CHAPTER

8

Contamination: bacteria and wild yeasts in a whisky fermentation

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During whisky production, the yeast *Saccharomyces cerevisiae* utilises fermentable carbohydrates to produce ethanol, carbon dioxide, and other metabolites, many of which contribute to whisky flavour. However, unlike in the brewing of beer, the wort is not boiled, and, as it is illegal in many countries to treat whisky fermentations with antibacterial agents including antibiotics, contamination by a variety of microorganisms cannot be avoided. This chapter deals with the diversity of contaminating microbes, specifically lactic acid bacteria and wild yeasts, that infect whisky production and their impact on fermentation performance and spirit character.

The lack of a boiling stage in distillery fermentations makes microbial contamination inevitable (Table 8.1). Primary routes of contamination are as follows. The grain supply harbours thermotolerant microbes, such as lactic acid bacteria (LAB), wild yeasts, and acetic acid bacteria. These microorganisms are able to survive the mashing temperature. Use of wooden washbacks provides shelter for any microbes present in previous fermentations and allows them to contaminate subsequent fermentations, as these vessels are virtually impossible to sterilise (Dolan, 1976). Contaminated process water used for cleaning and the yeast supply itself may contain very low levels of wild yeasts and LAB. The osmotic stress imposed upon contaminating microbes by wort has some antimicrobial activity; however, it is not until fermentation is underway that the hostility of the resulting environment begins to compromise all but the most adaptable and tolerant microbes. Acetic acid bacteria, such as *Acetobacter* spp. and aerobic yeasts, which are part of the malt microflora, are relatively unscathed by the mashing stage but are unable to survive during increasing anaerobic conditions. Enterobacteria, which may be introduced via contaminated process water, cannot tolerate the fall in pH that occurs due to acid production in distillery fermentations.

The brewery practice of repitching yeast between fermentations is not observed in the distilling industry and as such contamination with *Obesumbacterium* spp., common enteric contaminants of brewery fermentations (Priest and Barker, 2010), is not generally regarded as a problem. Furthermore, the more strongly acidic pH of distillery fermentations prevents the proliferation of such organisms. The primary yeast supply to modern distilleries is typically of high hygienic quality, with contaminants

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Table 8.1 Potential contaminants of distillery fermentations

Contaminant	Source	Process location	Problems caused
Lactic acid bacteria	Malt, grain dust, yeast supply	Throughout, but particularly in late fermentation	Yield reduction, acid and diacetyl production, off-flavours
Acetic acid bacteria	Plant material, process water	Wort, initial stages of fermentation, yeast supply	Acidic off-flavours
Enteric bacteria	Plant material, process water	Wort, initial stages of fermentation, yeast supply	Sulphide and diacetyl production, off-flavours
Wild yeasts	Malt, grain dust, yeast supply	Fermentative yeast occur throughout, aerobic yeast only in initial stages	Yield reduction, fusel oil and diacetyl production, off-flavours

in sufficiently small numbers to usually not be of any significance. However, if brewing yeast is pitched as a secondary yeast (for example, ale yeast from a brewery), another avenue of potential contamination is introduced. The use of ale yeast can introduce *Zymomonas* bacteria, which are responsible for rotten egg and fruity off-odours in beer (Van Vuuren and Priest, 2003), and as such would have a negative impact on any resulting spirit. *Pediococcus* spp. may also be introduced if secondary brewing yeasts are used. These bacteria are known to confer a so-called "sarcina sickness" to beer through the production of acids and diacetyl, and they can produce an extracellular slime responsible for "ropiness" in infected fermentations (Van Vuuren and Priest, 2003), thus contributing to off-flavours in the spirit, as well as compromising fermentation stability. Although both *Zymomonas* and *Pediococcus* have the potential to be serious contaminants in distillery fermentations where secondary yeasts are used, the presence of alcohol, the lack of available nutrients, and the low pH of such fermentations are often sufficient to restrict the effects of infection.

There are differences at this stage between the effect of contamination in malt distilleries and in grain distilleries. The use of 100% malt and wooden washbacks in malt distilleries would appear to render them more susceptible to contamination than larger, industrial-scale grain distilleries, which use pressure-cooked wheat or maize as 90% of the fermentable substrate. The decreased malt content and the use of stainless steel washbacks would appear to make grain distilleries more resilient to microbial infection. However, the large-scale industrial nature of such operations offers alternative routes of contamination, further complicating matters. Issues such as the recycling of process water, blockages in heat exchangers and piping, and larger, more numerous washbacks can all significantly increase the potential for contamination in a grain distillery.

The acidic, anaerobic, ethanolic, and nutritionally bereft environment of whisky fermentation results in the dominating microbes being limited to the pitched *Saccharomyces cerevisiae*, with contaminants represented primarily by LAB, which are part

of the malt microflora and are tolerant to a greater or lesser extent to the conditions discussed above (Van Beek and Priest, 2000). The range of LAB strains and species present in a distillery is typically stable but is subject to fluctuations in malt supply and distillery hygiene practices (Simpson et al., 2001).

The LAB are classified according to the details of their carbohydrate metabolism, specifically the metabolic products of this metabolism. Obligate homofermenting LAB, including *Lactococcus*, *Pediococcus*, *Streptococcus*, and some *Lactobacillus* species, use glycolysis to produce lactic acid as the sole end product (Figure 8.1). Obligate heterofermenting LAB, such as *Leuconostoc* and some *Lactobacillus* species, metabolise carbohydrates using the 6–phosphogluconate/phosphoketolase (6–PG/PK) pathway, producing lactic acid, carbon dioxide, and ethanol or acetic acid (Figure 8.2). Facultative heterofermenters, comprised primarily of various *Lactobacillus* species, are able to utilise glycolytic pathways and the 6-PG/PK pathway depending on environmental conditions (Axelsson, 2004). As LAB are in competition with yeast for carbohydrates during whisky fermentations, the presence and type of LAB present are of considerable importance to the whisky industry.

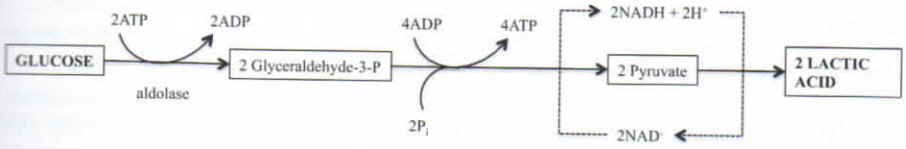

FIGURE 8.1

Homolactic fermentation.

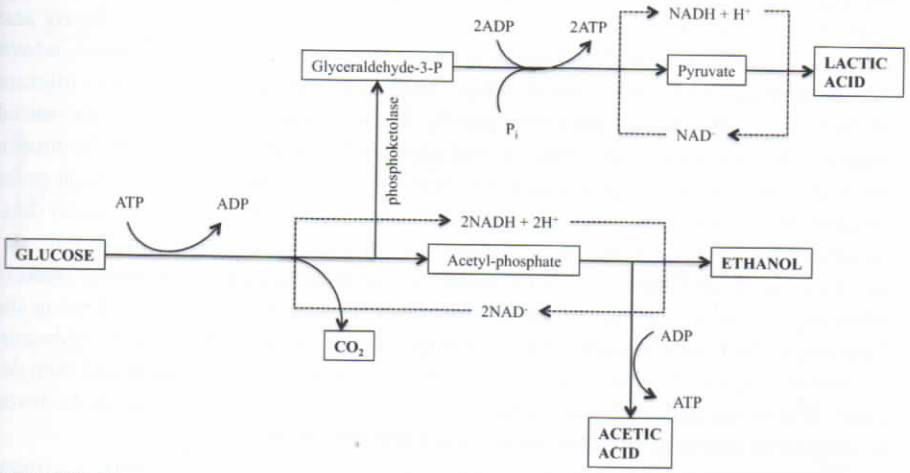

FIGURE 8.2

Heterolactic fermentation.

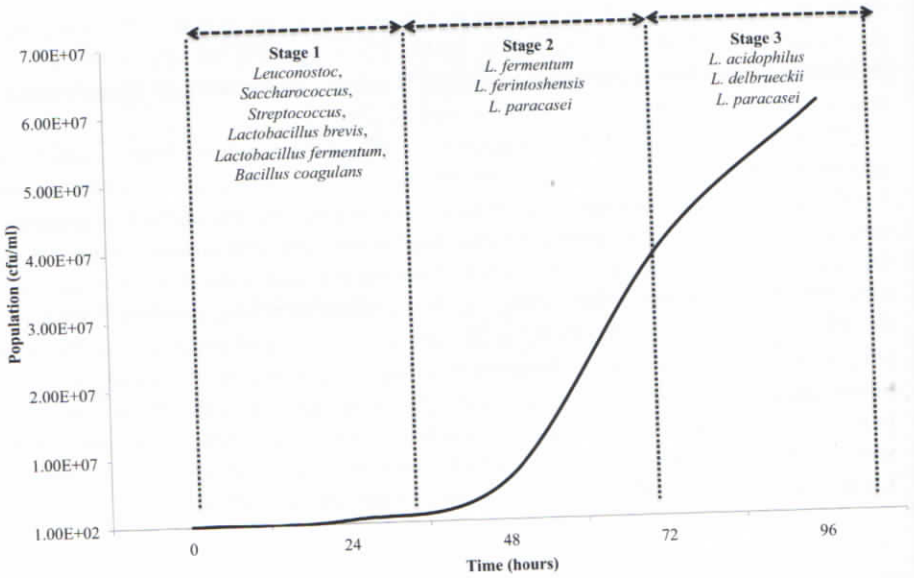

FIGURE 8.3
Evolution and growth of the LAB population throughout fermentation.

The LAB population evolution in whisky fermentations can be broken down into three stages (Figure 8.3). Initially, bacterial diversity is high, declining as fermentation proceeds (Van Beek and Priest, 2003). During the initial stage of fermentation (0 to 30–40 hours), bacterial growth is inhibited by rapid yeast growth and ethanol accumulation (Thomas et al., 2001; Van Beek and Priest, 2002). During this stage, the bacterial flora is primarily comprised of heterofermentative LAB, such as *Leuconostoc*, *Saccharococcus*, and *Streptococcus*, as well as *Lactobacillus brevis* and *Lactobacillus fermentum* (Van Beek and Priest, 2002) and *Bacillus coagulans*, which has been observed in the mash (Cachat, 2005). *Leuconostoc* spp. are less tolerant of high levels of ethanol and consequently do not persist much beyond the initial stages of fermentation. During the second phase of fermentation (30–40 to 70 hours), the yeast population enters a stationary phase and begins to decline, at which point ethanol production is between 80 and 90% complete. As the yeast population dies, lactobacilli such as *L. fermentum*, *L. paracasei*, and *L. ferintoshensis* proliferate, resulting in lactic and acetic acid accumulation, hastening yeast decline, and ultimately allowing for the dominance of lactobacilli (Van Beek and Priest, 2002). During the final stage (70 hours onward), bacterial populations, comprised primarily of homofermenters such as *L. acidophilus*, *L. delbrueckii*, and *L. paracasei*, peak and then decline. Due to the lack of fermentable sugars at this stage, these bacteria are believed to survive on nutrients liberated from dying and autolysing yeast cells.

The presence of LAB in whisky fermentations can be regarded as both detrimental and beneficial, depending on the degree of contamination and how that contamination is managed. High initial levels of LAB ($>10^6$ cells mL^{-1}) are known to reduce

ethanol yield, resulting in a loss of revenue (Makanjoula et al., 1992). Moreover, the presence of a large bacterial population at the start of a fermentation can result in the production of negative flavour characteristics (Van Beek and Priest, 2002). The primary method by which LAB inhibit ethanol production by yeast is through the production of lactic acid and, to a lesser extent, acetic acid. Lactic acid production diverts fermentable substrate away from alcohol production, as each sugar molecule used by LAB results in the loss of two ethanol molecules. Second, lactic acid accumulation lowers the pH, the effects of which are compounded if acetic acid is present (Narendranath et al., 2001). Furthermore, fatty acid production by LAB during distillery fermentations may have an inhibitory effect on yeast metabolism, particularly during the latter stages of fermentation (Lowe and Arendt, 2004).

A low initial LAB population (10^3 to 10^5 cells mL^{-1}) will be kept in check by extensive yeast growth and ethanol accumulation (Thomas et al., 2001) until the yeast population begins to decline and ethanol production ceases. At this point, the LAB population may be allowed to bloom (Figure 8.4). This secondary fermentation does not interfere with ethanol production and allows for the accumulation of desirable flavour and aroma compounds (Van Beek and Priest, 2002).

Although LAB are very much the dominating microbes during whisky fermentations, a background population of wild yeasts has been observed (Neri, 2006). The dominance of a distilling strain of *Saccharomyces cerevisiae* keeps the wild yeast population at a low level (10^1 to 10^4 cells mL^{-1}) which does not appear to evolve during the course of fermentation (Neri, 2006). The contaminating wild yeasts include

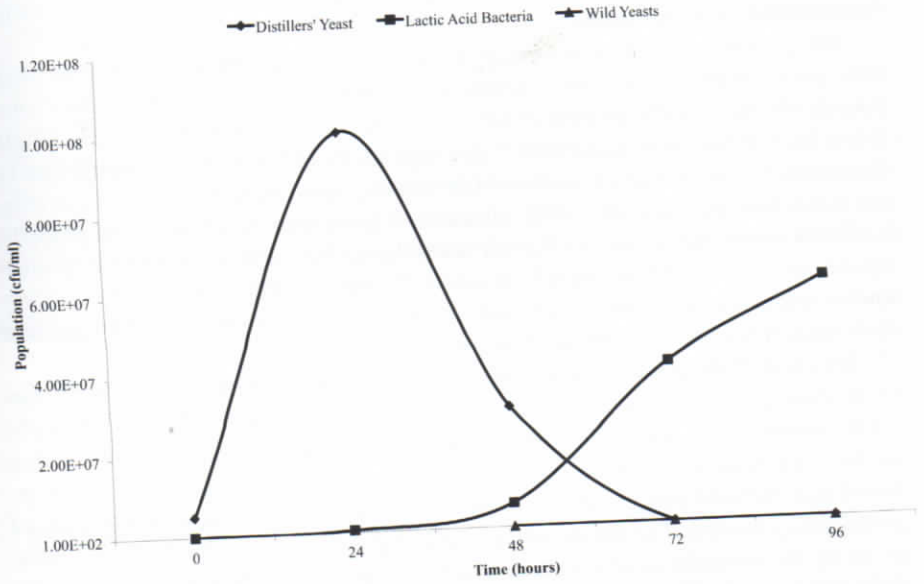

FIGURE 8.4

Microbial profile of a typical distillery fermentation.

various non-distilling strains of *S. cerevisiae*, *Pichia membranifaciens*, *Candida* spp., *Issatchenkia orientalis*, and *Torulaspora delbrueckii*.

Wild strains of *Saccharomyces cerevisiae* have been observed as brewery contaminants, with no perceived adverse effects on fermentation for limited populations. However, their biochemical similarity to distilling yeast means that competition for sugars and the production of potential off-flavours cannot be discounted (Campbell, 2003). It is interesting to note that variants of *S. cerevisiae,* originating during the later stages of fermentation, may represent mutations in the distilling strain (for example, respiratory deficient yeast) as a result of the hostile conditions that occur during prolonged fermentations (approximately 50 hours).

Significant *Pichia membranifaciens* contamination can lead to serious problems due to its aerobic metabolism in high ethanol concentrations (Campbell and Msongo, 1991). Such yeasts are common brewery contaminants (Campbell, 2003) and may be introduced to distillery fermentations through the use of secondary yeasts. *Issatchenkia orientalis*, which is an aerobic yeast known to produce off-flavours and to form a pellicle (scum-like layer) on the surface of contaminated beer fermentations, is a potential contaminant in distilleries using secondary yeasts (Campbell, 2003).

As with wild strains of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, a fermentative yeast, competes with distilling yeast for nutrients due to their biochemical similarity. It is a common contaminant in brewery fermentations, but few deleterious effects have been observed as a result of its presence. In high enough numbers, however, it is likely to contribute to off-flavour development (Campbell, 2003). It has been suggested that this background population may contribute to positive flavour profiles associated with sweet and creamy notes if present during the late lactic fermentation (Wilson, 2008).

The primary congeners in new-make spirit other than ethanol are higher alcohols, esters, aldehydes, ketones, organic acids, carbonyls, phenols, and sulphur compounds (Palmer, 1997), as well as fatty acids and lactones (Wanikawa et al., 2000). It has been extensively documented that high levels of LAB contamination can be detrimental to the perceived quality of the resulting spirit through a reduction in ethanol yield (Makanjoula et al., 1992). However, if fermentations are managed properly, LAB can contribute to positive flavour notes during late lactic fermentation, primarily due to the elevated production of esters (Campbell, 2003). What is less widely known is how the presence of such contamination affects congener quantity and how such differences are reflected in spirit quality.

The most abundant congeners in new-make spirit are the higher alcohols, primarily propanol, isobutanol, and amyl and isoamyl alcohols. As with ethanol yield, early LAB contamination will reduce the higher alcohol concentration; because higher alcohols are believed to confer positive green/grassy notes in whisky, it can be inferred that reduced concentrations of higher alcohols will result in a reduction of green/grassy aromas. The development of green/grassy notes in whisky is also influenced by the presence of wild yeasts, which produce higher alcohols and aldehydes (Wanikawa et al., 2002). Although available data show little correlation between wild yeast contamination and green/grassy flavour development (Neri, 2006), there

is some evidence to suggest a positive correlation between wild yeast contamination (*Torulaspora delbrueckii*) and green/grassy aroma when combined with late lactic fermentation, particularly in the presence of *Lactobacillus paracasei* and *L. plantarum* (Wilson, 2008), with elevated concentrations of acetaldehyde, propanol, and isobutanol. It is probable that additional contaminant-derived compounds contribute to green/grassy flavour development, including heptanol, octanol, nonanol, and various *cis-* and *trans-* isomers of nonenal (Wanikawa et al., 2002).

Late lactic fermentation is known to impart fruity and estery notes in whisky, due to lactic acid accumulation and subsequent increases in the ethyl lactate concentration (Van Beek and Priest, 2002). Contaminating homofermentative LAB will produce more lactic acid than heterofermenters. Late lactic fermentation with obligate homofermenters or facultative heterofermenters will result in an increased ethyl lactate concentration in the spirit, when compared to a lactic fermentation with obligate heterofermentors (Wilson, 2008). The influence of ethyl lactate, as well as ethyl acetate, ethyl hexanoate, and ethyl octanoate, on the development of fruity and estery notes is inconclusive on a laboratory scale (Wilson, 2008). It is likely that the perceived organoleptic benefits of late lactic fermentation are due to the complex interactions of numerous esters and similar compounds, occurring in both the fermentation and distillation stages on an industrial scale.

In addition to the general positive effects on spirit quality conferred by late lactic fermentation, small levels of specific compounds with low sensory thresholds can have an important organoleptic impact. One such group of compounds are lactones. The lactones known to be present in whisky include β-methyl-γ-octalactone, a cask derivative from maturation; γ-nonalactone derived from malt; and γ-decalactone and γ-dodecalactone, which are produced during fermentation and contribute to the development of pleasant sweet and buttery notes (Wanikawa et al., 2000). LAB (*Lactobacillus brevis*) and wild yeast (*Torulaspora delbrueckii*) contamination affects the accumulation of these potent flavour compounds, which are formed by yeasts through either *de novo* synthesis or the biotransfomation of hydroxy fatty acids (Wanikawa et al., 2000). In the latter stages of fermentation, LAB antagonise the yeast population by producing hydroxy fatty acids, specifically 10-hydroxypalmitic and 10-hydroxystearic acids, which are then oxidised by yeast to produce γ-decalactone and γ-dodecalactone, respectively. Production of these lactones is increased further if *T. delbrueckii* is present (Wilson, 2008). The production of diacetyl (butanedione) by *L. brevis*, which is also increased in the presence of *T. delbrueckii*, adds further complexity to the perception of a sweet/buttery aroma conferred by late lactic fermentation (Wilson, 2008).

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