

Yeast, cocoa beans and chocolate



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Yeast play a key role in the fermentation of many foods and beverages. The best known examples are bread, beer and wine, where understanding of the ecology, biochemistry, physiology and genomics of the yeast contribution is well advanced^{1,2}. Yeast also have prominent roles in the production of other well-known commodities, such as cheeses, salami-style meat sausages, and soy sauce, where their activities in the fermentation and maturation processes are attracting increasing research². Still, there are many other products where yeast have a significant role in fermentation, but aspects of their contributions and how these impact on product quality remain a mystery. Such products include many indigenous fermented foods of Asia, Africa and Central and South America³, and two economically important cash crops, cocoa beans and coffee⁴. Consider life without chocolate or good quality coffee! We have been studying cocoa bean fermentations in Indonesia⁵ and now in North Queensland, Australia. In this article, we review the role of yeast in the production of cocoa beans and chocolate.

Cocoa beans and chocolate

Cocoa beans are the principal raw material of chocolate manufacture. The beans originate as seeds in fruit pods of the tree *Theobroma cacao*, which is cultivated in plantations in tropical regions of the world⁶. Ivory Coast, Ghana, Indonesia and Brazil, are the leading producers of cocoa beans. However, cocoa beans can be successfully cultivated in North Queensland, where there is increasing interest in developing a cocoa-chocolate industry. Globally, the chocolate industry is valued at about US\$70 billion, annually, originating from about 3500 tonnes of fermented beans that sell for approximately US\$1500 per tonne⁷.

Cocoa pods are about 20 cm x 10 cm in size and contain 30–40 beans embedded in a white mucilaginous pulp (Figure 1). Fermentation is essential to release the beans from the pulp and to initiate the chemical changes necessary for the development of characteristic chocolate flavour and colour. Figure 2 outlines the steps in chocolate production. Cocoa pods are harvested from the tree, and the beans and pulp are manually or mechanically removed for fermentation. The fermented beans are dried and

sold to chocolate manufacturers who roast, mill and grind them to produce cocoa liquor, cocoa butter and cocoa powder for use in chocolate making⁸.

Individual beans (seeds) consist of an outer coat (testa) that envelopes two cotyledons (embryo). The seeds are surrounded by the mucilaginous pulp (endocarp). The pulp represents about 40% of the extracted material. It is a rich medium for microbial growth, containing 10–15% of fermentable sugars (glucose, fructose, sucrose), 1–2% pectin, and a relatively high content of citric acid (1–3%) that gives a pH of 3.2–3.8. The cotyledons are packed with cells containing lipids (55–60%), proteins (20%), starch, polyphenols, anthocyanins and alkaloids⁶.

Cocoa bean fermentation

In most countries, cocoa bean fermentations are conducted as traditional, village scale operations^{6,8}. In West Africa, the beans are massed into 25–1000 kg heaps on the ground, covered with banana leaves, and allowed to ferment. In some cases, they are fermented as a shallow layer on the surface of wooden trays. In South-East Asia, the beans are mostly fermented in approximately 1m³ wooden boxes. Fermentation requires four to six days, depending on the bean cultivar and frequency of mixing. Mixing aerates the beans and provides some physical uniformity, but its application is not consistent. Fermentation temperature is not controlled, but typically starts at about 25°C, and increases



Figure 1: Fresh cocoa
a. Cocoa pods cut showing beans surrounded by pulp
b. Close up of fermenting cocoa beans.

to 45–50 °C from heat generated by the fermentation. After fermentation, the beans are dried for about six days in the sun or in drying ovens.

Fermentation is an uncontrolled process conducted by indigenous micro-organisms that originate from the surfaces of cocoa pods, leaves, fermentation vessels, hands of workers, insects, etc. The microbial ecology of the process is complex and has been researched for over 100 years. Various species of yeast, acetic acid bacteria, lactic acid bacteria and *Bacillus* are involved^{5,6} but linkages between the growth and activity of individual species and chocolate quality are not clearly defined. The yeast, lactic acid bacteria and acetic acid bacteria grow very quickly in the pulp material, reaching maximum populations of about 10⁸ cfu/g within 24–72 hours. Subsequently, their populations decrease, eventually giving way to the growth of *Bacillus* species that are more tolerant of the higher temperatures (45–50 °C) that prevail in the later stages of fermentation.

Microbial activity during fermentation causes physical and chemical changes that impact on bean physiology and biochemistry and, ultimately, chocolate quality⁶. Degradation of pulp facilitates access of oxygen to the bean mass. The production and utilisation of acids, especially utilisation of citric acid by lactic acid bacteria and yeast, causes a decrease in pH of the bean cotyledons from about 7.0 to 5.0–5.5. Most significantly, ethanol produced by yeast, and acetic acid that is produced from oxidation of ethanol by the acetic acid bacteria, readily diffuse into the cotyledons. Ethanol and acetic acid combined with the temperature increase, kill the embryo of the beans leading to a breakdown in the cellular structure and organisation of the cotyledons⁶. These changes activate several groups of bean enzymes whose reactions are essential to the development of chocolate character⁹. Proteases break down globulin storage proteins of the bean into flavour-active peptides

and amino acids that participate in Maillard reactions during bean roasting. Polyphenol oxidases decrease bitterness and astringent properties by oxidising bean polyphenols, such as the catechins, anthocyanins and proanthocyanidins, and also contribute to colour development. Anthocyanidin pigments are glycosylated and are decolourised by glycosidase action. Invertase hydrolyses bean sucrose, giving glucose and fructose that contribute to Maillard reactions. Methyl pyrazines developed during bean roasting give specific chocolate aromas¹⁰.

The role of yeast

Beans and pulp that are aseptically extracted from undamaged, healthy cocoa pods are sterile and contain no detectable yeast. However, this is not the case in commercial operations, and freshly extracted material generally has yeast 10³–10⁵ cfu/g. The sugary-acid properties of the pulp present an excellent environment for the growth of yeast that quickly proliferate to 10⁷–10⁹ cfu/g. As the fermentation progresses, they eventually die off, being unable to tolerate the combined stresses of higher temperature and increased concentrations of ethanol and acetic acid (Figure 3). Although a diversity of species has been isolated from cocoa fermentations in different regions of the world, some consistent trends have emerged⁶. Essentially, three phases of yeast growth and activity are evident. Species of *Hanseniaspora* (anamorph, *Kloeckera*) dominate the first stage of fermentation. These yeast are not particularly ethanol tolerant and die off as more fermentative, ethanol producing species start to grow and dominate the middle stage of fermentation. Various strains of *Saccharomyces cerevisiae* become significant at this time. Several species of *Candida* are also found during this stage and become the dominant yeast in the final stages of fermentation. Notable species include *Candida pelliculosa* (*Pichia membranifaciens*) and *Candida krusei* (*Issatchenkia orientalis*). We have confirmed these observations in studies of

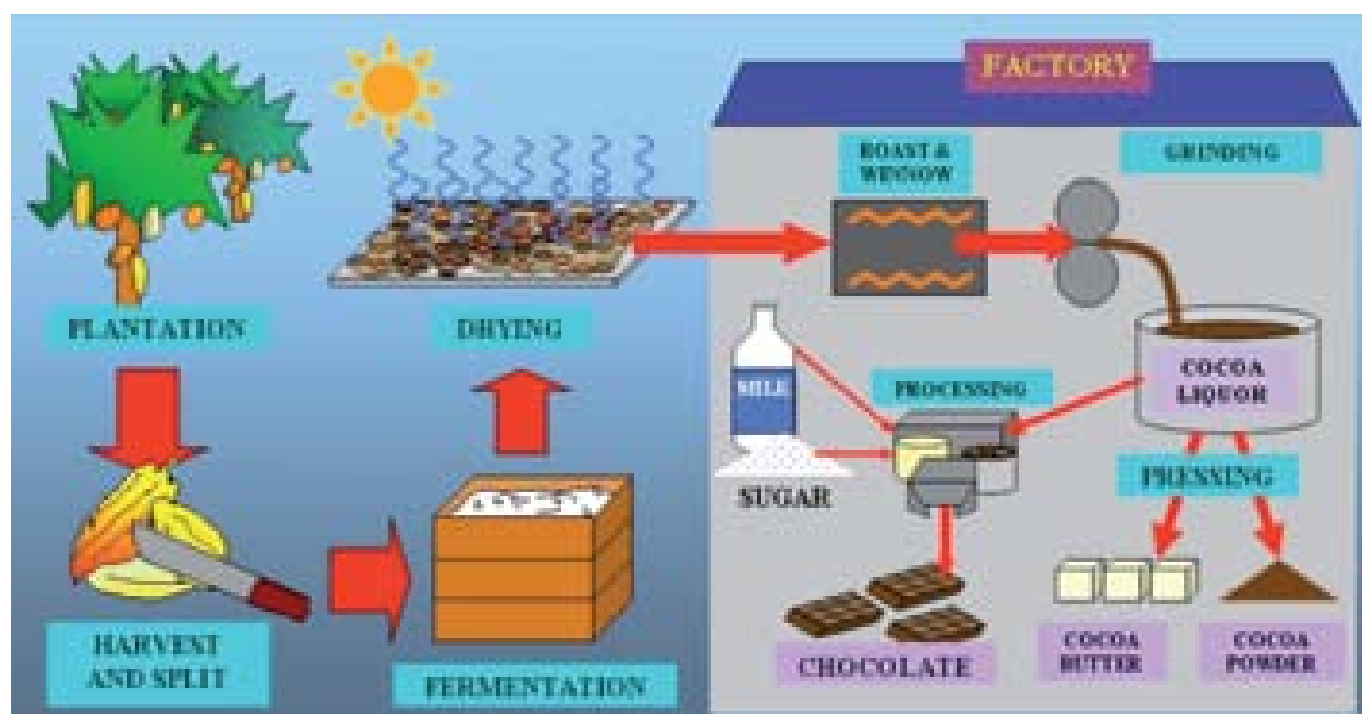


Figure 2.

cocoa beans fermented in Indonesia⁵, and in North Queensland. Culture-independent, molecular analyses of cocoa beans fermented in Ghana also support these conclusions¹¹. In those studies, PCR-denaturing gradient gel electrophoresis was used to examine DNA extracts of fermenting beans. While these analyses confirmed key contributions from the species already mentioned, they also showed that other novel yeast are present and require further study.

Yeast serve several functions during cocoa bean fermentation^{5,6}. Their main activity is metabolism of pulp sugars to produce ethanol, and an array of secondary metabolites. Ethanol concentrations of 5–10% are produced, and this has a selective effect on the species of bacteria that contribute to the fermentation. In particular, ethanol encourages the growth of acetic acid bacteria. As noted already, ethanol and acetic acid have key roles in killing the beans, and triggering endogenous enzymatic reactions that produce the precursors of chocolate character. Many of the secondary metabolites produced by yeast are flavour-active and are likely to be taken up by the cotyledons, to impact on chocolate flavour. Some yeast utilise the citric acid of the pulp, and this contributes to the equilibration of cotyledon pH, which impacts on endogenous enzyme activity⁹. Some strains of *Saccharomyces*, *Candida* and *Pichia* isolated from fermenting beans are pectolytic and are involved in pulp degradation⁶. After fermentation, the beans are dried and this is a stage when filamentous fungi can grow and compromise chocolate safety by producing mycotoxins. We have observed that fungal growth at this stage can be controlled by the species of yeast predominating in bean fermentation because they have anti-fungal properties.

Although the predominance and importance of yeast in cocoa fermentations are well recognised, we have little understanding of how individual species or strains influence bean quality and chocolate character, why certain species are consistently present, and whether or not contributions from all the different species are really necessary.

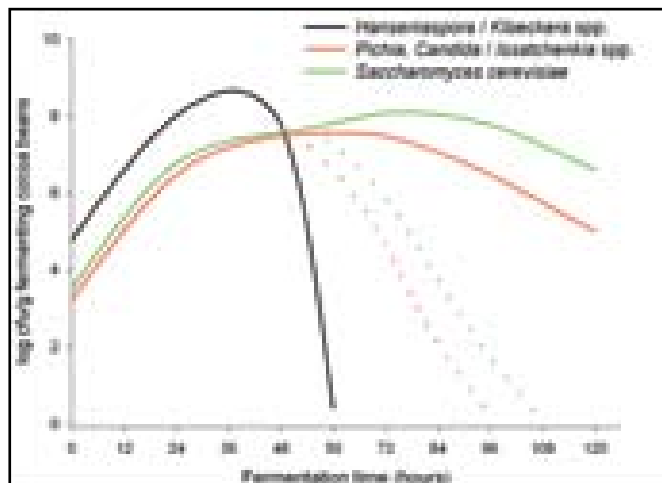


Figure 3 – Growth of yeast species during fermentation of cocoa beans. Note: The dotted line indicates death of the yeast, which occurs as the temperature of the bean mass approaches 50°C.

Controlled fermentations – the challenge

The quality of cocoa beans sold on the international market varies considerably. Lack of control over the fermentation is a major factor that leads to this inconsistency. The concept of controlling the fermentation by inoculating beans with starter cultures of specific microorganisms is not new, but has attracted little research⁶. The complexity in the yeast and bacterial ecology of the fermentation presents a challenge in selecting individual species or mixtures of species for development as starter cultures. Some studies have used pectinolytic strains of *S. cerevisiae* as starter organisms, and reported the production of beans that gave good quality chocolate⁶. However, indigenous yeast and bacteria also grew during these fermentations, thereby compromising interpretation of the data obtained. In commercial operations freshly extracted cocoa beans are invariably contaminated with indigenous microflora that are likely to compete with any inoculated organisms. This circumstance is not unusual, and a good analogy exists in the fermentation of grape juice into wine. Starter cultures of yeast are used extensively in wine production, and these successfully compete with indigenous micro-organisms that also occur in the grape juice¹².

In starting a new cocoa industry in North Queensland, we have the opportunity to develop a controlled, industrialised process based on the use of selected starter micro-organisms. Laboratory and small scale industrial fermentations have been conducted using different yeast species as starter cultures. These fermentations have been monitored for their microbial ecology, and changes in the chemical composition of the beans. Chocolate prepared from the beans has been examined for its sensory quality. Using this approach, we can demonstrate how different yeast species impact on cocoa bean quality, and chocolate character, and have developed an experimental protocol integrating microbiological, biochemical and sensory analyses that will advance scientific research of cocoa bean fermentations.

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