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Review

Fungi and mycotoxins in cocoa: From farm to chocolate

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ABSTRACT

Cocoa is an important crop, as it is the raw material from which chocolate is manufactured. It is grown mainly in West Africa although significant quantities also come from Asia and Central and South America. Primary processing is carried out on the farm, and the flavour of chocolate starts to develop at that time. Freshly harvested pods are opened, the beans, piled in heaps or wooden boxes, are fermented naturally by yeasts and bacteria, then dried in the sun on wooden platforms or sometimes on cement or on the ground, where a gradual reduction in moisture content inhibits microbial growth. Beans are then bagged and marketed. In processing plants, the dried fermented beans are roasted, shelled and ground, then two distinct processes are used, to produce powdered cocoa or chocolate. Filamentous fungi may contaminate many stages in cocoa processing, and poor practices may have a strong influence on the quality of the beans. Apart from causing spoilage, filamentous fungi may also produce aflatoxins and ochratoxin A. This review deals with the growth of fungal species and formation of mycotoxins during the various steps in cocoa processing, as well as reduction of these contaminants by good processing practices. Methodologies for fungal and mycotoxin detection and quantification are discussed while current data about dietary exposure and regulation are also presented.

Contents

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1. Introduction

Cocoa is an important crop, as it is the raw material from which chocolate is manufactured. Cocoa trees thrive best in very humid tropical climates, and are grown mainly in West Africa although significant quantities also come from Asia and Central and South America. Primary processing is carried out on the farm. Pods are harvested and opened by hand. The beans are piled in heaps or in wooden boxes, and allowed to undergo a natural fermentation. Microorganisms contaminate the beans from the outer surfaces of pods, workers' hands and tools, plant leaves, collection baskets, insects or residual mucilage in equipment. The desirable types are yeasts, and lactic or acetic acid bacteria, which secrete enzymes, alcohol and lactic and acetic acids (Ostovar and Keeney, 1973; Schwan and Wheals, 2004). These metabolic products lead to embryo death and production of important precursors of chocolate aroma (Voigt, 2013). After fermentation, which lasts several days, the beans are transferred to wooden sun drying platforms, or sometimes are dried on cement or on the ground, where a gradual reduction in moisture content and volatile acid production occurs, eventually stopping microbial growth and enzyme production. When the beans are fully dry, they are transferred to storage rooms, then later bagged and marketed. In processing plants, the dried fermented cocoa beans are roasted, shelled and ground, then two distinct processing lines produce powdered cocoa or chocolate.

Cocoa beans are susceptible to fungal contamination during many of these processing steps. Microbial growth is affected by intrinsic parameters of cocoa beans such as pH, by water activity and by the various organic acids produced during fermentation. Besides causing deteriorative alteration of sensorial properties, the presence of filamentous fungi in cocoa and chocolate is also a cause for concern due to the possibility of mycotoxin formation. Both aflatoxin and ochratoxin A have been reported from cocoa and chocolate.

The discovery of ochratoxin A in cocoa and by-products prompted international discussions. In 2012 the Codex Committee on Contaminants in Food elaborated a discussion paper on ochratoxin A in cocoa (Codex Alimentarius Commission, 2012). Information was gathered on the occurrence of ochratoxin A in cocoa and by-products to determine levels of contamination, the contribution of these products to ochratoxin A in the human diet, to elucidate the main factors responsible for ochratoxin A synthesis in cocoa and to reduce it during processing. A code of practice was formulated and is under discussion (Codex Alimentarius Commission, 2013).

This review focuses on the factors affecting the development of filamentous fungi and the potential for formation of aflatoxins and ochratoxin A at all processing stages. An update on international reports of mycotoxins in cocoa and chocolate is also provided.

2. Fungi and mycotoxins in farm processing of cocoa

2.1. Fermentation

Beans and pulp inside an intact, healthy cocoa pod are microbiologically sterile (Fig. 1, 1A–C) but when opened soon become contaminated with microorganisms, especially those that will contribute to the subsequent natural fermentation process.

During fermentation, the microbial population is dominated by yeasts in the first hours after which their level is surpassed by those of lactic acid bacteria, that in turn decline after 48 h of fermentation after which acetic acid bacteria develop, in a well established pattern of succession (Schwan and Wheals, 2004; Nielsen et al., 2013). Lima et al. (2011) present in detail the importance of fermentation and environmental factors in the development of a good quality product. The products of microbial metabolism strongly influence the microbial population that sequentially dominate this micro-environment. High amounts of alcohol are produced by yeasts and lactic and acetic acids by bacteria. Together with environmental factors including low pH, the action of the organic acids, elevated temperatures due to exothermic reactions and microaerophilic conditions, these fermentation products restrict the growth of filamentous fungi. Studies reported the presence of filamentous fungi especially in the last days of fermentation, in the surface (Fig. 1, 2A) (Schwan and Wheals, 2004; Copetti et al., 2013a) or when the cocoa mass is not turned regularly (Nielsen et al., 2013). Filamentous fungi can sometimes be seen in the cocoa inside the fermentation boxes when the mass is turned (Fig. 1, 2B).

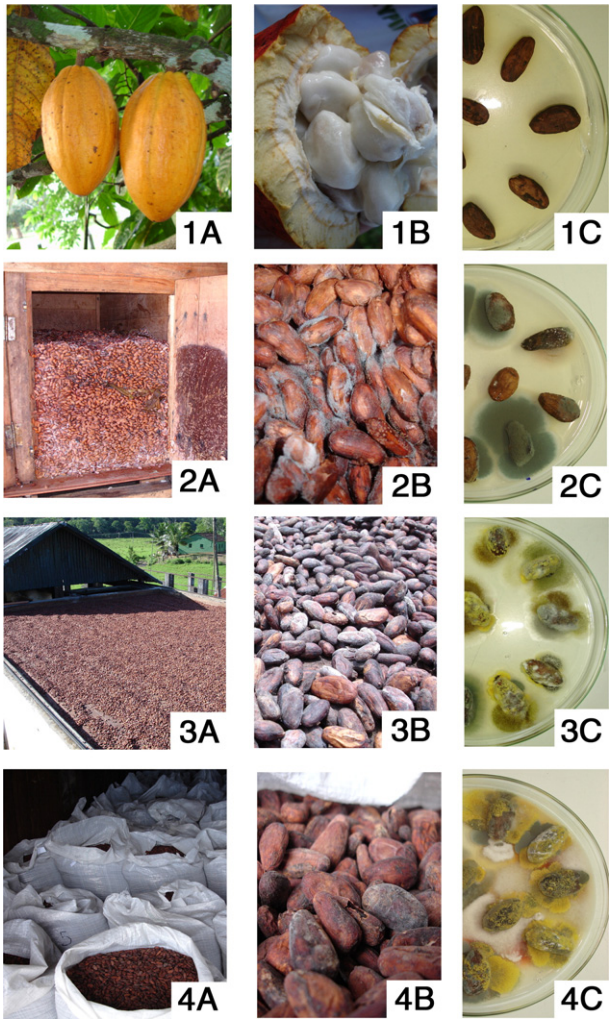

Fig. 1. 1A, cocoa pod; 1B, cocoa beans surrounded by pulp; 2A, 2B, fungal presence during fermentation; 3A, sun drying of cocoa beans; 3B, mouldy cocoa beans at drying; 4A, storage of cocoa beans; 4B, mouldy cocoa beans in storage; 1C, 2C, 3C, 4C, mycological evaluation of cocoa beans by direct plating in DG18, 1C, before fermentation; 2C, during fermentation; 3C, during drying; 4C, during storage.

The role of filamentous fungi during cocoa fermentation is not well understood. It is known that some species can cause hydrolysis of the pulp, produce acids or off flavours and so alter the taste of the cocoa beans (Ardhana and Fleet, 2003; Schwan and Wheals, 2004). Extensive fungal development at the end of fermentation may cause increased deterioration in the drying phase (Gilmour and Lindblom, 2008).

After studying the microbial ecology of cocoa fermentation in wooden boxes in Indonesia, Ardhana and Fleet (2003) observed the presence of *Penicillium citrinum* and an unidentified basidiomycete in the first 36 h of fermentation. Both fungi showed strong polygalacturonase activity, suggesting their role in the degradation of pulp in the early stages of fermentation. The presence of *Aspergillus versicolor*, *Aspergillus wentii* and *Penicillium purpurogenum* was also reported (Ardhana and Fleet, 2003).