Abstract

Zoi Papalexandratou

Freshly harvested cocoa beans have an astringent, bitter, and unpleasant flavour. Post-harvest curing, including fermentation and drying of the cocoa-pulp bean mass out of the cocoa pods, represents the first step of chocolate production. The fermentation step is still a spontaneous microbiological process carried out mainly in heaps and boxes, or on platforms. It starts after the opening of the cocoa pods at the fields, when the mucilaginous pulp that surrounds the cocoa beans is contaminated by microorganisms present in the direct environment. Up to now, three main fermentation phases have been distinguished, although overlap occurs. They include consecutively the growth of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). In parallel, complex enzymatic reactions are initiated in the beans that are responsible for the formation of the colour and flavour precursors of well-fermented dry cocoa beans.

The present study dealt with a comparison of the microbial species diversity, community dynamics, and metabolite kinetics of cocoa bean fermentations carried out in different important cocoa-producing regions worldwide, namely in Ghana, Ivory Coast, Brazil, Ecuador, and Malaysia, thereby focusing primarily on the impact of the cocoa variety, the method of fermentation (heap, box, platform), and/or local operational practices on the farm on the quality of fermented (dry) cocoa beans and chocolates produced thereof. Therefore, a multiphasic approach was performed, based on extensive microbiological analyses, both culture-dependently and culture-independently in combination with metabolite target analysis of samples taken during the whole cocoa bean fermentation and/or drying process, followed by chocolate making from the concomitant fermented dry beans and sensory evaluation of the chocolates made.

The multiphasic approach mentioned above unravelled the consecutive succession of yeasts (mainly *Hanseniaspora opuntiae*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae*), enterobacteria (mainly *Tatumella* spp.), LAB (mainly *Fructobacillus tropeaoli*-like, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Leuconostoc pseudomesenteroides*), and AAB (mainly *A. pasteurianus* and *Acetobacter senegalensis*) during cocoa bean fermentation, independent of fermentation method, cocoa variety or cocoa-producing region, provided the cocoa bean fermentations were carried out with care (well-maintained plantations and equipment, mature cocoa pods, separation of healthy and infected pods and beans, removal of the placenta, regular mixing of the fermenting cocoa pulp-bean mass, and controlled drying). Whereas yeasts were responsible for ethanol production out of glucose, heterofermentative LAB produced lactic acid out of glucose and citrate and reduced fructose to mannitol, and AAB oxidised ethanol into acetic acid and overoxidised acetic acid and lactic acid into carbon dioxide and water. In addition to yeasts, enterobacteria may be held responsible for pectin degradation. Also, they may be held responsible for citric acid conversion, besides LAB, and for gluconic acid production. From fermentations carried out with high-quality raw materials and characterised by a restricted microbial species diversity, successfully fermented cocoa beans and good chocolates produced from the concomitant fermented dry beans could be obtained. It could be assumed that the prevailing species *H. opuntiae*, *S. cerevisiae*, *Lb. fermentum*, and *A. pasteurianus* were responsible for it. Hence, selected strains of these species may be proposed as starter culture for uniform, fast, and successfully controlled cocoa bean fermentations.

To conclude, a multiphasic analysis approach of the cocoa bean fermentation process worldwide revealed that a restricted yeast and bacterial species diversity participates in this fermentation and that this microbiota is independent of the cocoa-producing region, cocoa variety, and cocoa fermentation method. Moreover, a restricted yeast and bacterial species diversity seems to be required for successful cocoa bean fermentations. This means that high-quality fermented dry cocoa may be obtained in any cocoa-producing region, provided the fermentations are carried out with care. This may be obtained by applying good agricultural (good maintenance of the plantations, careful cocoa pod/bean selection) and operational farming practices (clean equipment, regular mixing, control of drying conditions). Finally, although spontaneous cocoa bean fermentations last long (5-6 days), they can lead, with low cost, to superior raw materials for chocolate production when correct practices are applied.