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Typical texture and flavour in certain cheese varieties (for a review, see Law, 1982).

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**Mixed cultures for cottage cheese**

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milk and are commonly used for flavour development. The pathways involved are shown in Fig. 1. The citrate 'fermentation' does not yield energy for the LAB and they only metabolize it in the presence of a truly fermentable carbohydrate (lactose in milk) (Cogan, 1983). However, the reduction of some of the diacetyl to acetoin may serve as an alternative pathway for NADH oxidation.

In theory, Str. lactis subsp. diacetylactis could be used alone to make cottage cheese since it produces both lactic acid and diacetyl. However, this organism metabolizes citrate as soon as it begins to multiply (Cogan, 1982), with concomitant production of CO₂ (Fig. 1). This can cause the curds to float in the vat so that efficient whey separation becomes impossible. L. cremoris, on the other hand, grows much more slowly than the streptococci so that in mixed culture with non-citrte fermenting group N streptococci, they only produce CO₂ and diacetyl late in the manufacturing process after separation of curds and whey. Also, diacetyl production by the leuconostoc is maximal at low pH and high citrate concentration, the very conditions prevailing in cheese curds (Cogan et al., 1981). Citrate inhibits diacetyl reductase (Cogan, 1983) so that the flavour compound can accumulate to well above threshold levels.

Mixed cultures for Emmenthal cheese and related types

These varieties are well known for their long-keeping qualities which they owe to the very high cooking temperature (50°C) used early in the manufacturing process. The cooking stage not only aids the syneresis (drying out) of thermophilus the curd to give a low moisture cheese, but it also destroys or multiply 4h after pressing (Moquot, 1979). Both the streptococcus and lactobacillus in any one culture is not these organisms for acidification, though the proportion of lactobacilli in the starter, Turner et al. (1983) consider that the function of Lb. helveticus in Emmental manufacture is the fermentation of this sugar, rather than lactose, to \( \text{D}(-) \) and \( \text{L}(+) \) lactic acid. Without the lactobacilli, or with galactose-negative variants of lactobacilli, the pH of the cheese does not fall sufficiently to control the maturation process.

The composition of the starter culture therefore has an important influence on the properties of the cheese as it is acidified in press. However, the changes in temperature which follow the removal of the curd from the hot vat (>50°C) to the press room (20°C) also have important consequences for the development of the third component of the starter culture, the propionibacteria which produce pockets of CO₂, known as 'eyes' (Moquot, 1979). The best growth of propionibacteria, and therefore the biggest eyes, occurs near the centre of the cheese. This is due to a sequence of inter-related events, initially dependent on a temperature gradient created as the cheese cools more quickly near its surface than in the centre. Although the starter LAB are tolerant of high temperatures, they grow and metabolize best at temperatures reached at the cheese periphery (30-40°C) within a few hours of pressing. Thus the temperature gradient, by its effect on the growth of the LAB, produces a lactate gradient, exaggerated by the diffusion of lactose from the centre to the periphery where it is utilized more rapidly. P. shermanii begins to multiply 4-6 weeks after the cheese has been made and by this time the lactate concentration is inhibitory near the periphery. The surface salting of the cheese also helps to create relatively unfavourable growth conditions in this region so that the propionibacteria multiply most rapidly in the centre of the cheese. These organisms metabolize lactate via a double cyclic pathway involving pyruvate, catalytic amounts of methylmalonyl CoA and a biotin-dependent transcarboxylation (Allen et al., 1964; Fig. 2). Although the fermentation is named from the end product, propionic acid, it is the CO₂ produced from pyruvate which has the most visible effect on the cheese; much of it (approx. 50 litres in a standard-sized cheese) is retained by the cheese structure in the 'eyes', the largest being characteristically near the cheese centre where the propionibacteria grow best (up to 10⁹ g⁻¹). The correct size and distribution of the eyes is as important to the grade and value of the cheese as is its flavour, emphasizing the vital importance of the correct balance of the mixed cultures used in its manufacture.

The overall flavour of Emmental cheese is thought to be derived from propionic acid (sweet), acetic acid (sour) and the savoury flavours of amino acids and peptides. Although there are some differences in the specificities of the proteolytic and peptidolytic enzymes of the streptococi,
lactobacilli and propionibacteria, their relative importance as products of a mixed culture is not clearly understood (Law & Kolstad, 1983).

**Mixed floras on surface-ripened cheeses**

Some varieties of soft and semi-soft cheeses owe their characteristic flavours to a surface growth of microorganisms (Adda et al., 1982). In the case of Camembert cheese, the white mould, *Penicillium camemberti*, is the only deliberately added part of the flora, but adventitious moulds and yeasts also appear to be involved in maturation, especially in cheeses made from raw milk (Schmidt & Lenoir, 1980). *Geotrichum candidum* is the dominant mould, whereas yeast floras are more variable. All of these surface organisms (including the penicillium) utilize lactate as an energy source, and in doing so, cause the pH at the surface of the cheese to rise from 4.6 to > 6.0. The presence of the yeasts and moulds accelerates the neutralization process, compared with that measured in cheeses ripened with *Pen. camemberti* alone. Also, *G. candidum* produces high levels of glutamic and aspartic acid deaminases which generate ammonia (Greenberg & Ledford, 1979). This compound not only aids neutralization near the cheese surface, but also contributes to the flavour profile. All members of the cheese-surface flora produce lipases, proteinases, peptidases and amino acid-catabolizing enzymes, most of which function best at the neutral pH which is ensured by their collective metabolite activities (Hemme et al., 1982; Lenoir, 1984).


**Fermented milks: new developments in the biochemistry of the starter cultures**

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The commercial yoghurts of today are usually made by fermenting milk with mixed cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. With the interactions between these starters known, it has become possible to make yoghurt using single starters and to develop new products of desirable organoleptic qualities using other species (Marshall et al., 1982).

Acetaldehyde is an important flavour compound in both cheese and fermented milks. It is produced during the manufacture of yoghurt in far greater quantities than any other flavour volatile (Pette & Lolkema, 1950; Bottazzi & Vescovo, 1969). The conventional starter organisms associated with yoghurt manufacture are each capable of producing acetaldehyde in sufficient quantity for well-flavoured single starter products to be made (Marshall et al., 1982), but milks fermented with *Lb. acidophilus* and/or species of bifidobacteria have little flavour (Vedamuthu, 1974). Acetaldehyde can arise within the bacterial cell from threonine metabolism and both *Lb. bulgaricus* and *Lb. acidophilus* have a threonine aldolase which converts threonine to glycine with the release of acetaldehyde. *Lb. bulgaricus* differs from *Lb. acidophilus* in not possessing an alcohol dehydrogenase to reduce the intracellular acetaldehyde. This organism therefore excretes this unwanted metabolite into the milk medium whereas *Lb. acidophilus* metabolizes it to ethanol and the resulting fermented milk lacks the typical aroma and flavour of conventional yoghurt (Marshall & Cole, 1983). To enable milk to be fermented