

The Commercial and Community Significance of Yeasts in Food and Beverage Production

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1.1 Introduction

The history of yeast association with human society is synonymous with the evolution of bread, beer and wine as global food and beverage commodities, originating some 5,000 years ago. The microbial science of these products commenced in the mid-1600s with the first observations of yeast cells being reported by Antonie van Leeuwenhoek (The Netherlands). The significance of these findings laid dormant until the classic studies of Pasteur (France) and Hansen (Denmark) during 1850–1900, which heralded the beginnings of the disciplines of microbiology and biochemistry. Subsequent studies by Guilliermond (France) and Kluver (The Netherlands) in the early 1900s established yeasts as a unique group of microorganisms that had a major role in food and beverage production (Rose and Harrison 1969; Rose 1977). Since the 1950s, several classic texts have specifically highlighted the commercial and social significance of yeasts in foods and beverages (Cook 1958; Rose and Harrison 1970, 1993; Phaff et al. 1978; Skinner et al. 1980; Spencer and Spencer 1990; Reed and Nagodawithana 1991; Deak and Beuchat 1996; Boekhout and Robert 2003).

Today, the impact of yeasts on food and beverage production extends beyond the original and popular notions of bread, beer and wine fermentations by *Saccharomyces cerevisiae* (Table 1.1). In a positive context, they contribute to the fermentation of a broad range of other commodities, where various yeast species may work in concert with bacteria and filamentous fungi. Many valuable food ingredients and processing aids are now derived from yeasts. Some yeasts exhibit strong antifungal activity, enabling them to be exploited as novel agents in the biocontrol of food spoilage. The probiotic activity of some yeasts is another novel property that is attracting increasing interest. Unfortunately, there is also a darker side to yeast activity. Their ability to cause spoilage of many commodities, with major economic loss, is well known in many sectors of the food and beverage industries, while the public health significance of yeasts in foods and beverages is a topic of emerging concern. This chapter defines the scope and diversity of the many beneficial and

Table 1.1 The commercial and community significance of yeasts in food and beverage production

- Production of fermented foods and beverages.
- Production of ingredients and additives for food processing.
- Spoilage of foods and beverages.
- Biocontrol of spoilage microorganisms.
- Probiotic and biotherapeutic agents.
- Source of food allergens.
- Source of opportunistic, pathogenic yeasts.

detrimental aspects of yeasts in foods and beverages. Subsequent chapters in this book will provide more detailed and specific coverage of these concepts.

1.2 The Informative Process

To effectively exploit and manage the growth and activities of yeasts in foods and beverages, a structured process of knowledge development and understanding is needed, the concepts of which are outlined in Table 1.2 (Fleet 1999). Obtaining this information is a challenging task, and requires the collaborative interaction of microbiologists, chemists, biochemists, molecular biologists, and food scientists. Application of molecular technologies to the detection and identification of yeasts in food and beverage ecosystems (Fernandez-Espinar et al., Chap. 3) and to determining the genetic bases of their biochemical and physiological responses to these habitats (Walker and van Dijck, Chap. 5; Barrio et al., Chap. 6; Bond and Blomberg, Chap. 7; Dickinson and Kruckeberg, Chap. 8) have greatly facilitated acquisition of this fundamental knowledge and understanding. Moreover, there is now a broad recognition and acceptance that many yeasts species other than *Saccharomyces cerevisiae* are intimately and significantly involved in food and beverage production Romano et al. (Chap. 2) and that their individual contributions can be moderated and impacted by interactions with bacteria and filamentous fungi (Viljoen, Chap. 4). For most commodities, the chain of knowledge linking yeast ecology, yeast activity, product chemistry and product quality is very incomplete. Wine could be singled out where most progress has been made in this context.

1.3 Production of Fermented Foods and Beverages

Most individuals, whether they have a scientific or nonscientific background, have a positive image of yeasts because of their well-known association with the production of bread, beer, wine and other alcoholic beverages. Some will know that there are differences between baker's yeast, brewer's yeast, wine yeast and distiller's yeast, and the more learned will know these as either *S. cerevisiae*, *S. bayanus*, or *S. pastorianus*, according to current taxonomic classifications (Vaughan-Martini and Martini 1998; Kurtzman 2003). However, there is increasing awareness that many species other than those of *Saccharomyces* make positive contributions to the fermentations of foods and beverages, and the diversity of these associations are described in Chap. 2.

Table 1.2 Information needed to exploit and manage yeasts in food and beverage production

- Taxonomic identity of species and strains that contaminate and colonize the food throughout the total chain of production and sale.
- Growth profiles of individual species and strains throughout the chain of production and sale.
- Physical location and spatial distribution of species within the product.
- Biochemical, physiological and molecular explanation of how yeasts colonize the product and change its chemical and physical properties.
- Impact of intrinsic, extrinsic and processing factors on yeast growth and metabolic activity in the product.
- Correlation between growth and activity of individual species/strains, and product quality and safety.

In addition to *S. cerevisiae* and *S. bayanus*, it is now well established that various species of *Hanseniaspora*, (*Kloeckera*), *Candida*, *Pichia*, *Metschnikowia*, *Kluyveromyces*, *Schizosaccharomyces* and *Issatchenkia* can make positive contributions to the fermentation of wine from grapes and cider from apples (Fleet 1998, 2003a; Pretorius 2000). *Dekkera* (*Brettanomyces*) species, in addition to *S. pastorianus* and *S. cerevisiae* are significant in the production of some styles of beer (Dufour et al. 2003), while *Schizosaccharomyces pombe* can be important in rum fermentations (Fahrasmane and Ganou-Parfait 1998).

Although the microbiology of dairy products is generally dominated by discussions of lactic acid bacteria, there is now substantial literature describing the important role of yeasts in flavour and texture development during the maturation stage of cheese production, and in the production of fermented milks such as kefir and koumiss (Fleet 1990; Frohlich-Wyder 2003). The most predominant and important species in these associations are *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Kluyveromyces marxianus* and *S. cerevisiae*, but *Galactomyces geotrichum*, *Candida zeylanoides* and various *Pichia* species are also significant. In addition to lactic acid bacteria, micrococci and staphylococci, yeasts also play an important role in the fermentation of meat sausages and the maturation of hams. *D. hansenii*, other *Debaryomyces* species, *Y. lipolytica* and various *Candida* species are involved, and contribute to flavour and colour development in these products (Lucke 1998; Samelis and Sofos 2003).

Yeasts other than *S. cerevisiae* are found in the fermentation of various cereal products, including sourdough breads, where their activities impact on product flavour and rheology. Prominent contributors are *S. exiguus*, *C. humicola*/*C. milleri*, *Torulaspota delbrueckii*, various *Pichia* species and other species of *Candida* (e.g. *C. krusei*/*Issatchenkia orientalis*) (Jenson 1998; Meroth et al. 2003; Hammes et al. 2005). Coffee beans and cocoa beans (chocolate) undergo natural, indigenous fermentations in the primary stages of their processing, where the growth and activities of a diversity of *Hanseniaspora*, *Candida*, *Pichia*, *Issatchenkia*, *Kluyveromyces* and *Saccharomyces* species have been reported. Essentially, these yeasts assist in degradation of bean pulp and contribute to the production of chocolate flavour precursors (Schwan and Wheals 2003, 2004). *Zygosaccharomyces rouxii*, *C. versatilis*, and *C. etchellsii* are important osmotolerant species that play a key role in soy sauce fermentation (Hanya and

Nakadai 2003). Finally, a vast range of traditional, fermented products are produced in Africa, Asia and Latin America, where, along with bacteria, a diversity of yeast species make important contributions (Steinkraus 1996; Nout 2003).

With very few exceptions (e.g. beer), most fermented foods and beverages involve a mixed ecology of yeasts, bacteria, filamentous fungi in some cases, and their viruses. Consequently, complex microbial interactions are likely to be involved (Chap. 4). The ultimate goal is to understand which species are important to product quality and process efficiency, and to develop operational parameters that maximize their positive contributions. Novel bioreactor technologies could be developed to improve process efficiency (Strehaiano et al., Chap. 9) and targets for genetic improvement of strains could be identified (Verstrepen et al., Chap. 13).

1.4 Yeasts as Sources of Ingredients and Additives for Food Processing

Because yeasts have a positive image with consumers, they are considered as a safe source of ingredients and additives for food processing (Demain et al. 1998). Preparations of baker's and brewer's yeasts have been available for many years as dietary, nutrient supplements because of their high contents of B vitamins, proteins, peptides, amino acids and trace minerals. Also, yeasts are often considered as an alternative source of protein for human consumption (Peppler 1970; Harrison 1993). Many products are now derived from yeasts and, according to Stam et al. (1998), about 15–20% of the global industrial production of yeasts is used for this purpose. Abbas (Chap. 10) describes the production of antioxidants, aromas, flavours, colours and vitamins by yeasts. Other detailed accounts of these topics may be found in Halasz and Laszity (1991) and Reed and Nagodawithana (1991).

Flavour ingredients based on yeast extracts, yeast autolysates and dried yeast preparations represent the most commercially significant products extracted from yeasts, and are used extensively in the food industry as a source of savoury, roasted, nutty, cheesy, meaty and chicken flavours. In addition, some extracts are specifically enriched in their contents of glutamic acid and nucleotides that function as strong flavour enhancers (Dziezak 1987; Nagodawithana 1992; Kollar et al. 1992; Stam et al. 1998). While baker's and brewer's yeasts have been the traditional sources of these products, their diversity and functionality are being expanded by the use of other yeasts such as *C. utilis* (*Pichia jadinii*) and *K. marxianus* (Lukondeh et al. 2003). Yeasts are frequently mentioned as potential sources of high value aroma and flavour substances such as vanillin (*S. cerevisiae*, *Rhodotorula glutinis*), citronellol, linalool and geraniol (*K. marxianus*), and γ and δ -decalactones (*Sporidiobolus sulmonicolor*, *Y. lipolytica*) (Hagedorn and Kaphammer 1994; Vandamme and Soetaert 2002).

The yeast cell wall, composed principally of β -(1 \rightarrow 3) and β -(1 \rightarrow 6)-glucans and mannoprotein, represents about 20–30% of the cell dry weight (Fleet 1991; Nguyen et al. 1998). The β -glucans have gelling, thickening and fat-sparing functional properties that offer a range of applications in food processing (Seeley 1977) and, moreover, they have been reported to have anticancer, (Bohn and Be Miller 1995) immunomodulating (Sandula et al. 1999) and cholesterol-lowering activities

(Bell et al. 1999). They also absorb mycotoxins and could offer a method for removing these substances from beverages such as wine (Yiannikouris et al. 2004; Bejaoui et al. 2004).

Food colorants such as astaxanthin and other carotenoid pigments (Lyons et al. 1993; Johnson and Schroeder 1995) and a diversity of vitamins (Reilly 1991; Sauer et al. 2004) can also be derived from yeasts.

1.5 Spoilage of Foods and Beverages by Yeasts

There is an extensive literature on yeasts as food and beverage spoilage agents and, no doubt, this reflects the enormous commercial and economic significance of this problem. Stratford (Chap. 11) gives an updated account of this topic. Earlier, comprehensive discussions include those of Ingram (1958), Walker (1970), Deak (1991), Fleet (1992), Tudor and Board (1993), Thomas (1993), Deak and Beuchat (1996) and Loureiro and Querol (1999). Yeast spoilage is a constant threat and widespread problem in the food and beverage industries that can only be managed by employing educated staff and implementing effective quality assurance programs.

Yeast spoilage is very predictable, principally occurring in those products where bacterial growth is either retarded or prevented by the intrinsic, extrinsic and processing that prevail. Without this competition, yeasts will grow and spoil the product. Typically, high-acid, low-pH foods, products with high sugar (e.g. more than 10% w/v) or high salt (more than 5% NaCl) content, and products preserved with weak organic acids (e.g. sorbic, benzoic, acetic) are prone to yeast spoilage. Fruits, fruit juices, and fruit drinks, fruit pulp, fruit juice concentrates, sugar and flavour syrups, confectionery products, alcoholic beverages, carbonated beverages, vegetable salads with acid dressings, salt- and acid-based sauces, fermented dairy products and fermented or cured (salted) meat products represent prime candidates for yeast spoilage (Walker 1970; Tudor and Board 1993; Deak and Beuchat 1996). Some yeasts (e.g. *Cryptococcus* and *Rhodotorula* spp.) grow better than bacteria at subfreezing temperatures, and will spoil frozen meat, poultry, seafood and other products stored for lengthy periods. Some high-fat, low-water-activity commodities such as margarine and butter can support the surface growth of yeasts (e.g. *Y. lipolytica*). While there is significant diversity in the yeast species associated with food and beverage spoilage, some specific associations are frequent and often predictable. For example, these include *Z. rouxii* in very high sugar products, *D. hansenii* in salted meat products, *Z. bailii* in products preserved with weak organic acids and *Y. lipolytica* in high-fat products. However, an open and enquiring outlook should be maintained, because new spoilage and food processing species may be present and await discovery – for example, *Z. lentus* (Steels et al. 1999) and *Tetrapisispora fleetii* (Kurtzman et al. 2004).

Controlling the growth and activity of spoilage yeasts requires good understanding of their physiology (Chap. 5), biochemistry (Chaps. 8, 9) and genetic responses (Chaps. 6, 7). Unfortunately, there remain large gaps in this knowledge, especially for yeasts other than *S. cerevisiae*. Factors affecting growth and survival, and being able to predict yeast response to these factors, are particularly important at the practical levels of quality control and assurance. For most yeasts, the growth and survival limits, and inactivation kinetics for basic technological parameters such as temperature,

pH, sugar concentration and salt concentration, are not well defined, and require more careful, systematic investigation (Praphailong and Fleet 1997; Betts et al. 2000). Also, new food and beverage processing technologies such as high hydrostatic pressure, exposure to low- and high-intensity electric fields, and treatment with novel antimicrobial plant extracts (Gould 2000) are emerging and new information on the growth, survival and inactivation responses of individual yeast species to these factors is needed. Finally, the ecological origin or source of spoilage yeasts remains a mystery for many species and requires further research. The first line of defense in controlling food spoilage by microorganisms is the prevention of contamination, but the importance of microbial ecology in quality assurance programs is often underestimated.

1.6 Yeasts as Biocontrol Agents

In Chap. 4, Viljoen notes that most food and beverage habitats present complex ecosystems where a diversity of microbial species and interactive responses are likely to occur and impact on product quality. These responses can be beneficial, antagonistic or neutral to individual species within the product. Antagonistic interactions have given rise to the concept of biocontrol, whereby one species could be deliberately exploited to inhibit the growth and survival of another, less desirable species. During the last 20 years, several yeast species that exhibit strong antagonistic activity against filamentous fungi have been discovered. These yeasts have been investigated as potential agents for the biocontrol of fungi that cause pre- and postharvest spoilage of fruits and vegetables (e.g. *Botrytis*, *Penicillium*, *Aspergillus*, *Rhizopus* spp.), thereby enabling a lesser use of chemical fungicides (Fleet 2003b; Punja and Utkhede 2003; Spadaro and Gullino 2004). *C. oleophila* and *Pseudozyma flocculosa* have been commercialized for such use, and other species with biocontrol potential include *Metschnikowia pulcherrima*, *P. guilliermondii*, *C. sake*, *Sporobolomyces roseus*, *Aureobasidium pullulans* and various *Cryptococcus* species (Fleet 2003b). *P. anomala* has been well studied for its biocontrol of fungi that spoil cereal silages (Druvefors et al. 2002). Various mechanisms have been proposed to explain the antagonistic activity of yeasts towards other fungi, and these include production of killer toxins and other inhibitory proteins and peptides, competition for nutrients and space, production of fungal cell wall lytic glucanases and chitinases, production of toxic metabolites such as ethanol, acetaldehyde, ethyl acetate and fatty acids, and induction of fungal resistance or defense reactions within the plant (Punja and Utkhede 2003).

It should not be forgotten that some yeasts influence the growth and survival of other yeasts by the simple mechanisms of ethanol and killer toxin production (Shimizu 1993). These properties may also impact on bacteria and filamentous fungi (Fleet 1999, 2003a), and highlight the fact that yeasts probably have much greater potential as biocontrol agents than currently recognized. More research is needed on this topic. Ethanol and killer toxin production are significant properties in the selection and commercialization of yeasts for wine production (Degre 1993).

1.7 Public Health Significance of Yeasts in Foods and Beverages

With respect to the field of food safety, yeasts have an impeccably good record and this topic is discussed by Fleet and Roostita in Chap. 12. Unlike bacteria, viruses and some filamentous fungi, yeasts are rarely associated with outbreaks of foodborne gastroenteritis or other foodborne infections or intoxications. As part of normal, daily food consumption, humans are unknowingly and inadvertently ingesting large, viable populations of a diversity of yeast species without adverse impact on their health (e.g. yeasts in many cheeses, fermented and cured meats, fruits and fruit salads, home-brewed beer and wine). Nevertheless, an open mind and vigilance on yeasts and foodborne disease are required – several bacterial species (e.g. *Escherichia coli*) not considered to be serious foodborne pathogens 25 years ago, are now classified in the high risk category.

There is a significant body of “lay” and “alternative” literature that connects yeast presence in foods to the onset of a broad range of allergic and hypersensitive reactions in humans. Migraines, respiratory problems, chronic fatigue syndrome, dysfunctional gut syndrome, irritable bowel syndrome and gut dysbiosis are prominent among these disorders (Crook 1986; Eaton 2004). The linkage between human disorder, food and yeast is largely based on dietary observations – when the suspect food is removed from the diet, the disorder disappears, and returns when the food is reintroduced into the diet. The underlying mechanisms of the human response require systematic, scientific research and could reflect adverse reactions to the yeast cells themselves, or metabolites they have produced (e.g. proteins, biogenic amines, sulphur dioxide).

Unlike many bacteria and viruses, yeasts are not known as aggressive infectious pathogens. However, some yeast species fall into the category of opportunistic pathogens. *C. albicans* and *Cryptococcus neoformans* are prominent in this context, and cause a range of mucocutaneous, cutaneous, respiratory, central nervous, systemic and organ infections in humans (Hazen and Howell 2003). Usually, healthy, immunocompetent individuals are not at risk of such infections. Generally, individuals with weakened health and immune function are at greatest risk, and include cancer and AIDS patients, hospitalized patients and those undergoing treatments with immunosuppressive drugs, broad-spectrum bacterial antibiotics and radio-chemotherapies. The increased frequency of such individuals in the community in recent years has led to a significant increase in the reporting of yeast infections. Moreover, increasing numbers of yeast species, other than *C. albicans* and *Cryp. neoformans* have been associated with these infections and are now considered in the list of opportunistic pathogens (Hazen 1995; Hobson 2003; Georgiev 2003). These include yeast species that are frequently found in foods such as *C. krusei*, *I. orientalis*, *P. anomala*, *K. marxianus*, *S. cerevisiae* and various *Rhodotorula* species. Murphy and Kavanagh (1999) have drawn specific attention to the pathogenic potential of *S. cerevisiae*.

Epidemiological statistics suggest that many yeast infections in hospitalized patients originate from yeast contamination of catheters (Douglas 2003; Kojic and Daroviche 2004), and there is increasing concern that foods could be a significant

source of yeasts in the general hospital environment. It is also possible that foods could be a source of yeasts that colonize the intestinal tract, from where they translocate to the blood system, resulting in fungaemia and distribution to infect various organs (Cole et al. 1996). Greater understanding of the yeast ecology of the human gastrointestinal tract is needed.

1.8 Probiotic Yeasts

Probiotics are viable microorganisms that are beneficial to the host when consumed in appropriate quantities. Lactic acid bacteria are widely recognized as the main probiotic species but there is increasing interest in adding other organisms to the probiotic list, including yeasts (Klaenhammer 2001). Live *S. cerevisiae* preparations have been used as supplements to animal and poultry feeds for many years, and have been reported to improve the growth and health of these hosts (Lyons et al. 1993). Also, there is an expanding interest in using yeasts as probiotics in the aquaculture industry (Gatesoupe 1995). With respect to humans, *S. cerevisiae* var. *boulardii*, has been successfully used over the last 20 years as an oral, biotherapeutic agent to treat patients with severe cases of diarrhea and other gastrointestinal disorders (McFarland and Bernasconi 1993; Czerucka and Rampal 2002). The yeast colonizes the intestinal tract and, in this context, acts in a probiotic function. Research to establish its credentials as a probiotic agent that can be added to foods is still in progress (van der Aa Kuhle et al. 2005). However, there are significant concerns about its public health safety because of increasing reports of its association with cases of fungaemia (Cassone et al. 2003). Generally, the concept of using yeasts as human probiotics is at an early stage of development and further research is required. Apart from health benefit and safety issues, probiotic yeasts will also require certain technological properties for use in foods, namely, to remain viable in the food, not to grow in and spoil the food, and not to adversely affect sensory acceptability of the food (Heenan et al. 2004).

1.9 Future Prospects

As mentioned already, harnessing and exploiting the activities of yeasts in food and beverage production requires fundamental knowledge of their ecology, physiology, biochemistry and molecular biology. This knowledge provides the base for genetic improvement strategies (Chap. 13) and the discovery of novel bioreactor and biocatalytic technologies (Chap. 9) that are likely to drive the next generations of product and process development.

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