Yeasts: an underestimated role in cheese production

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Introduction

The fermentation of milk into cheese is a textbook example of industrial microbiology. Lactic acid bacteria ferment milk lactose into lactic acid, and milk proteins and fat are coagulated into a curd which is further processed into the final cheese.

The microbiology, biochemistry and molecular biology of milk fermentation have been researched to enormous depth[1,2]. But this fermentation represents only a fragment of the science of cheese production. While most cheeses start with milk fermentation as the basic operation, their unique and distinguishing characters only develop with further processing of the curd. Generally, this involves heating, cutting, pressing, salting (brining) and, finally, maturation. During maturation, the curd is stored under conditions of controlled temperature and humidity for periods lasting a few weeks to many months, depending on the cheese. It is during maturation that yeasts assert their influence and impact, especially for the higher value, mould-ripened soft cheeses such as camembert, brie and blue-veined varieties[3].

Yeast ecology of maturation

Cheese maturation is ecologically and biochemically very complex. At the commencement of maturation, the curd presents a specialised habitat for microbial growth – a semi-solid, low moisture substrate, with low pH (approximately 4.5), high salt content (1.5-4% NaCl w/w depending on the cheese type), high levels of lactic acid (1-2%), high contents of protein and lipid, and little fermentable sugar.

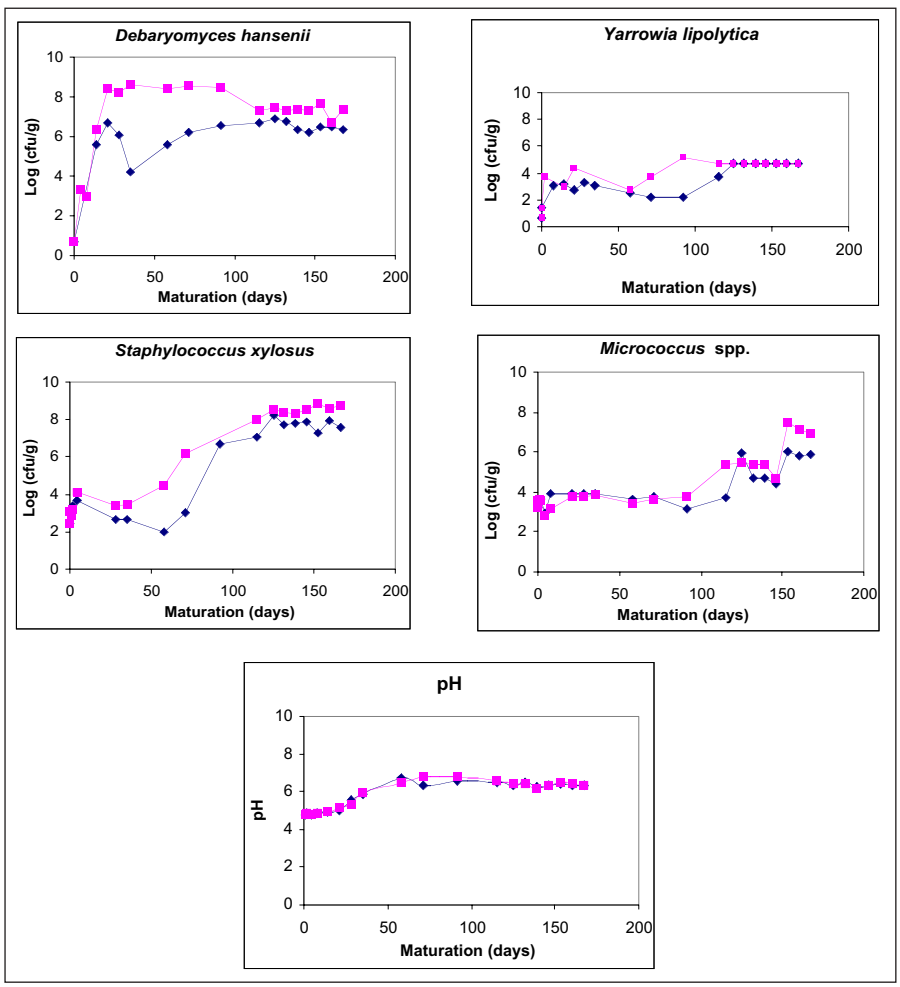
The initial microbial load is dominated by lactic acid bacteria which carry over from milk fermentation, and by any mould species such as *Penicillium camemberti* and *Geotrichum candidum* in camembert and brie cheeses, and *Penicillium roqueforti* in blue-veined cheeses, that are deliberately inoculated as maturation flora.

Also, there is significant 'natural' contamination that originates from the processing environment (e.g. brine solution, air, contact with equipment surfaces)[2]. Yeasts constitute part of this natural load and mostly come from the brine mixture. Essentially, curd properties select for the initial growth of yeasts, salt-tolerant lactic acid bacteria and inoculated moulds.

In the case of yeasts, initial loads of 10^2-10^3 cfu/g quickly grow to populations of 10^8-10^9 cfu/g (Figure 1), and remain at this level throughout maturation and, indeed, until the time the cheese is consumed[3,4].

For cheeses produced throughout the world, *Debaryomyces hansenii* (anamorph *Candida famata)* is the most prevalent and predominant yeast species, followed by *Yarrowia lipolytica (Candida lipolytica).* Why? – because these two species have

Figure 1. The growth of yeasts and bacteria during the maturation of an Australian produced blue-veined cheese – outer curd, ■; inner curd, ◆[4].



unique abilities to grow in the presence of high concentrations of salt, at low temperatures, and utilise lactic acid. Other physiological and biochemical traits (see later) may also contribute to their frequent association with cheeses[5,6]. *Kluyveromyces marxianus,* various *Candida* species, *Pichia membranifaciens* and, to a lesser extent, *Saccharomyces cerevisiae* are also associated with cheese maturation, but more so in varieties with lower salt contents.

How do yeasts impact on cheese quality?

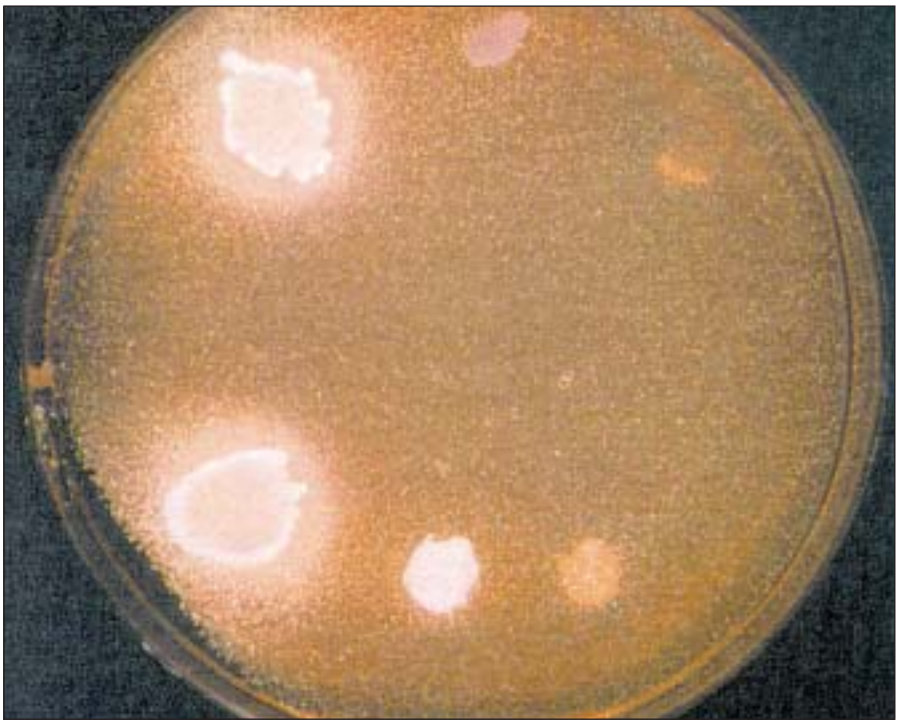
There are several ways in which yeasts contribute positively or negatively to cheese quality[2,3] Stimulation of bacterial growth is probably the most significant. In conjunction with moulds, yeasts utilise lactic acid, causing the pH of the curd to progressively increase from 4.5 to 6.5-7.5 (Figure 1). Acid inhibition of bacteria is removed and, as the pH increases above 5.5, a diversity of salt-tolerant bacterial species begins to grow and contribute to the maturation flora. As yeast cells die, they autolyse and release nutrients (e.g. vitamins, amino acids) that also encourage bacterial growth (Figure 2).

Species of staphylococci (e.g. *Staphylococcus xylosus)* and micrococci (e.g. *Micrococcus varians)* frequently predominate (Figure 1), but various corynebacteria (e.g. *Brevibacterium linens),* lactic acid bacteria (e.g. *Enterococcus, Lactobacillus, Leuconostoc* spp.) and some Gram negative bacteria (e.g. *Acinetobacter* spp., *Enterobacteriaceae)* may be significant, depending on the salt content of the cheese.

Like the yeast, these bacteria originate as 'natural' contaminants. These bacteria can produce various proteolytic and lipolytic enzymes that hydrolyse curd proteins and fat but, overall, it is not clear how they contribute to cheese quality. The increase in pH may also encourage the growth of pathogens, and this is one reason why soft cheeses have been responsible for foodborne diseases caused by *Listeria monocytogenes* and enterotoxigenic strains of *Escherichia coli*[1,2].

Individual species of yeasts may have particular influences on cheese flavour and texture, but these linkages need further research. *Yarrowia lipolytica* is a very potent producer of extracellular proteases and lipases. *Kluyveromyces marxianus* can ferment residual lactose in the curd to produce ethanol, and other volatile, secondary metabolites that contribute to flavour. Volatile sulphur compounds that give cabbage and garlic-like flavours are produced by *D. hansenii, Y. lipolytica* and *K. marxianus.* Yeast autolysis contributes flavour, and most yeasts accumulate high levels of flavour impacting polyols such as glycerol and arabitol when grown in the presence of high concentrations of salt. Not all these activities are positive and, in some cases, cheese spoilage could be the outcome[3,4].

Figure 2. Spot-on-lawn culture showing that *Debaryomyces hansenii* (lawn) stimulates the growth of *Staphylococcus xylosus* (spot culture)[4].



Future challenges

Maturation is a time consuming and costly process that develops cheese individuality and value, but also introduces spoilage and safety risks. For many soft and semi-soft cheese varieties, yeasts are important contributors to the ecology and biochemistry of maturation. Mostly, this is an uncontrolled, indigenous contribution that needs to be better understood and managed through process control and the development of novel yeast strains that could be used as maturation starter cultures.

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