *Leuconostoc* sp.

Difficulties in achieving consistent flavour production from cottage cheese starters have lead some investigators to look for synthetic



General flavour characteristics common to many surface ripened varieties have been attributed to 3-methyl-1-butanol, phenylethanol and phenol.<sup>15</sup> These are found in Camembert, but specific flavour characteristics are thought to be conferred by oct-1-en-3-ol and by complex sulphur compounds. Some of these are probably produced directly from amino acid metabolism; for example, 3-methylthiopropanol, methional and methanethiol are possible products of methionine. Non-enzymic reactions are important in producing additional compounds such as bis(methylthio) methane (methanethiol + formaldehyde) and thioesters of short chain fatty acids, though there is recent evidence for microbial formation of the latter.<sup>16</sup> Musty, earthy flavour notes in both Camembert and Blue cheeses appear to be due to aromatic hydrocarbons of the alkyl and alkenyl benzene types, produced via unknown pathways.

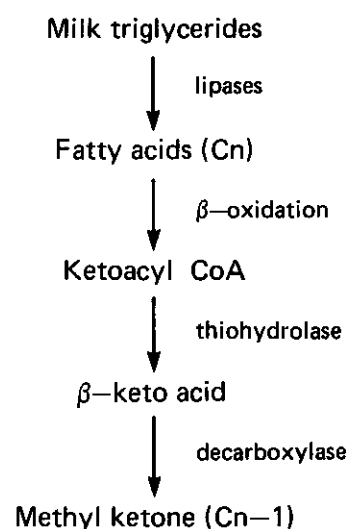
### Semi-hard Cheeses

This category covers a wide range of varieties from the relatively bland, acid-flavoured English Territorials (e.g., Caerphilly, Cheshire) to the very strongly flavoured "surface smear" cheeses such as Limburg and Bel Paise. The distinguishing characteristic of the surface smear cheeses lies in their initial flora of lactate-utilizing yeasts which raises the pH and provides growth factors for the multiplication of yellow/orange pigmented strains of *Brevibacterium linens*. This organism is very proteolytic<sup>17</sup> and can break down methionine to methanethiol<sup>18</sup> to give the characteristic putrid smell of the cheese surface. Katz and coworkers also claimed that sulphides of carbonyl compounds (e.g., acetyl methyl disulphide) have aromas characteristic of Limburg cheese and described how they can be synthesized.<sup>19</sup>

### Blue-vein Cheese

This category is typified by Danish Blue, Stilton, Roquefort and Gorgonzola. Their curds are inoculated with spores of *Penicillium roqueforti* which germinate in the cheese when air is admitted by "spiking" with wires or metal rods. As it grows within the body of the cheese, the mould produces fatty acids and methyl ketones which provide the overriding flavour notes. The rate of fatty acid release by *Penicillium* lipases governs the rate of ketone formation.<sup>20</sup>

The ketones are derived from fatty acids via partial  $\beta$ -oxidation (fig. 2). The  $\beta$ -ketoacyl CoA formed by enzymic dehydrogenation of the fatty



n = 6, 8, 10 or 12

**Figure 2. Formation of methyl ketones by mycelium and spores of penicillium roqueforti**

acid  $\beta$ -hydroxyacyl CoA derivatives is deacylated by thiohydrolase to form free  $\beta$ -keto acid. The decarboxylase which then catalyses the formation of corresponding methyl ketones is most active in mycelia and  $\beta$ -ketolauric acid (yielding 2-undecanone) is its preferred substrate, but the preponderance of heptan-2-one in cheese is due to the preference of the preceding thiohydrolase for  $\beta$ -keto-octanyl CoA. Roquefort cheese is traditionally made with ewe's milk and is said to lose some of its characteristic flavour notes if made with cow's milk. Hall and Kosikowski suggested that the relatively high levels of caproic acid and nonan-2-one in the ewe's milk cheese were responsible for the difference.<sup>21</sup>

In addition to its role in producing fat-derived flavours, *P. roqueforti* also produces proteinases and peptidases whose concerted action generates high levels of peptide and free amino acid N. The amino acids can reach concentrations as high as 10% of total cheese N and they buffer the cheese to approximately pH 6.5, aiding other enzyme activities and providing background flavour.<sup>20</sup>

Flavour simulation studies suggest that  $\delta$ -lactones contribute to cheese flavour.<sup>22</sup> Jolly and Kosikowski increased the quality of Blue cheese flavour by increasing the concentrations of  $\delta$ -tetradecalactone and  $\delta$ -dodecalactone.<sup>23</sup> Two mechanisms for  $\delta$ -lactone formation are possible. Traces of  $\delta$ -hydroxy acid may be present in milk

glycerides and they can be released by lipases during cheese ripening. The free acids may spontaneously undergo ring closure to form lactones, or they may be converted enzymically.<sup>24</sup> Alternatively, esterified  $\delta$ -keto acids in milk glycerides may be released by lipases, reduced to hydroxy-acids, then converted to lactones. Boldingh and Taylor cited examples of yeasts and moulds which could produce lactones by this pathway.<sup>24</sup>

Blue cheese flavours for addition to other foods (e.g., salad dressings, cheese dips) can be formulated from saturated aliphatic fatty acids ( $C_4$ - $C_{10}$ ), lactones ( $C_4$ - $C_{22}$ ), phenol and methyl ketones ( $C_2$ - $C_{13}$ )<sup>25</sup> or generated rapidly by fermentation of lipolysed fats (milk fat, maize oil or coconut oil) with *P. roqueforti*.<sup>26-28</sup> Ney and coworkers claimed that the mushroom-flavoured volatile, oct-1-en-3-ol enhanced the flavour of Blue cheese formulations.<sup>29</sup>

### Hard Cheeses

The three principal types of hard cheeses are represented by Cheddar, Emmental and Italian hard cheese. Cheddar is made with the same mesophilic starters used for the other varieties discussed so far, but thermophiles must be used for the high-cook Emmental and Italian cheeses. Strains of *Str. thermophilus* and *Lb. bulgaricus*, *helveticus* or *casei* are most commonly used.

### Cheddar Cheese

The flavour of Cheddar cheese is the most difficult to describe and identify despite several decades of intense research effort by bacteriologists, chemists and biochemists to elucidate the pathways and end products involved in its formation. The flavour of very young Cheddar cheese is similar to that of other internally-salted varieties made with mesophilic starters; it can be described as acid, slightly buttery and salty. The flavour compounds at this stage of manufacture are largely derived from the carbohydrate fermentation of the starter streptococci; these organisms are regarded by taxonomists as homofermentative with lactose and they produce mainly lactic acid. However, they do possess alternative pathways of pyruvate metabolism which are expressed in the cheese vat to a degree which allows production of acetic acid, ethanol and acetaldehyde (fig. 1 and Law 1981—review<sup>2</sup>). The importance of these pathways was demonstrated by Czulak and coworkers who showed that pyruvate dehydrogenase activity (a key step in diverting pyruvate to the flavourful metabolites) in starter streptococci

was inhibited in cheese made with milk containing high levels of polyunsaturated fat.<sup>30</sup> The cheeses were low in acetate, acetaldehyde and diacetyl and had rather bland flavour.

The important role of diacetyl in Cheddar flavour aroma is supported by the analytical work of Manning and Robinson who identified it as one of eight compounds which contributed to typical aroma in low-boiling Cheddar cheese distillates.<sup>31</sup> However, acetic acid has a less clear function since its concentration can vary considerably between cheeses without concomitant variability in the quality or intensity of typical flavour.<sup>32</sup> It probably adds to the sharp mouth-feel of cheese conferred by the high lactic acid concentration, but overproduction of acetic acid can lead to vinegar-like off-flavours. Claims that ratios of acetic acid to other fatty acids are important determinants of Cheddar flavour<sup>33</sup> have not been confirmed.<sup>32</sup>

Amounts of volatile fatty acids other than acetic increase during Cheddar cheese maturation due to the weak esterase and lipase activities of the milk flora and the starter bacteria.<sup>34</sup> Although these volatile fatty acids are included in many synthetic cheese flavour formulations,<sup>25,35</sup> evidence for their contribution to typical Cheddar cheese aroma/flavour is equivocal and contradictory. Studies with enzyme-modified cheese suggest that increased free volatile fatty acids in lipase-treated American Cheddar increase its flavour intensity, provided that high rancidity-inducing lipase levels are avoided.<sup>36,37</sup>

The normal levels of fatty acids found in cheese (approximately 500 ppm) represent amounts well above typical flavour and aroma thresholds, ranging from 0.3-100 ppm<sup>38</sup> so that they would be expected to contribute to the overall organoleptic qualities of Cheddar cheese. However, claims that fatty acids are important because low-fat or fat-free cheeses do not develop flavour<sup>33</sup> are oversimplistic since deviations from an optimum fat percentage in the product alters its characteristics markedly and may have indirect effects on flavour retention and perception. Fat-free "Cheddar" is so unlike normal Cheddar as to be irrelevant to the discussion.

Patton claimed that volatile fatty acids ( $C_2$ - $C_8$ ) were the "backbone" of Cheddar aroma because blocking agents for carboxylic functional groups impaired the aroma of cheese fat distillates.<sup>39</sup> However, Manning and Price argued that side-reactions could have occurred in these experiments which would leave the results open to different interpretations.<sup>40</sup> These investigators

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showed that the removal of volatile fatty acids from Cheddar cheese head space did not affect its aroma at all and concluded that these acids were only important in the background taste of the cheese. Further evidence against the importance of fatty acids comes from the analysis of New Zealand Cheddars of which twenty-three out of forty-one contained no acids higher than C<sub>4</sub>.<sup>41</sup> At present we can only conclude that mixtures of alkanolic acids with carbon chains from C<sub>2</sub> to C<sub>8</sub> or C<sub>10</sub> can impart cheese-like flavours either to naturally maturing cheese or in flavour mixtures for process cheese, but that their contribution to the aroma and the special character of Cheddar cheese is unproven.

Other fat-derived flavour compounds implicated in Cheddar flavour include ketones and lactones. The odd-numbered methyl ketones do not appear to be vital flavour compounds since they are absent from mature flavoured experimental cheeses made with only starter bacteria.<sup>32</sup> Pentanone concentrations in normal cheese are a good index of cheese age,<sup>42</sup> but the compound is not necessarily involved in flavour. Butanone is normally present in Cheddar cheese and was cited by Scarpellino as a component of desirable flavour,<sup>43</sup> but it is never found at concentrations higher than its threshold<sup>44</sup> and tends to disappear as cheese ages.<sup>45</sup>

Although lactones have been shown to improve Blue cheese flavour, their contribution to Cheddar flavour is less clear and no definitive evidence exists linking lactone concentrations in maturing cheese with flavour quality or intensity. However, they are regarded as important by some flavourists, as evidenced by their inclusion in synthetic cheese flavour formulations.<sup>25,46</sup>

The proteins of fresh curds (mainly caseins) provide a second source of flavour compounds as they are degraded slowly by the proteinases and peptidases of the starter bacteria. Many of the individual free amino acids released by this process have distinctive tastes and their predominance in certain foods is thought to confer characteristic flavour (e.g., methionine in uni, glycine in crab<sup>47</sup>) but it is generally accepted that their role in Cheddar cheese is one of conferring a savoury background flavour as a complex mixture.<sup>48,49</sup>

Peptides are often cited rather loosely as contributing various flavour notes to cheese, but evidence is not definitive and the range of flavours associated with synthetic peptides (sour, bitter, tasteless) is hardly the basis for high expectations of this class of compounds. Nevertheless, the fact remains that it is possible

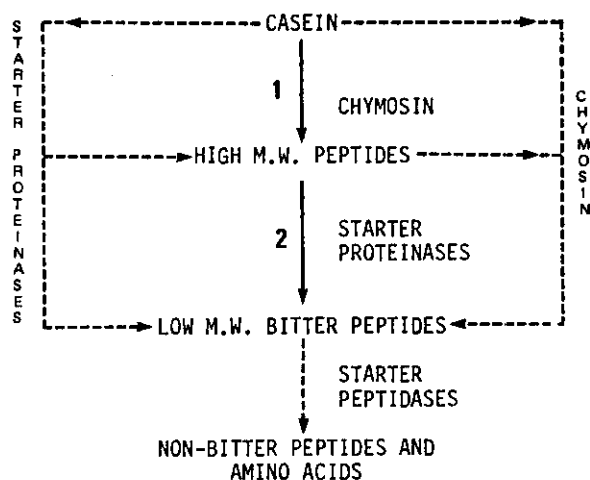
to intensify flavour and/or accelerate flavour development in Cheddar cheese by adding exogenous proteinases to speed up casein breakdown.<sup>37,50</sup>

The correlation between increased typical flavour intensity and increased proteolysis only holds good over a limited range and the choice of proteinase type is critical. It is the author's experience that neutral proteinases are ideal and that acid proteinases produce excessive amounts of bitter peptides even at low concentrations in cheese.

There are two possible reasons for the intensification of cheese flavour through progressive proteolytic action. First, the direct result of increasing free amino acid concentrations in cheese is to increase its savoury taste and provide substrates for the release of volatile sulphur compounds. The importance of such compounds has long been suspected and their unique position within the wide spectrum of Cheddar flavour volatiles was demonstrated by McGugan and coworkers.<sup>51</sup> They showed that both flavourless cheese produced without starter bacteria and mature normal cheese contained the same neutral volatile compounds in similar quantities, with the notable exception of sulphur compounds.

Later, in a series of papers beginning in 1973, Manning accumulated evidence suggesting that methanethiol (derived from methionine by nonenzymic reactions) was the key compound in Cheddar aroma (see Law 1981 for review<sup>2</sup>). Hydrogen sulphide was also considered important but its concentration was not critical unless very large amounts were present, when "sulphide" flavour defects became noticeable. Attempts to synthesize Cheddar aroma with these compounds have been unsuccessful probably because their extreme volatility causes their concentrations in synthetic mixtures to change rapidly and become unbalanced; the individual aromas of both compounds are extremely unpleasant. Presumably the matrix of cheese is such that these volatiles are bound or dissolved and they are only released when the cheese is masticated.

McGugan and coworkers suggested that the binding equilibria of flavour and aroma compounds to cheese proteins was an important factor in determining overall cheese flavour intensity.<sup>52</sup> These investigators suggested that the correlation between flavour intensity and proteolysis may be due to the progressive weakening of flavour binding as cheese proteins are degraded to smaller fragments.



**Figure 3. Lowrie and Lawrence (1972) model for bitterness development in Cheddar cheese. Bold arrows indicate the important stages (1 and 2). Broken lines indicate stages of lesser importance.**

Proteolysis in cheese has an important negative influence on flavour quality, related to the caseinolytic action of the starter streptococci and the residual rennet. The bitter defect in cheese is caused by an accumulation of peptides containing a high proportion of hydrophobic side chains.<sup>53-55</sup> Opinions differ as to the importance of proteolysis by mesophilic starters in the production of bitter defects in cheese. Early hypotheses suggested that bitter peptides were produced by chymosin and that the so-called "bitter" starters were those which had insufficient peptidase activity to break down the bitter peptides to nonbitter peptides and amino acids.<sup>56</sup>

The situation is more complex. While it is true that chymosin produces bitter peptides from casein, the starter proteinases can also do this and, indeed, can produce small bitter peptides from nonbitter, casein derived peptides (fig. 3).<sup>57,58</sup> It is suggested that this latter process was the single most important determinant in bitterness development, and that "fast" starters which multiplied at relatively high cooking temperatures during Cheddar manufacture were the most likely to give bitter cheese, simply because the resultant high cell numbers contributed large quantities of bitter peptide-producing proteinases.

Lowrie and coworkers supported this hypothesis with experimental evidence that bitter starters could be made to produce non-bitter cheese if their number in curds were restricted by controlled bacteriophage infections or by higher cooking temperatures.<sup>58</sup> Conversely

the slow nonbitter starters made bitter cheese if they were allowed to multiply to high cell numbers by altering the manufacturing process. Direct evidence for the involvement of starter cell wall proteinases in bitterness development was provided recently by the observation that proteinase-deficient variants of "fast" starters produce less bitterness in cheese than their parent strains even when total starter cell populations are high.<sup>59</sup>

The factors controlling bitter defects in Gouda cheese appear to be more complex, since the starters generally reach high populations in curds at the relatively low cooking temperatures used for this variety. Stadhouders and Hup showed that factors influencing the retention of chymosin in Gouda curd (e.g., cooking temperature, initial milk pH) also influenced the tendency of the cheese to become bitter.<sup>60</sup> They emphasized that some starter strains produce more bitter peptide-degrading peptidases than others. It is not known whether these are specific peptidases confined to nonbitter stains or general peptidases present at different levels. Chiba and Sato identified both di-peptidase and amino-peptidase activity in debittering fractions of cell-free extracts from starter streptococci but individual enzymes were not isolated.<sup>61</sup> It appears, then, that proteolysis by mesophilic starters is important in producing the bitter defect in cheese but its contribution is different depending on the cheese variety in question.

The fruity defect is relatively common in Cheddar cheese and although it can be pleasant at low intensities it usually becomes unpleasantly strong in mature cheese. The defect occurs in cheeses which contain high ethanol levels (147-1527 ppm).<sup>62</sup> Esterase activity in the cheese catalyses the reaction between volatile fatty acids and ethanol to produce esters whose flavour/aroma is reminiscent of pear drops. Ethyl butyrate and ethyl hexanoate (caproate) appear to be the main fruity esters in Cheddar cheese.<sup>62,63</sup> The available microbiological evidence suggests that the fruity defect can be caused by the starter streptococci themselves, by heterofermentative nonstarter lactic acid bacteria, or by enzymes from psychrotrophic bacteria.

### Emmental and Gruyère

These varieties are manufactured using thermophilic starters for acid production. The typical holes in the cheese are produced as a result of CO<sub>2</sub> formation from lactic acid by propi-

onicbacteria growing in the cheese. Biede and Hammond identified three major flavour fractions in mature cheese:

- water-soluble volatiles (acetic, propionic, butyric acid and diacetyl) giving the basic sharpness and general cheesy note
- water-soluble nonvolatiles (amino acids, especially proline, peptides, lactic acid, salt, CA<sup>2+</sup> and Mg<sup>2+</sup>) providing a mainly sweet flavour note
- oil-soluble fraction (short chain fatty acids other than those in the water-soluble volatile fraction)<sup>64,65</sup>

This last fraction contained "nutty" flavour notes thought to be due to alkyl pyrazines.<sup>66,67</sup> The sweet Emmental-like flavour can be produced in Cheddar cheese by including a proline-producing strain of *Lb. bulgaricus* in the starter.<sup>68</sup> Mitchell produced an Emmental-like flavour in processed cheese spread using 500 ppm acetic acid, 5000 ppm propionic acid and 1200-1500 ppm proline but the flavour was not that of high quality cheese.<sup>69</sup>

The flavour of Gruyère cheese is augmented by the growth of an aroma-producing surface flora of yeasts, micrococci and *B. linens*. Their concerted action produces methanethiol and thioesters of acetic and propionic acid which together can account for the mildly putrid cheese aroma of this variety.<sup>16,70</sup>

Many more volatiles are found in this variety (alcohols, carbonyls, hydrocarbons, alkyl pyrazines) whose additional effects on typical flavour and aroma are impossible to discount or verify at present. However, a Gruyère aroma defect likened to musty potatoes has been identified as emanating from one alkyl pyridine, 3-methoxy-2-propylpyridine, formed from amidated valine and glyoxal by condensation.

### Italian Hard Cheeses

These varieties are typified by Provolone, Romano, Grana and Fontina. They are low moisture cheeses produced by high cook processes using thermophilic starters. Although there are differences in flavour and texture between them, the general common flavour note is the "piquant" aromatic aroma/taste conferred by volatile fatty acids in relatively high concentrations. This is achieved traditionally by using lipase-containing rennet paste or pre-gastric lipase from sheep. More recently, fungal sources of lipases have been investigated as alternatives.<sup>71</sup>



Artificial "Italian cheese" flavour can be imparted to fresh curds or processed cheese by adding  $\beta$ -phenyl propionic acid (100 ppm) and iso-valeric acid (20-300 ppm), or a mixture of butyric acid, caproic acid and caprylic acids (600-10,000 ppm) to give Fontina or Provolone flavour respectively.<sup>72,73</sup>

### References

1. D. A. Forss, *J. Dairy Res.* **46**, 691 (1979)
2. B. A. Law, *Dairy Sci. Abs.* **43**, 143 (1981)
3. B. A. Law, in *Economic Microbiology* Vol. 7, A. H. Rose, Ed., Academic Press, London (1982; in press)
4. J. G. Davis, in *Cheese* Vol. III (1976) Churchill, London
5. H. Mulder, *Neth. Milk Dairy J.* **6**, 157 (1952)
6. F. V. Kosikowski and G. Moquot, in *Advances in Cheese Technology* F.A.O. Agric. Stud. No. 38 (1958)
7. R. C. Lindsay, E. A. Day and W. E. Sandine, *J. Dairy Sci.* **48**, 863 (1965)
8. T. W. Keenan, R. C. Lindsay and E. A. Day, *Appl. Microbiol.* **14**, 802 (1966)
9. P. F. Fox, *Dairy Sci. Abs.* **40**, 727 (1978)
10. R. C. Lindsay, E. A. Day and L. A. Sather, *J. Dairy Sci.* **50**, 25 (1967)
11. W. J. Bergemann and P. D. Harkes, *Neth. Pat.* 7,305,105 (1973)
12. M. Moinas, M. Groux and I. Horman, *Le Lait* **53**, 601 (1973)
13. M. Moinas, M. Groux and I. Horman, *Le Lait* **55**, 414 (1975)
14. J. P. Dumont, S. Roger, P. Cerf and J. Adda, *Le Lait* **54**, 501 (1974)
15. J. P. Dumont, S. Roger and J. Adda, *Le Lait* **56**, 595 (1976)
16. A. Cuer, G. Dauphin, A. Kergomard, J. P. Dumont and J. Adda, *Agr. Biol. Chem.* **43**, 1782 (1979)
17. H. Foissy, *Milchwissenschaft* **33**, 221 (1978)
18. M. E. Sharpe, B. A. Law, B. A. Phillips and D. G. Pitcher, *J. Gen. Microbiol.* **101**, 345 (1977)
19. I. Katz, A. O. Pittet, R. A. Wilson and W. J. Evers, *U.S. Pat.* 4,045,587 (1977)
20. J. E. Kinsella and D. Hwang, *Biotechnol. Bioeng.* **18**, 927 (1976)
21. R. Hall and F. V. Kosikowski, XVIII Int. Dairy Congress IE, 385 (1970)
22. N. P. Wong, R. Ellis, D. E. La Croix and J. A. Alford, *J. Dairy Sci.* **56**, 636 (1973)
23. R. C. Jolly and F. V. Kosikowski, *J. Agric. Fd. Chem.* **23**, 1175 (1975)
24. J. Bolding and R. J. Taylor, *Nature (London)* **194**, 909 (1962)
25. G. J. Henning, *U.S. Pat.* 3,520,699 (1970)
26. B. K. Dwivedi and J. E. Kinsella, *J. Food Sci.* **39**, 620 (1974)
27. F. V. Kosikowski and R. C. Jolly, *U.S. Pat.* 4,133,895 (1979)
28. B. J. Amu and B. Jarvis, *Brit. Pat.* 1,361,817 (1974)
29. K. H. Ney, I. Poetoe, G. Wirotama, G. Freytag, *U.S. Pat.* 3,865,952 (1975)
30. J. Czulak, L. A. Hammond and J. F. Horwood, *Australian J. Dairy Tech.* **29**, 124 (1974)
31. D. J. Manning and H. M. Robinson, *J. Dairy Res.* **40**, 63 (1973)
32. B. A. Law, M. J. Castañón and M. E. Sharpe, *J. Dairy Res.* **43**, 117 (1976)
33. J. A. Ohren and S. L. Tuckey, *J. Dairy Sci.* **52**, 598 (1969)
34. J. Stadhouders and H. A. Veringa, *Neth. Milk Dairy J.* **27**, 77 (1973)
35. K. H. Ney, I. P. G. Wirotama and W. G. Freytag, *Neth. Pat.* 7,204,792 (1972)
36. F. V. Kosikowski and T. Iwasaki, *J. Dairy Sci.* **58**, 963 (1975)
37. V. K. Sood and F. V. Kosikowski, *J. Dairy Sci.* **62**, 1865 (1979)
38. R. E. Baldwin, M. R. Cloninger and R. C. Lindsay, *J. Food Sci.* **38**, 528 (1973)
39. S. Patton, *J. Dairy Sci.* **46**, 856 (1963)
40. D. J. Manning and J. C. Price, *J. Dairy Res.* **44**, 357 (1977)
41. R. C. Lawrence, *New Zealand J. Dairy Sci. Tech* **2**, 55 (1967)
42. D. J. Manning, *J. Dairy Res.* **46**, 523 (1979)
43. R. J. Scarpellino, *Dis. Abs.* **22**, 421 (1961)
44. J. E. Kinsella, *Chem. Ind. (London)* **2**, 36 (1969)
45. A. R. Keen, N. J. Walker and M. F. Peberdy, *J. Dairy Res.* **41**, 249 (1974)
46. A. Y. Smith, P. Dietrich and W. Pickenhagen, *Brit. Pat.* 1,495,227 (1977)
47. J. Kirimura, A. Shimizu, A. Kimizuka, T. Ninomiya and N. Katsuya, *J. Agric. Fd. Chem.* **17**, 689 (1969)
48. T. F. Fryer, *Dairy Sci. Abs.* **31**, 471 (1969)
49. H. M. Liebich, D. R. Douglas, E. Bayer and A. Zlotkis, *J. Chromatogr. Sci.* **8**, 355 (1970)
50. B. A. Law and A. S. Wigmore, *J. Dairy Res.* **49** (in press, 1982)
51. W. A. McGugan, S. G. Howsam, J. A. Elliot, D. B. Emmons, B. Reiter and M. E. Sharpe, *J. Dairy Res.* **35**, 237 (1968)
52. W. A. McGugan, D. B. Emmons and E. Larmond, *J. Dairy Sci.* **62**, 398 (1979)
53. V. R. Harwalker, *J. Dairy Sci.* **55**, 735 (1972)
54. B. C. Richardson and L. K. Creamer, *New Zealand J. Dairy Sci. Tech.* **8**, 46 (1973)
55. T. Matoba and T. Hata, *Agr. Biol. Chem.* **36**, 1423 (1972)
56. J. Czulak, *Australian J. Dairy Tech.* **14**, 117 (1959)
57. R. J. Lowrie and R. C. Lawrence, *New Zealand J. Dairy Sci. Tech.* **7**, 51 (1972)
58. R. J. Lowrie, R. C. Lawrence and M. F. Peberdy, *New Zealand J. Dairy Sci. Tech.* **9**, 116 (1974)
59. O. E. Mills and T. D. Thomas, *New Zealand J. Dairy Sci. Tech.* **51**, 131 (1980)
60. J. Stadhouders and G. Hup, *Neth. Milk Dairy J.* **29**, 335 (1975)
61. Y. Chiba and Y. Sata, *Jap. J. Dairy Fd. Sci.* **29**, 161 (1980)
62. W. A. McGugan, J. A. Blais, M. Boulet, R. N. Giroux, J. A. Elliot, and D. B. Emmons, *Can. Inst. Food Sci. Tech. J.* **8**, 196 (1975)
63. D. D. Bills, M. E. Morgan, L. M. Libbey and E. A. Day, *J. Dairy Sci.* **48**, 1168 (1965)
64. J. L. Biede and E. G. Hammond, *J. Dairy Sci.* **60** (Suppl. 1), 41 (1977)
65. J. L. Biede and E. G. Hammond, *J. Dairy Sci.* **62**, 227 (1979)
66. J. P. Dumont, G. Pradel, S. Roger and J. Adda, *Le Lait* **56**, 18 (1976)
67. D. Sloot and H. J. Hofman, *J. Agr. Food Chem.* **23**, 358 (1975)
68. G. T. Lloyd, J. F. Horwood and I. Barlow, *Australian J. Dairy Tech.* **35**, 137 (1980)
69. G. E. Mitchell, *Australian J. Dairy Tech.* **36**, 21 (1981)
70. J. P. Dumont and J. Adda, *J. Agr. Food Chem.* **26**, 364 (1978)
71. H. T. Huang and J. G. Dooley, *Biotech. Bioeng.* **18**, 909 (1976)
72. W. G. Freytag, K. H. Ney and I. P. G. Wirotama, *Brit. Pat.* 1,470,256 (1977)
73. Hindustan Lever Ltd., *Indian Pat.* 136,717 (1976)