Camembert and Brie are ripened by a mould called *Penicillium camembertii*, which changes the **texture of the cheese** more than its flavour. This fungus grows on the surface of the cheese extruding enzymes which digest the curds to a softer consistency from the outside towards the centre (Fig. 17.42).

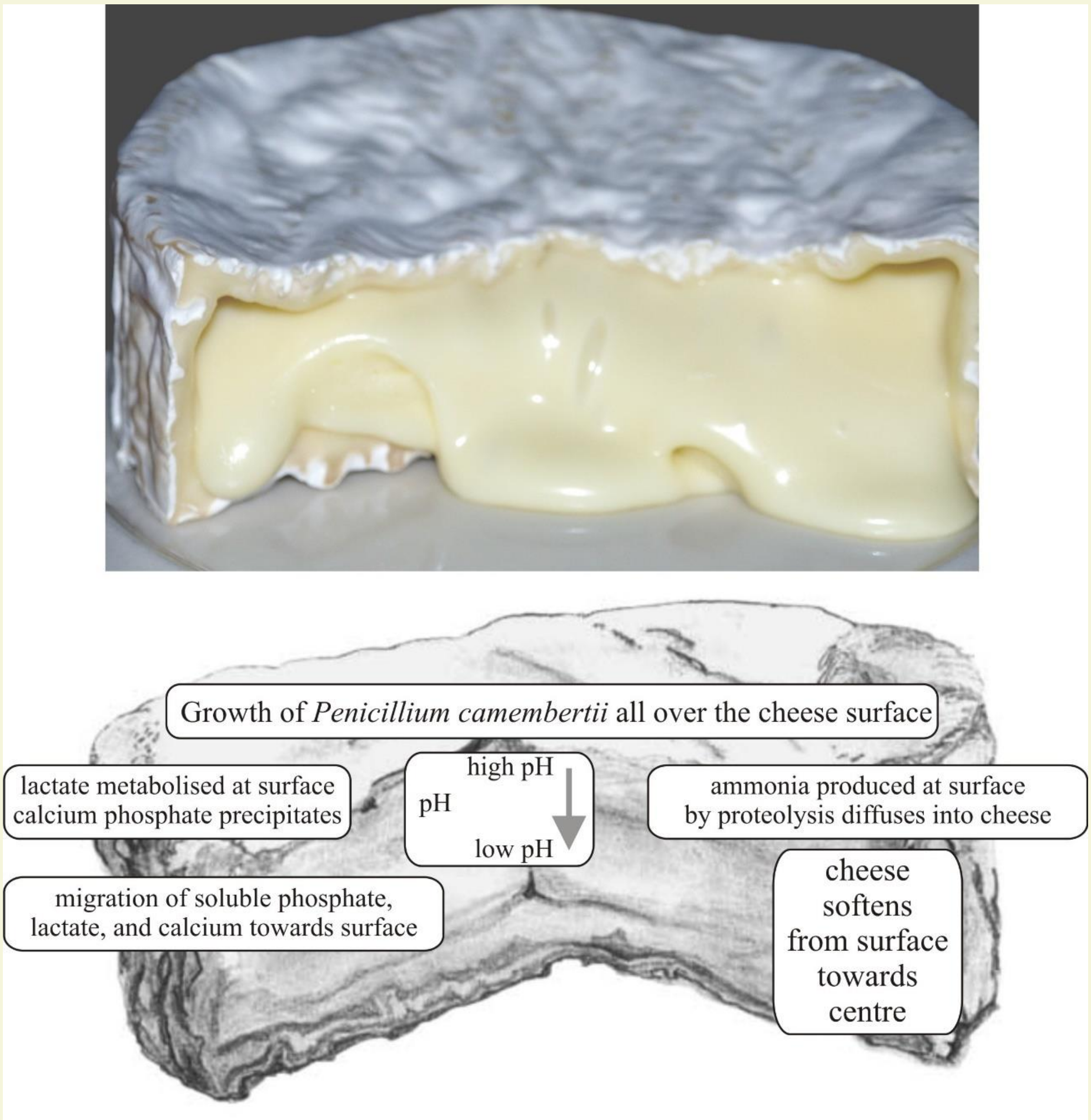

Fig. 17.42. A mature Camembert cheese ready for eating (top). The sketch below is a schematic representation of the changes that occur during ripening of such a cheese as a consequence of the growth of *Penicillium camembertii* over the surface of the cheese. Photograph by David Moore, schematic modified and redrawn from McSweeney, 2004.

Mushrooms are a useful, and acceptable, addition (‘meat extender’) to meat content in beef sausage recipes (Al-Dalain, 2018), but *Penicillium nalgiovense* is a filamentous fungus (Ascomycota) which is the most widely used as a starter culture for cured and **fermented meat products**. Use of moulds in the production of fermented sausages is known from 18th century Italy where fermented and air-dried sausage was a popular peasant food because it could be stored safely at room temperature for long periods. Today **salami** is widely produced in southern-European countries using a range of meats, including beef, goat, horse, lamb, pork, poultry, and/or venison. The chopped meat is mixed with minced animal fat, cereals, herbs and spices, and salt and allowed to ferment for a day. This mixture gives the salami sausage its typical marbled appearance when cut. The mixture is then stuffed into a casing, treated by dipping or spraying with a suspension of the *P. nalgiovense* starter culture at a concentration of about 10^6 spores ml^{-1}, and finally hung to cure.

The fungus does a number of jobs as it grows over the sausage and into the meat and the rest of the mixture. Basically, it **imparts flavour** and **prevents spoilage** during the curing process, but this is achieved by:

- proteolysis, lactate oxidation, amino acid degradation, lipolysis, and fatty acid metabolism during the maturing process creating the desirable flavour;
- with the surface of the sausage colonised by a specific, usually proprietary, mould the air-exposed sausage is protected against spoilage by other undesirable species of yeasts, moulds and bacteria;
- the surface covering of the mould mycelium controls the drying process and ensures a smooth and uniform surface appearance of the product.

17.25 Soy sauce, tempeh and other food products

Fungi are used in the processing of several food products that enjoy large markets in Asia. In these the fungus is mainly responsible for the characteristic odour, flavour, or texture and may or may not become part of the final edible product, though many microorganisms may be involved in these microbial ecosystems. Growing fungi on water-soaked seeds of plants is a popular way to produce soy sauce and various other fermented foods (Moore & Chiu, 2001; Hutkins, 2006; Montet & Ray, 2015; Wolfe & Dutton, 2015; Joshi, 2016).

Soy sauce has been used in China for more than 2,500 years so it is one of the world's oldest condiments. It is a seasoning agent with a salty taste and a distinctly meaty aroma, although it is made by a complex fermentation process of a combination of soybeans and wheat in water and salt, in which carbohydrates are fermented to alcohol and lactic acid and proteins broken down to peptides and amino acids.

This traditional brewing, or fermentation, method can take 6 months or more to produce the finest soy sauce, which should be a dark brown, transparent, salty liquid with balanced flavour and aroma. The brown colour is a result of sugar caramelisation during the 6 to 8 month maturation process.

There is an alternative non-brewed method: an acid hydrolysis that often produces opaque liquor with a harsher flavour but takes only two days to make. We note the alternative but will not discuss it further.

The raw materials are:

- **soybeans** (seeds of *Glycine max*; also known as soya or soja beans) are mashed prior to mixing them with other ingredients;
- **pulverised wheat grains** are mixed with the crushed soybeans (soy sauce produced by chemical hydrolysis generally does not use wheat);
- **salt** (specifically NaCl) is added at the start of second fermentation to 12-18%; this obviously contributes to flavour but it also establishes a selective environment for the lactic acid bacteria and yeasts to complete the fermentation; high salt concentration also serves as a preservative.

Manufacturing through the traditional brewed method occurs in three steps (Luh, 1995):

- **Koji-making**: in which the crushed soybeans and wheat are blended together in water that is boiled until the grains are thoroughly cooked and softened. The mash, as it is known, is allowed to cool to about 27°C and fermented with the filamentous ascomycetes *Aspergillus oryzae* and *A. sojae*. Depending on the size of the factory, the soybeans may be fermented in fist-sized balls (the traditional method) or on trays. The culture of soy, wheat, and mould is known as **koji**. The main function of these moulds is to break down the proteins in the mash; this process takes 3 days and the fungi (in what is known as the **seed starter**) are often closely-guarded **proprietary strains** because this step has a vital role in determining the flavour of the final soy sauce, and methods are now being developed to use genomics for strain improvement (Zhong *et al*., 2018).
- **Brine fermentation**: when the substrate has become overgrown with the mould fungus the koji is transferred to fermentation tanks and mixed with water and salt to produce a mash called **moromi**. **Lactic acid bacteria** (*Pediococcus halophilus*), and 30 days later **yeast** (*Saccharomyces rouxii*), are then added to complete the fermentation. The moromi ferments for several months, during which the soy and wheat paste is digested into a semi-liquid, reddish-brown 'mature mash' that contains over 200 flavour compounds produced as the bacteria and yeast enzymatically digest the protein and other residues to produce amino acids and derivatives.
- **Refinement**: the raw soy sauce is separated from the wheat and soy residue after 6 to 9 months of moromi fermentation by pressure-filtration through cloth. The filtrate is pasteurised (which forms additional flavour compounds) and bottled ready for sale. Soy sauce press cake is a valuable by-product as an animal feed.

Clearly, three major groups of microorganisms are involved in soy sauce fermentation: *Aspergillus* fungi involved in the koji production, and communities of halotolerant bacteria and yeasts are responsible for the moromi fermentation. Enzymes produced by all these various microbes hydrolyse the raw materials during the complex soy sauce fermentation process. Present-day manufacture is done in large factories using carefully controlled production conditions. Soybeans are now steamed instead of being boiled and temperature and humidity controls are applied during koji production. But in the traditional method in China, moromi is fermented in the open air; and there are significant differences between the products produced in different seasons of the year (Cui *et al*., 2014).

Soy sauce is the liquid produced as soybeans are fermented, several other traditional soyfoods are made mainly from **whole soybeans** (Moore & Chiu, 2001; Hutkins, 2006). Soybeans are highly nutritious, containing large amounts of protein and other nutrients, and the traditional soyfoods offer real health benefits. Traditional soyfoods are classified into nonfermented and fermented products (Liu, 2008). **Non-fermented soyfoods** include soymilk, **tofu**, soy sprouts, yuba (soymilk film), okara (soy pulp), vegetable soybeans, soynuts and toasted soy flour. **Fermented soyfoods** include the bacterial products **natto** (fermented with the rice straw bacterium *Bacillus natto*), **soy yoghurt** (soymilk fermented by bacteria), and the fungus-dependent products:

- **sufu**, which is pickled tofu made by fermenting with the zygomycete fungi *Actinomucor elegans*, *Mucor racemosus*, or *Rhizopus* spp.
- **Soy nuggets**; large whole soybeans soaked and steamed and mixed with roasted wheat- or glutinous-rice flour before fermentation with the koji mould *Aspergillus oryzae*. After several days of incubation, the resultant soybean koji is packed in kegs with

saltwater and various spices, seeds, and/or root ginger slivers (and occasionally rice wine), then aged for several months. The soy nuggets are then sun dried.

- **Tempeh** is a fermented food made by the controlled fermentation of cooked germinated soybeans with the filamentous zygomycete *Rhizopus oligosporus*. Fermentation by *Rhizopus* binds the soybeans into a protein-rich compact white cake that can be used as a meat substitute. Tempeh has been a favourite food and staple source of protein in Indonesia for several hundred years (http://www.tempeh.info/)
- **Miso** is possibly the **most important fermented food in Japanese cuisine**. It is basically fermented soybean paste but rice and several cereal grains and even other seeds can be combined into an extremely wide variety of miso that differ according to the combination of ingredients. The basic production process is essentially the same for all recipes: rice, barley or soybeans are steamed, cooled, and inoculated with the koji mould *Aspergillus oryzae*. When the koji has become established it is added, as a seed culture, to a mixture of washed, cooked, cooled and crushed soybeans, water and salt. This is placed in vats and allowed to ferment for 12 to 15 months to allow the proteins in the mixture to be broken down slowly and naturally, forming a paste that is flavoursome and nutritious.

Another fermented product of this sort, **ang-kak** is a bright reddish purple fermented rice product popular in China and the Philippines which is fermented using *Monascus* species (Ascomycota, order Eurotiales). In Petri dish cultures, the mycelium is white in early growth stages, however, it rapidly changes to a rich pink and subsequently to a distinctive yellow-orange colour (illustrated in Pattanagul *et al*., 2007). *Monascus purpureus* produces the characteristic pigments and ethanol which are used for red rice wine and food colouring. The pigments are a mixture of red, yellow and purple polyketides and about ten times more pigment is obtained from solid state fermentation than from submerged liquid fermentation.

The rice is first soaked in water until the grains are fully saturated and may then be directly inoculated or steamed prior to inoculation. Inoculation is done by mixing *M. purpureus* spores or powdered red yeast rice together with the processed rice. The mix is then incubated at room temperature for 3 to 6 days. At the end of this time, the rice grains should have turned bright red in the centre and reddish purple on the outside. **Red rice** is sold as a pasteurised wet aggregate in jars, as whole dried grains, or as a ground powder (to be used as a red food colourant) (Lin *et al*., 2008; Pattanagul *et al*., 2007; Panda *et al*., 2010).

17.26 Chapter 17 References and further reading

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