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Short communication

Filamentous fungi producing ochratoxin a during cocoa processing in Cameroon

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Abstract

Ochratoxin A (OTA) is the main mycotoxin occurring in cocoa. A study was conducted in Cameroon to assess how filamentous fungi and toxigenesis were affected by the type of cocoa post-harvest treatment (boxes or heaps). The filamentous fungi isolated were almost identical when fermentation was carried out in boxes or heaps, with the presence of abundant black *Aspergillus* filamentous fungi: *A. niger* and *A. carbonarius*. Filamentous fungi were more abundant at the end of the harvesting season. Factors affecting bean integrity (poor handling, deferred processing) resulted in a qualitative and quantitative increase in contamination, when the total number of filamentous fungi could reach a maximum value of $5.5 \pm 1.4 \times 10^7$ CFU g^{-1} and black Aspergilli a maximum value of $1.42 \pm 2.2 \times 10^7$ CFU g^{-1}. A toxigenesis study showed that *Aspergillus carbonarius* was the main OTA-producing strain isolated. Its maximum production could reach 2.77 μg g^{-1} on rice medium. *Aspergillus niger* strains did not always produce OTA and their toxigenesis was much lower. Fermented dried cocoa from poor quality pods was the most contaminated by OTA: up to 48 ng g^{-1}.

Keywords: OTA; Cocoa; Filamentous fungi; *Aspergillus carbonarius*; *Aspergillus niger*

1. Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite produced by several species of *Aspergillus* and *Penicillium* genera. It attracts particular attention through the damage it does to the organism of humans and animals (Abarca et al., 1998). It has nephrotoxic (Mantle and McHugh, 1993), immunotoxic, teratogenic and carcinogenic properties (Kuiper-Goodman and Scott, 1989; Kuiper-Goodman, 1996; Höhler, 1998). OTA has been associated with Balkan Endemic Nephropathy (BEN) and tumour development in the urinary tract (Mantle and McHugh, 1993). Following experiments on animals, the International Agency for Research on Cancer (IARC, 1993) classed OTA as carcinogenic for humans (group 2B).

Ochratoxin A is mainly produced by *Aspergillus carbonarius*, *A. niger* and *A. ochraceus* in tropical zones, and by *Penicillium verrucosum* and *P. nordicum* in temperate zones (Pitt et al., 2000; Abrunhosa, et al., 2001; O'Callaghan et al., 2003). Studies have been conducted to determine the degree of OTA contamination in several foodstuffs and drinks (Thirumala-Devi et al., 2001; Pittet et al., 1996; WHO, 2001; Gareis and Scheuer, 2000; Skaug, 1999; Blanc et al., 1998; Hurst and Martin, 1998; Jorgensen, 1998). Given its existence in several consumer products, consumer exposure to OTA is increasing. In order to protect consumers, the European Union has drawn up a standard defining tolerable contamination limits. Other products, such as cereals, coffee and wine are already covered by international regulation (European

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Commission, 1995; Règlement (CE) n°472/2002), which is not yet the case for cocoa. Given the extent of cocoa consumption worldwide, the European Union will not delay in defining maximum contamination limits.

World cocoa production is estimated at 3 592 000 tons (ICCO, 2006). Fermentation is the main stage in cocoa post-harvest processing. It is generally carried out in a traditional manner by spontaneous fermentation. First of all, there is colonization by yeasts, followed by lactic bacteria, and then by acetic bacteria, which are finally replaced by aerobic sporulated bacilli (Schawn and Wheals, 2004; Thompson et al., 2001; Lopez and Dimick, 1995; Lehrian and Patterson, 1983). Recent studies dealed with yeast (Jespersen et al., 2005) and bacteria (Camu et al., 2007) populations associated with cocoa fermentations. Their succession and respective implication during fermentation were investigated using molecular-based methods and the understanding of the process was subsequently improved (Nielsen et al., 2007). However, very few studies exist on cocoa filamentous fungi during technological treatments. The main species isolated during natural fermentation of cocoa in Indonesia comprise *Penicillium citrinum*, *Kloeckera apis*, *Saccharomyces cerevisae*, *Candida tropicalis*, *Lactobacillus cellobiosus*, *Lactobacillus plantarum* and *Acetobacter pasteurianus* (Ardhana and Fleet, 2003). In the Dominican Republic, a predominance of yeasts of the genera *Kloeckera* and *Candida* is found at the beginning of fermentation, followed by *Lactobacillus pentosus*, *Lactobacillus paracasei* subsp. *paracasei* and *Lactobacillus brevis* as the lactic bacteria and *Acetobacter lovaniensis* as the main acetic bacterium (Lagunes-Gálvez et al., 2007). Whilst several studies have been carried out on the evolution of filamentous fungi in coffee and its relation with OTA content during post-harvest processing (Suàrez-Quiroz et al., 2004; Wilkens and Jörissen, 1999; Studer-Rohr et al., 1995; Micco et al., 1989), that is not the case for cocoa. A microbiological analysis on cocoa samples from 9 producing countries led to the isolation of *Aspergillus fumigatus* and *Rhizomucor pusillus* (Niles, 1981). There have been no studies on filamentous fungi and OTA-producing species in cocoa depending on the type of post-harvest processing.

High contamination frequencies have been found in cocoa samples and by-products. Burdaspal and et Legarda (2003) showed that OTA was found in 99.7 % of chocolate and cocoa powder samples. Contamination of 81.3% was also described in cocoa by-products by Miraglia and Brera (2002). Tafuri et al. (2004) found OTA contamination of between 0.22 and 0.77 μg kg^{-1} in 10 samples of cocoa powder found on the Italian market. A study involving 46 cocoa samples of different origins found that 63 % of samples were contaminated by OTA, with an average contamination of 1.71 μg kg^{-1} (Amézqueta et al., 2004). A maximum content of 100 μg kg^{-1} was obtained with cocoa contaminated artificially (Hurst and Martin, 1998). Shelling by hand helped to reduce contamination levels in cocoa beans by more thane 95% (Amézqueta et al., 2005).

The purpose of our study was to list and identify the fungi that colonize cocoa beans at different stages of processing, depending on the type of post-harvest process, and to study their potential for producing OTA.

2. Materials and methods

2.1. Cocoa

The cocoa pods (*Theobroma cacao* L.) were harvested by hand during the 2005 cocoa season in the Kumba region of Cameroon.

2.2. Cocoa fermentation

Two types of fermentation were studied: box fermentation, where the beans were placed in wooden boxes measuring 45 cm × 45 cm × 45 cm, and heap fermentation where the beans were tipped onto banana leaves placed on the ground. Fermentation was carried out in each case using 50 kg of beans. The heap was then covered with other banana leaves. After harvesting in the field, the pods were opened either immediately or later. Depending on the type of fermentation, pod condition or delay in pod opening, four treatment variants were investigated (Fig. 1): heap fermentation of beans from whole pods that had been opened immediately (T1), box fermentation of beans of the same type (T2), heap fermentation of beans from whole pods opened after 10 days (T3) heap fermentation of beans from damaged pods also opened after 10 days (T4). In the last case, the pods were partially opened after harvesting. Natural drying (in the sun) was carried out for between 5 and 10 days. Three fermentations of each type were performed during the cocoa season: at the beginning (September–October), in the middle (October–November) and at the end of the season (November–December).

2.3. Sampling

Cocoa samples were taken at different stages of processing (Fig. 1). They involved unfermented beans (A), fermented

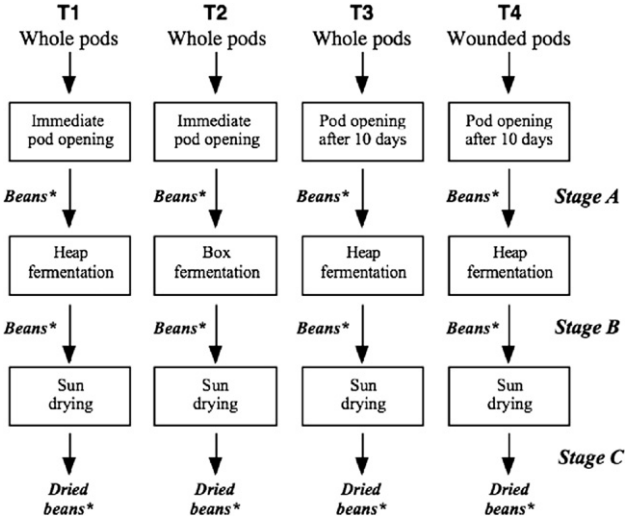

Fig. 1. Cocoa post-harvest processing (* sampling). T1: Heap fermentation of beans from whole pods with immediate pod opening. T2: Box fermentation of beans from whole pods with immediate pod opening. T3: Heap fermentation of beans from whole pods with pod opening deferred by 10 days. T4: Heap fermentation of beans from wounded pods with pod opening deferred by 10 days. A, B, C: Sampling stages.