Enzymatic Decontamination as an Approach for Reduction of Human Exposure to Mycotoxins in Rural Subsistence Farming Communities in Africa

JOHANNA F. ALBERTS, HESTER-MARIÉ BURGER, JOHN P. RHEEDER, GORDON S. SHEPHARD and WENTZEL C.A GELDERBLOM

Mycotoxicology Research Group, Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, PO Box 1906, Bellville, South Africa

Introduction

Mycotoxins are produced by food-borne mycotoxigenic fungi and are important environmental and carcinogenic agents occurring in many parts of the world (Marasas *et al.*, 2008). Of the different mycotoxins, aflatoxin produced by *Aspergillus* spp. and the fumonisins, produced by *Fusarium* spp. are common mycotoxin contaminants of grain crops and are known to adversely affect human and animal health. The majority of Africa's grain supplies are at risk to be contaminated by mycotoxins, which further exacerbates the existing food insecurity. In southern Africa, 80% of farms are smallholdings that provide their households with subsistence crops. Subsistence crops are cultivated for home consumption, i.e. for food preparation and beer brewing, as well as for informal trading. As mycotoxins exhibit a variety of biological effects and are implicated in many human diseases, the prevention of chronic exposure, particularly in developing countries such as southern Africa, is of critical importance [International Agency for Research on Cancer (IARC), 2012]. Young children are especially vulnerable, with chronic exposure resulting in adverse health effects such as growth impairment. Co-contamination of food and co-exposure of vulnerable populations to multiple mycotoxins have been widely documented in low socio-economic areas in Africa, and is of particular concern. Strict regulations of mycotoxins in food exist in developed countries with high standards of monitoring food quality to protect against the adverse effects on human and animal health. In Africa, only 15 countries have mycotoxin regulations, which is mainly related to aflatoxin contamination of major dietary staples [Food and Agricultural Organization (FAO), USA, 2003]. Developing countries are often confronted with food insecurity due to severe climatic conditions, poor socioeconomic status and economic instability, and lack of the necessary agricultural expertise regarding crop management pre- and postharvest (Alberts

albertsh@cput.ac.za

et al., 2017). Apart from the economic losses encountered, these conditions increase the risk of mycotoxin exposure on a daily basis and the associated adverse health effects. The fate of poor quality food is also a concern, as it might still be consumed by humans and/or animals in times of food scarcity. These scenarios require ingenious ways to reduce mycotoxin exposure in low socio-economic rural communities where access to sophisticated removal techniques is not available or practically viable. Population groups that are the worst affected include subsistent maize growing farmer communities where mycotoxin levels are not monitored, as mono-cereal crops are cultivated and consumed locally. The World Health Organisation (WHO, 2006) made several recommendations for mycotoxin reduction and control relevant to rural farming communities, involving approaches such as the implementation of simple and practical mycotoxin reduction techniques at household level to effectively reduce exposure.

Biologically based interventions for control of mycotoxigenic fungi and mycotoxins

The lack of effective and environmentally safe chemical control methods against fungal growth and mycotoxin production in food crops, has led to investigations into biologically safe alternatives to prevent these contaminants from entering the food chain (Alberts *et al.*, 2016). Biological pesticides and methods involving natural resources such as plants, microorganisms, genetic factors thereof and clay minerals are popular alternatives being evaluated. Mycotoxigenic fungal infection and mycotoxin production are effectively reduced by several natural and biological methods, pre- and postharvest. Pre-harvest control methods include breeding for resistant maize cultivars; introduction of biocontrol microorganisms; application of phenolic plant extracts; and expression of antifungal proteins and mycotoxin degrading enzymes in transgenic maize cultivars. Postharvest approaches include the sequestering of mycotoxins from food by natural clay adsorbents and enzymatic degradation of mycotoxins. However, these technological approaches are currently mainly aimed at commercialization; application in animal feed; and in areas with established infrastructure, i.e. developed countries.

Enzymatic degradation of mycotoxins postharvest

Enzymatic degradation of mycotoxins in food sources postharvest is a new research field with plenty of scope for novel developments and improvement of the safety aspects of treatment methods (Alberts *et al.*, 2016; 2017). The focus is on targeted modification of the chemical structures by enzymatic cleavage or conversion of chemical bonds/groups that play a key role during cytotoxicity (Alberts *et al.*, 2006; 2009; Gelderblom *et al.*, 1993; Heinl *et al.*, 2010). Enzymatic detoxification of aflatoxin B_1 (AFB_1) mainly focuses on cleavage of the double bond of the furofuran ring through oxidation. The furofuran ring is actively involved in oxidation of AFB_1 to AFB_1-8,9-epoxide, a highly reactive precursor of malignant transformation (Minto and

Townsend, 1997; Mishra and Das, 2003). Microbial manganese peroxide and oxidase enzymes catalyse cleavage of the 8,9-unsaturated carbon-carbon bond of AFB_1 through oxidation and hydrolysis to AFB_1-8,9-dihydriol(Wang *et al.*, 2011; Yehia, 2014; Wu *et al.*, 2015). Treatment with laccase enzymes resulted in effective reduction of the mutagenic properties of AFB_1 (Alberts *et al.*, 2009). With regards to the fumonisins, the free amino group plays a pivotal role in its toxicological effects *in vitro* and *in vivo*, while the tricarballylic acid moiety is suggested as a requirement for effective absorption of the fumonisins from the gut of rats (Gelderblom *et al.*, 1993). Microorganisms and enzymes capable of degrading FB_1 include carboxylesterase and amino oxidase enzymes of *Exophiala spinifera* ATCC 74269, *Rhinocladiella atrovirens* ATCC 74270 as well as carboxylesterase and aminotransferase enzymes of Bacterium ATCC 55552 and *Sphingopyxis macrogoltabida* MTA144 (Blackwell *et al.*, 1999; Duvick *et al.*, 1998a; 1998b; Heinl *et al.*, 2010; Hartinger *et al.*, 2011). Detoxification is achieved through de-esterification of fumonisin B_1 (FB_1) and subsequent deamination of hydrolysed FB_1 (HFB_1) with the formation of 2-keto HFB_1. Recently, a commercial fumonisin esterase FumD (EC 3.1.1.87), FUMzyme® (BIOMIN, Austria) of bacterial origin (Sphingopyxis macrogoltabida MTA144), capable of effectively hydrolysing FB_1 was developed. The enzyme activity is specific and irreversible, while the hydrolysis product, HFB_1, exhibited considerable less cytotoxicity when evaluated in pig intestine, as indicated by sphinganine/sphingosine ratios in the liver and plasma; intestinal immune response; the absence of hepatotoxicity; and the intestinal morphology (Oswald *et al.*, 2012). FUMzyme® has been registered safe for humans, animals, and the environment by the European Food Safety Authority (EFSA). Although enzymatic detoxification has become a promising approach, application is presently directed towards the animal feed industry (Moll *et al.*, 2011).

Community-based interventions for reduction of mycotoxins postharvest

Approaches such as culturally sensitive, simple, practical and affordable mycotoxin reduction techniques at household level, for subsistence farming communities in developing countries, are becoming increasingly important, i.e. hand-sorting, winnowing, washing, flotation and dehulling of grains (Alberts *et al.*, 2017). These methods are especially applicable to the preparation of maize-based food in rural subsistence farming households. Hand sorting of maize kernels results in 69-71% reduction in fumonisins levels and lower mass loss than dehulling (Van der Westhuizen *et al.*, 2010; Desjardins *et al.*, 2000; Fandohan *et al.*, 2006; Afolabi *et al.*, 2006; Kimanya *et al.*, 2008; Matumba *et al.*, 2015). Dehulling and shelling of maize are common practices in West Africa (Fandohan *et al.*, 2006), with the removal of the pericarp, an effective way to reduce mycotoxin contamination. Mechanical shelling and dehulling of maize by various methods (shelling by hand; handle-operated shellers; motorized shellers) result in 57-65% reduction in fumonisin levels; however, it causes damage to maize kernels and considerable mass loss (Fandohan *et al.*, 2006; Matumba *et al.*, 2015).

Maize washing complementary to sorting results in additional 13-15% reduction in fumonisin levels (Van der Westhuizen *et al.*, 2011; Fandohan *et al.*, 2006; Matumba *et al.*, 2015). In South Africa a simple, practical and culturally sensitive hand-sorting and washing intervention method was developed and implemented for reduction of fumonisin exposure in a subsistence maize-farming community. The two-step maize kernel water wash method developed by Van der Westhuizen *et al.* (2010; 2011) involved visually sorting of maize kernels followed by a 10 min water wash method in a rural subsistence maize farming community in the Eastern Cape Province of South Africa, resulting in an overall decrease of 84% in fumonisins; 62% reduction in the probable daily intake (PDI); and 52% reduction in urinary excretion of FB_1.

Integration of community-based approaches with technological advances

Several commercial products for biological control of mycotoxigenic fungal diseases and the mycotoxins have been developed for application alone, in combination or as part of an integrated control strategy (Alberts *et al.*, 2016; 2017). Application is, however, limited in low socio-economic rural subsistence farming communities due to a lack of infrastructure, resources and access to technologies. Certain technologies such as enzymatic methods and montmorillonite clay adsorbents could in future find application in combination with community-based reduction methods. There are, however, challenges regarding integration and sustainability of technological and community-based mycotoxin reduction strategies into remote rural subsistence farming communities, including (i) the availability of grains (food security), (ii) the traditional use of mouldy grains (beer-making, specific grain-based dishes or animal feed), (iii) cultural beliefs and practices, (iv) education level, and (v) the socioeconomic status of the specific population (Kimanya *et al.*, 2008; Fandohan *et al.*, 2006). Advantages of interventions involving practical methods usually take the form of improved health outcomes rather than market outcomes (Wu and Khlangwiset, 2010a; 2010b). Public health interventions should be culturally sensitive; be implemented through educational campaigns; and must have financial and infrastructural support to be feasible in remote rural areas where they are most needed.

Future approaches

Different approaches utilizing enzymatic detoxification to safeguard human populations in rural subsistence communities are envisaged. In order to reduce mycotoxin exposure of rural farming communities to mycotoxins, detoxifying enzymes could be introduced during the routine water washing of grains prior to food preparation (Van der Westhuizen *et al.*, 2011), resulting in increased reduction of mycotoxin levels. For a commercial application, the enzymes could be incorporated during dry milling of maize (Burger *et al.*, 2013). Enzymes could be introduced during the initial moisture conditioning step and mycotoxin levels monitored in milling fractions throughout the milling process. Enzymatic reduction of mycotoxin levels in milling fractions could have an important commercial effect, as well as an impact on food security, i.e. an increased amount of certain milling fractions will be available for incorporation into animal feed, and the amount of grain wastage generated during milling will be reduced.

Enzymatic detoxification of mycotoxins in grains could make an important contribution to ensure food security in poverty challenged environments where food has become a scarce commodity due to severe climatic conditions, including prolonged droughts. The advantages of enzymatic approaches could include (i) limitation of mycotoxin exposure, (ii) reduction of the amount of contaminated food wastage, (iii) reduction of the amount of contaminated food that will enter the food chain in times of food insecurity, and (iv) commercial advantages for the animal feed industry.

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