Ecology of Fungal Food Spoilage

2

Food is not commonly regarded as an ecosystem, perhaps on the basis that it is not a "natural" system. Nevertheless an ecosystem it is and an important one, because food plants and the fungi that colonise their reproductive parts (seeds and fruit), have been co-evolving for millennia. The seed and nut caches of rodents have provided a niche for the development of storage fungi. Fallen fruit, as they go through the cycle of decay and desiccation, have provided substrates for a wide range of pathogenic and spoilage fungi also. Humans have aided and abetted the development of food spoilage fungi through the setting up of vast and varied food stores. It can be argued, indeed, that rapidly evolving organisms, such as haploid asexual fungi, are moving into niches created by man's exploitation of certain types of plants as food.

Food by its very nature is expected to be nutritious: therefore food is a rich habitat for microorganisms – in contrast with the great natural systems, soil, water and plants. Given the right physicochemical conditions, only the most fastidious microorganisms are incapable of growth in foods, so that factors other than nutrients usually select for particular types of microbial populations.

Perhaps the most important of these factors relates to the biological state of the food. Living foods, particularly fresh fruits, vegetables, and also grains and nuts before harvest, possess powerful defence mechanisms against microbial invasion. The study of the spoilage of such fresh foods is more properly a branch of plant pathol-

ogy than food microbiology. The overriding factor determining spoilage of a fresh, living food is the ability of specific microorganisms to overcome defence mechanisms. Generally speaking, then, spoilage of fresh foods is limited to particular species. Such specific relationships between fresh food and fungus are discussed in Chap. 11 and under particular species.

Other kinds of foods are either moribund, dormant or nonliving, and the factors which govern spoilage are physical and chemical. There are eight principal factors:

1. Water activity;
2. Hydrogen ion concentration – pH;
3. Temperature – of both processing and storage;
4. Gas tension, specifically of oxygen and carbon dioxide;
5. Consistency – solid or liquid;
6. Nutrient status;
7. Specific solute effects; and
8. Preservatives.

Each will be discussed in turn below.

2.1 Water Activity

Water availability in foods is most readily measured as water activity. Water activity (a_w), is a physico-chemical concept, introduced to

microbiologists by Scott (1957), who showed that a_w effectively quantifies the relationship between moisture in foods and the ability of microorganisms to grow on them.

Water activity is defined as a ratio:

$$a_w = p / p_o$$

where p is the partial pressure of water vapour in the test material and p_o is the saturation vapour pressure of pure water under the same conditions.

Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal. If a sample of food is held at constant temperature in a sealed enclosure until the water in the sample equilibrates with the water vapour in the enclosed air space (Fig. 2.1a), then

$$a_w (food) = ERH(air)/100.$$

Conversely, if the ERH of the air is controlled in a suitable way, as by a saturated salt solution, at equilibrium the a_w of the food will be numerically equal to the generated ERH (Fig. 2.1b). In this way a_w can be experimentally controlled, and the relationship of a_w to moisture (the sorption isotherm) can be studied. For further information on water activity, its measurement and significance in foods see Duckworth (1975), Pitt (1975), Troller and Christian (1978) and Rockland and Beuchat (1987). For sorption isotherms of a wide range of foods see Iglesias and Chirife (1982).

For an understanding of the mathematical relationships underlying the shape of sorption isotherms see Timmermann et al. (2001).

In many practical situations, a_w is the dominant environmental factor governing food stability or spoilage. A knowledge of fungal water relations will then enable prediction both of the shelf life of foods and of potential spoilage fungi. Although the water relations of many fungi will be considered individually in later chapters, it is pertinent here to provide an overview.

Like that of all other organisms, growth of fungi is profoundly affected by the availability of water. On the a_w scale, life as we know it exists over the range 0.9999+ to 0.60 (Table 2.1) or possibly as low as 0.585 (Stevenson et al. 2017). Growth of animals is virtually confined to 1.0–0.99 a_w; the permanent wilt point of mesophytic plants is near 0.98 a_w; and most microorganisms cannot grow below 0.95 a_w. A few halophilic algae, bacteria and archaea can grow in saturated sodium chloride (0.75 a_w) and even lower in mixed salt solutions (Stevenson et al. 2015), but are confined to salty environments. Ascomycetous fungi and conidial fungi of ascomycetous origin comprise most of the organisms capable of growth below 0.9 a_w. Fungi capable of growth at low a_w, in the presence of extraordinarily high solute concentrations both inside and out, must be ranked as among the most highly evolved organisms on earth. Even among the fungi, this evolutionary

Fig. 2.1 The concept of water activity (a_w) (**a**) the relationship between a_w and equilibrium relative humidity (ERH); (**b**) one method of controlling a_w by means of a saturated salt solution, which generates a specific ERH at a specific constant temperature
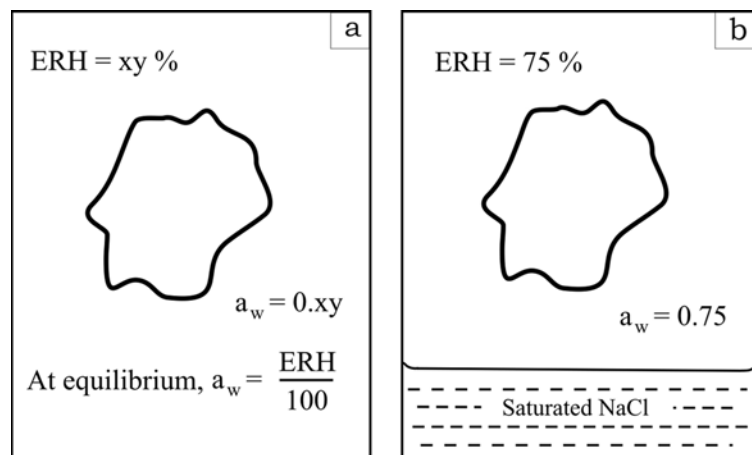
Table 2.1   Water activity and microbial water relations in perspective[a]

a_w	Perspective	Foods	Moulds	Yeasts
1.00	Blood Plant wilt point Seawater	Vegetables Meat, milk Fruit		
0.95	Most bacteria	Bread	Basidiomycetes Most soil fungi	Basidiomycetes
0.90		Ham	Mucorales *Fusarium*	Most ascomycetes
0.85	*Staphylococcus aureus*	Dry salami	*Rhizopus* *Cladosporium*	*Zygosaccharomyces rouxii* (salt)
0.80			*Aspergillus flavus* Xerophilic Penicillia	*Zygosaccharomyces bailii*
0.75	Salt lake Halophiles	Jams Salt fish Fruit cake	Xerophilic Aspergilli *Wallemia* *Eurotium*	*Debaryomyces hansenii*
0.70		Confectionery Dried fruit Dry grains	*Xerochrysium* *Eurotium halophilicum*	
0.65			*Xeromyces bisporus*	*Zygosaccharomyces rouxii* (sugar)
0.60	DNA disordered			

[a]Modified from data of J.I. Pitt as reported by Brown (1974). Water activities shown for microorganisms approximate minima for growth reported in the literature

path must have been of the utmost complexity: the ability to grow at low a_w is confined to only a handful of genera (Pitt 1975).

The degree of tolerance to low a_w is most simply expressed in terms of the minimum a_w at which germination and growth can occur. Fungi able to grow at low a_w are termed xerophiles: one widely used definition is that a xerophile is a fungus able to grow below 0.85 a_w under at least one set of environmental conditions (Pitt 1975). Xerophilic fungi will be discussed in detail in Chap. 9.

Information about the water relations of many fungi remains incomplete, but where it is known it has been included in later chapters.

2.2   Hydrogen Ion Concentration

At high water activities, fungi compete with bacteria as food spoilers. Here pH plays the decisive role. Bacteria flourish near neutral pH and fungi cannot compete unless some other factor, such as low water activity or a preservative, renders the environment hostile to the bacteria. As pH is reduced below about 5, growth of bacteria becomes progressively less likely. Lactic acid bacteria are exceptional, as they remain competitive with fungi in some foods down to about pH 3.5. However, most fungi are little affected by pH over a broad range, commonly 3–8 (Wheeler et al. 1991). Some conidial fungi are capable of growth down to pH 2, and yeasts down to pH 1.5. However, as pH moves away from the optimum, usually about pH 5, the effect of other growth limiting factors may become apparent when superimposed on pH. Figure 2.2 is an impression of the combined influence of pH and a_w on microbial growth. The diagram is schematic: wide variation exists among organisms.

For heat processed foods, pH 4.5 is critical: the heat processing necessary to destroy the spores of *Clostridium botulinum* also destroys all fungal spores. In acid packs, below pH 4.5, less severe processes may permit survival of heat resistant fungal spores (Sect. 2.3).

Fig. 2.2 A schematic diagram showing the combined influence of water activity and pH on microbial growth
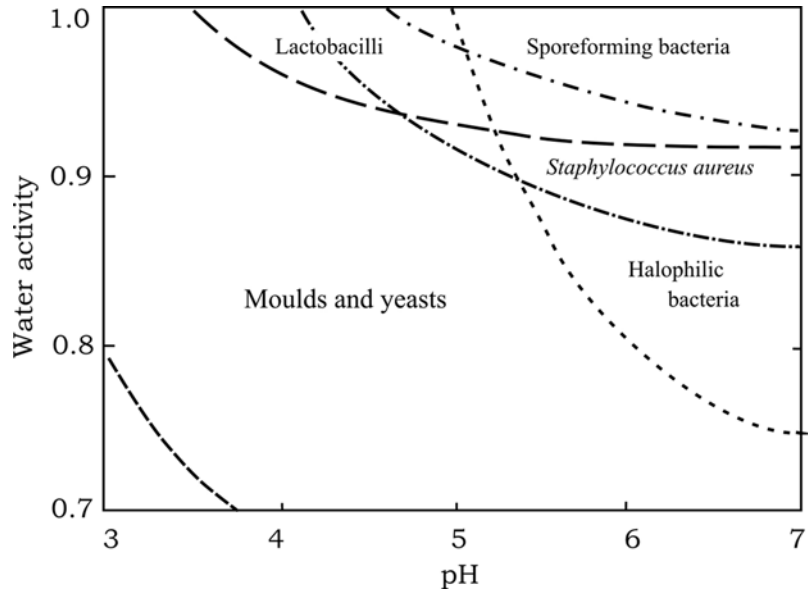
2.3   Temperature

The influence of temperature in food preservation and spoilage has two separate facets: temperatures reached during processing and those existing during storage.

As noted above, heat resistant fungal spores may survive pasteurising processes given to acid foods. Apart from a few important species, little information exists on the heat resistance of fungi. Much of the information that does exist must be interpreted with care, as heating menstrua and conditions can vary markedly, and these may profoundly affect heat resistance. High levels of sugars are generally protective (Beuchat and Toledo 1977). Low pH and preservatives increase the effect of heat (Beuchat 1981a, b; Rajashekhara et al. 2000) and also hinder resuscitation of damaged cells (Beuchat and Jones 1978).

Ascospores of filamentous fungi are more heat resistant than conidia (Pitt and Christian 1970; Table 2.2). Although not strictly comparable, data of Put et al. (1976) indicate that the heat resistance of yeast ascospores and vegetative cells is of the same order as that of fungal conidia.

Among the ascomycetous fungi, *Byssochlamys* species are notorious for spoiling heat processed

fruit products (Olliver and Rendle 1934; Richardson 1965). The heat resistance of *B. fulva* ascospores varies markedly with isolate and heating conditions (Beuchat and Rice 1979): a D value between 1 and 12 min at 90 °C (Bayne and Michener 1979) and a *z* value of 6–8 °C (King et al. 1969; Kotzekidou 1997) are practical working figures. The heat resistance of *B. nivea* ascospores is marginally lower (Beuchat and Rice 1979; Kotzekidou 1997). Species of *Talaromyces*, *Hamigera* and *Thermoascus* seem to be emerging more commonly as heat resistant spoilage species in fruit juices and purees. D values of 1.6–2.6 min at 82 °C have been reported for *Talaromyces trachyspermus*, and 2.7–4.1 min at 88 °C for *T. bacillisporus* in blueberry and grape juice (Tranquillini et al. 2017). *Hamigera avellanea* was more heat resistant than *Talaromyces* species, with reported D values up to 0.43–1.25 min at 93 °C, and *Thermoascus crustaceus* the most heat resistant with D_{95} values between 1.11 and 2.53 min in blueberry or grape juice (Scaramuzza and Berni 2014).

Ascospores of *Neosartorya fischeri* have a similar heat resistance to those of *B. fulva*, but have been reported less frequently as a cause of food spoilage, although they occur commonly in raw materials such as berries. Heat resistant fungi are discussed further in Chap. 4.

Table 2.2   Comparative heat resistance of ascospores and conidia[a]

Fungus	Spore type	Initial viable count/ml	Survivors (%)		
			50 °C	60 °C	70 °C
Eurotium amstelodami	Ascospores	5.0×10^2	93	85	3
	Conidia	7.3×10^2	107	0.3	0
Eurotium chevalieri	Ascospores	1.0×10^3	103	62	21
	Conidia	8.9×10^2	128	0.1	0
Xeromyces bisporus	Ascospores	1.0×10^3	93	30	0.3
Aspergillus candidus	Conidia	3.8×10^2	102	0	0
Wallemia sebi	Conidia	7.1×10^2	42	0	0

[a]Heated at temperatures shown for 10 min. Data from Pitt and Christian (1970)

Food products may be stored under refrigeration, where temperature is expected to play a preservative role, or at ambient temperatures, in which case prevention of spoilage relies on other parameters. Food frozen to −10 °C or below appears to be microbiologically stable, despite some reports of fungal growth at even lower temperatures. The lowest reliably reported temperatures for fungal growth are in the range −7 to 0 °C, for species of *Fusarium*, *Cladosporium*, *Penicillium* and *Thamnidium* (Pitt and Hocking 1997). Nonsterile food stored at ca 5 °C in domestic refrigerators, where conditions of high humidity prevail, will eventually be spoiled by fungi of these genera. At high a_w and neutral pH, psychrophilic bacteria may also be important (mostly *Pseudomonas* species).

Thermophilic fungi, i.e. those which grow only at high temperatures, are rarely of significance in food spoilage. If overheating of commodities occurs, however, in situations such as damp grain, thermophiles can be a very serious problem.

Thermotolerant fungi, i.e. species able to grow at both moderate and high temperatures, are of much greater significance. *Aspergillus flavus* and *A. niger*, able to grow between ca 8 and 45 °C, are among the most destructive fungi known.

2.4   Gas Tension

Food spoilage moulds, like almost all other filamentous fungi, have an absolute requirement for oxygen. However, some species appear to be efficient oxygen scavengers, so that the total amount of oxygen available, rather than the oxygen tension, determines growth. The concentration of oxygen dissolved in the substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Miller and Golding 1949). For example, *Penicillium expansum* has been reported to grow quite normally in only 2.1% oxygen over its entire temperature range (Golding 1945), and many other common food spoilage fungi are inhibited only slightly when grown in nitrogen atmospheres containing approximately 1.0% oxygen (Hocking 1990). *Paecilomyces variotii* (=*Byssochlamys spectabilis*) produced normal colonies when grown in our laboratory at 25 °C under 650 mm of vacuum.

Most food spoilage moulds appear to be sensitive to high levels of carbon dioxide, although there are notable exceptions. When maintained in an atmosphere of 80% carbon dioxide and 4.2% oxygen, *Penicillium roqueforti* still grew at 30% of the rate in air (Golding 1945), provided that the temperature was above 20 °C. At 25 °C, in 40% CO_2 and 1% O_2, *P. roqueforti* grew at almost 90% of the rate in air (Taniwaki et al. 2001a). *Xeromyces bisporus* was reported to grow in high levels of carbon dioxide (Dallyn and Everton 1969), but Taniwaki et al. (2009) could not repeat those observations.

Byssochlamys species are particularly tolerant of reduced oxygen and/or elevated carbon dioxide levels. Growth of *B. nivea* was little affected by replacement of nitrogen in air by carbon dioxide, and growth in carbon dioxide – air mixtures was proportional only to oxygen concentration, at least up to 90% carbon dioxide (Yates et al. 1967). Both *B. nivea* and *B. fulva* were capable of

growth in atmospheres containing 20%, 40% or 60% carbon dioxide with less than 0.5% oxygen, though inhibition increased with increasing carbon dioxide concentration (Taniwaki et al. 2001, 2009). Indeed *Byssochlamys* species can be correctly described as facultative anaerobes, growing under similar conditions to the obligate anaerobe *Clostridium sporogenes* (Taniwaki et al. 2009). *B. fulva* is capable of growth in 0.27% oxygen, but not in its total absence (King et al. 1969). It is also capable of fermentation in fruit products, but presumably only if some oxygen is present.

At least some species of *Mucor*, *Rhizopus* and *Fusarium* are able to grow and ferment in bottled liquid products and sometimes cause fermentative spoilage. Growth under these conditions may be yeast-like. Species of *Mucor*, *Rhizopus* and *Amylomyces* used as starter cultures in Asian fermented foods can grow under anaerobic conditions, demonstrated by growth in an anaerobe jar with a hydrogen and carbon dioxide generator (Hesseltine et al. 1985). Other authors have reported growth under anaerobic conditions of *Mucor* species, *Absidia spinosa*, *Geotrichum candidum*, *Fusarium oxysporum* and *F. solani* (= *Neocosmospora solani*) (Stotzky and Goos 1965; Curtis 1969; Taniwaki 1995; Taniwaki et al. 2009). The yeast-like fungus *Moniliella acetoabutans* can cause fermentative spoilage under totally anaerobic conditions (Stolk and Dakin 1966).

As a generalisation, however, it is still correct to state that food spoilage problems due to filamentous fungi usually occur under aerobic conditions, or at least where oxygen tension is appreciable, due to leakage or diffusion through packaging.

In contrast, *Saccharomyces* species, *Zygosaccharomyces* species and other fermentative yeasts are capable of growth in the complete absence of oxygen. Indeed, *S. cerevisiae* and *Z. bailii* can continue fermentation under several atmospheres pressure of carbon dioxide. This property of *S. cerevisiae* has been harnessed by mankind for his own purposes, in the manufacture of bread and many kinds of fermented beverages. *Z. bailii*, on the other hand, is notorious for its ability to continue fermenting at reduced water

activities in the presence of high levels of preservatives. Fermentation of juices and fruit concentrates may continue until carbon dioxide pressure causes container distortion or explosion. The closely related species *Z. rouxii* is a xerophile, and causes spoilage of low moisture liquid or packaged products such as fruit concentrates, jams and dried fruit. The difference in oxygen requirements between filamentous fungi and fermentative yeasts is one of the main factors determining the kind of spoilage a particular commodity will undergo.

2.5   Consistency

Consistency, like gas tension, exerts considerable influence over the kind of spoilage to which a food is susceptible. Generally speaking, yeasts cause more obvious spoilage in liquid products, because single celled microorganisms are able to disperse more readily in liquids. Moreover, a liquid substrate is more likely to give rise to anaerobic conditions and fermentation is more readily seen in liquids. In contrast, filamentous fungi are assisted by a firm substrate and ready access to oxygen, but may grow on the surface of liquids.

The foregoing is not intended to suggest that yeasts cannot spoil solid products nor moulds liquids: merely that all other factors being equal, fermentative yeasts have a competitive advantage in liquids and cause more obvious spoilage under these conditions. In solid foods, filamentous fungi have the advantage of producing hyphae, capable of penetrating and utilising substrates, while yeasts (and bacteria) are confined to growing on surfaces only.

2.6   Nutrient Status

As noted in the preamble to this chapter, the nutrient status of most foods is adequate for the growth of any spoilage microorganism. Generally speaking, however, it appears that fungal metabolism is best suited to substrates high in carbohydrates, whereas bacteria are more likely to spoil proteinaceous foods. Lactobacilli are an exception.

Most common mould species appear to be able to assimilate any food-derived carbon source with the exception of hydrocarbons and highly condensed polymers such as cellulose and lignin. Most moulds are equally indifferent to nitrogen source, using nitrate, ammonium ions or organic nitrogen with equal ease. Some species achieve only limited growth if amino acids or proteins must provide both carbon and nitrogen. A few isolates classified in *Penicillium* subgen. *Biverticillium* (= *Talaromyces* in current taxonomies) are unable to utilise nitrate (Pitt 1979).

Some xerophilic fungi are known to be more demanding. Ormerod (1967) showed that growth of *Wallemia sebi* was strongly stimulated by proline. Xerophilic *Chrysosporium* species (= *Bettsia* and *Xerochrysium*) and *Xeromyces bisporus* also appear to require complex nutrients, but the factors involved have not been defined (Pitt 1975).

Yeasts are often fastidious. Many are unable to assimilate nitrate or complex carbohydrates; a few, *Zygosaccharomyces bailii* being an example, cannot grow with sucrose as a sole source of carbon. Some require vitamins. These factors limit to some extent the kinds of foods susceptible to spoilage by yeasts. This point is worth emphasising. Certain foods (or nonfoods) lack nutrients essential for the growth of *Z. bailii*. Addition of nutrient, for whatever reason, can turn a safe product into a costly failure. Two cases from our own experience illustrate this point. In the first, a highly acceptable (and nutritious) carbonated beverage containing 25% fruit juice was eventually forced from the Australian market because it was impractical to prepare it free of occasional *Z. bailii* cells. Effective levels of preservative could not be added legally and pasteurisation damaged its flavour. Substitution of the fruit juice with artificial flavour and colour removed the nitrogen source for the yeast. A spoilage free product resulted, at the cost of any nutritional value and a great reduction in consumer acceptance.

The other case concerned a popular water-ice confection, designed for home freezing. This confection contained sucrose as a sweetener and a preservative effective against yeasts utilising sucrose. One production season the manufacturer decided, for consumer appeal, to add glucose to the formulation. The glucose provided a carbon source for *Z. bailii*, and as a result several months production, valued at hundreds of thousands of dollars, was lost due to fermentative spoilage.

2.7   Specific Solute Effects

As stated earlier, microbial growth under conditions of reduced water availability is most satisfactorily described in terms of a_w. However the particular solutes present in foods can exert additional effects on the growth of fungi. Scott (1957) reported that *Aspergillus amstelodami* (= *A. montevidensis*) grew 50% faster at its optimal a_w (0.96) when a_w was controlled by glucose rather than magnesium chloride, sodium chloride or glycerol. Pitt and Hocking (1977) showed a similar effect for *A. chevalieri*, and reported that the extreme xerophiles *Chrysosporium fastidium* (= *Bettsia fastidia*) and *Xeromyces bisporus* grew poorly if at all in media containing sodium chloride as the major solute. In contrast Pitt and Hocking (1977) and Hocking and Pitt (1979) showed that germination and growth of several species of *Aspergillus* and *Penicillium* were little affected when medium a_w was controlled with glucose–fructose, glycerol or sodium chloride.

Zygosaccharomyces rouxii, the second most xerophilic organism known, has been reported to grow down to 0.62 a_w in fructose (von Schelhorn 1950). Its minimum a_w for growth in sodium chloride is reportedly much higher, 0.85 a_w (Onishi 1963).

Some fungi are halophilic, being well adapted to salty environments such as salted fish. *Phialosimplex halophilus* and *Polypaecilum pisce* grow more rapidly in media with NaCl as controlling solute, rather than glucose or glycerol (Andrews and Pitt 1987; Wheeler et al. 1988). Such fungi have been called halophilic xerophiles to distinguish them from obligately halophilic bacteria.

2.8   Preservatives

Obviously, preservatives for use in foods must be safe for human consumption. Under this constraint, food technologists in most countries are limited to the use of weak acid preservatives: benzoic, sorbic, nitrous, sulphurous, acetic and propionic acids – or, less commonly, their esters. In the concentrations permitted by most food laws, these acids are useful only at pH levels up to their pK_a plus one pH unit. Classical theory indicates that their effect is due to entry to cells as the undissociated acid, followed by dissociation and disruption of cell metabolism, however, that has been disputed recently (Stratford and Anslow 1998; Stratford et al. 2013). For earlier studies of the mechanism of action of weak acid preservatives see Warth (1977, 1991), Brul and Coote (1999) and Stratford and Lambert (1999).

The use of chemical preservatives in foods is limited by law in most countries to relatively low levels, and to specific foods. A few fungal species possess mechanisms of resistance to weak acid preservatives, the most notable being *Zygosaccharomyces bailii*. This yeast is capable of growth and fermentation in fruit based cordials of pH 2.9–3.0, of 45 °C Brix and containing 800 mg/L of benzoic acid (Pitt and Hocking 1997). The yeast-like fungus *Moniliella acetoabutans* can grow in the presence of 4% acetic acid, and survive in 10% (Pitt and Hocking 1997).

Of the filamentous fungi, *Penicillium roqueforti* appears to be especially resistant to weak acid preservatives, causing spoilage of preserved European bread (Suhr and Nielsen 2004). Weak acid resistance has been suggested as a useful aid to isolation and identification (Engel and Teuber 1978).

2.9   Conclusions: Food Preservation

It is evident from the above discussion that the growth of fungi in a particular food is governed largely by a series of physical and chemical parameters, and definition of these can assist greatly in assessing the food's stability. The situation in practice is made more complex by the fact that such factors frequently do not act independently, but synergistically. If two or more of the factors outlined above act simultaneously, the food may be safer than expected. This has been described by Leistner and Rödel (1976) as the "hurdle concept". This concept has been evaluated carefully for some commodities such as fermented sausages and is now widely exploited in the production of shelf stable bakery goods and acid sauces.

For most fungi, knowledge remains meagre about the influence of the eight parameters discussed here on germination and growth. However, sufficient information is now available that some rationale for spoilage of specific commodities by certain fungi can be attempted, especially where one or two parameters are of overriding importance. This topic is considered in later chapters devoted to particular commodities.

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