International Journal of Food Microbiology 165 (2013) 57–64

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

The mycotoxin distribution in maize milling fractions under experimental conditions

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ARTICLE INFO

Article history:
Received 30 November 2012
Received in revised form 21 March 2013
Accepted 29 March 2013
Available online 11 April 2013

Keywords:
Mycotoxin distribution
Whole maize
Milling fractions
Fumonisins
Deoxynivalenol
Zearalenone

ABSTRACT

Mycotoxin contamination of maize and maize-based food and feed products poses a health risk to humans and animals if not adequately controlled and managed. The current study investigates the effect of dry milling on the reduction of fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA) in maize. Five composite samples, constructed to represent different mycotoxin contamination levels were degermed yielding degermed maize and the germ. The degermed maize was milled under laboratory conditions and four major milling fractions (SPECIAL, SUPER, semolina (SEM) and milling hominy feed) collected. The whole maize, degermed maize and total hominy feed (germ + milling hominy feed) were reconstructed to ensure homogenous samples for mycotoxin analyses. For comparison, commercial dry milling fractions (whole maize, SPECIAL, SUPER and total hominy feed), collected from three South African industrial mills, were analysed for the same mycotoxins and hence a more accurate assessment of the distribution between the different milling fractions. The distribution of the mycotoxins during the experimental dry milling of the degermed maize differs, with FB mainly concentrated in the SPECIAL, DON in the SEM whereas ZEA was equally distributed between the two milling fractions. Distribution of mycotoxins between the fractions obtained during commercial dry milling generally provided similar results with the total hominy feed containing the highest and the SUPER milling fractions the lowest mycotoxin levels although variations existed. Although milling is an effective way to reduce mycotoxins in maize, kernel characteristics and resultant fungal colonisation may impact on the distribution of specific mycotoxins among the different milling fractions. Differences in industrial dry milling practices and problems encountered in sampling bulk maize remain a large problem in assessing mycotoxin contamination in milling fractions intended for human consumption.

1. Introduction

Mycotoxins are secondary metabolites and natural contaminants produced by various food-borne fungi that can infect food commodities during pre-and post-harvest periods, storage or during food processing. Dietary exposure to mycotoxins is a global problem that impacts on human and animal health, as well as the food industry and international trade. The rapid changes encountered in the globalisation of world economies, climate and food sufficiency highlights the need for quality control parameters and safety evaluation of food contaminants that may adversely affect human and animal health (Dimitri and Oberholzer, 2006).

Some of the mycotoxins considered to be of importance to human health are: aflatoxins (AF) produced by *Aspergillus* spp., ochratoxin A (OTA) produced by *Aspergillus* spp. and *Penicillium* spp., deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FB) produced by *Fusarium* spp. (Binder et al., 2007). A large range of agricultural commodities are affected including peanuts, maize, wheat, nuts, beans, cassava, oats, barley and rice. Aflatoxins and OTA are also known to contaminate milk and dairy products (Prandini et al., 2009; Pattono et al., 2011). Mycotoxins commonly co-occur in agricultural commodities such as maize and can exhibit synergistic, additive, potentiating or antagonistic biological effects upon ingestion (Eaton and Klaassen, 2001; Scudamore and Patel, 2009; Waśkiewicz et al., 2012). This has relevance to the livestock industry where the co-occurrence of mycotoxins could exert far greater adverse health effects (Binder et al., 2007; Streit et al., 2012). Due to their thermal and chemical stability, mycotoxins can only be partly removed by food processing and/or decontamination procedures (Bullerman and Bianchini, 2007; Munkvold, 2003). Complete elimination of mycotoxins is impossible and susceptibility of food commodities to ambient temperatures, rainfall, relative humidity and moisture content plays an important role affecting fungal infestation (Lattanzio et al., 2007). The selection, cleaning and grading of unprocessed maize for food or feed production are important initial decontamination steps, although storage conditions can still result in an increase in mycotoxin

Abbreviations: FB, fumonisins; DON, *deoxynivalenol*; ZEA, zearalenone; SEM, semolina.

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http://dx.doi.org/10.1016/j.ijfoodmicro.2013.03.028

contamination prior to food processing and production (Bennett and Richard, 1996). Food processing that may reduce mycotoxin contamination includes sorting, trimming, cleaning, dehulling, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalisation (tortilla process) and extrusion (Bullerman and Bianchini, 2007).

Milling of maize is a physical process regarded as the first step in the production of maize-based products (Castells et al., 2008) by *removing* the outer structures, the hull, pericarp (bran), germ and tip cap to expose the endosperm, which is then utilised to produce various milling fractions such as the grits, germ, meal and flour (Alexander, 1987). The milling fractions mostly utilised as human foodstuffs are the flaking grits and flour, whereas the bran and germ milling fractions are used for animal feed and oil extraction, respectively. In South Africa dry milling is mostly utilised to produce food products such as samp, maize grits, maize rice, unsifted, sifted, coarse, SUPER and SPECIAL maize meal. Apart from the physical breaking of each kernel, the efficiency of the degerming process will also affect the yield and composition of the grits and again impact on the levels of mycotoxins. Inadvertently, differences will exist between whole maize batches and mills, as well as the overlapping of kernel components constituting the different milling fractions. Currently the terminology used for describing the various milling end-products differs globally and restricts comparisons of the corresponding mycotoxin contamination between different countries (Scudamore and Patel, 2009). In this regard utilising particle sizes of each milling fraction could be a simple solution to describe a milling fraction or product (Scudamore and Patel, 2009). Due to the complexity of the milling process the mycotoxins may be distributed, yielding higher or lower levels in the various milled products (Bullerman and Bianchini, 2007).

The *level* of mycotoxin contamination in whole maize and the distribution thereof between the resultant fractions produced during food processing, remains a food safety challenge. Most studies in this regard are focused on the distribution of mycotoxins in industrial dry milling whereas studies utilising laboratory or small-scale milling to *investigate* distribution are limited (Stoloff and Dalrymple, 1977; Park, 2002; Broggi et al., 2002; Brera et al., 2006; Bullerman and Bianchini, 2007; Castells et al., 2008; Pietri et al., 2009; Vanara et al., 2009). Studies investigating the effect of industrial dry milling on FB1 showed *overall* a reduction in contamination levels in the resultant milling fractions intended for human consumption, i.e. milling fractions derived from the endosperm including the maize meal, flour and grits. The milling fractions retaining the hull, pericarp, germ and tip cap intended for animal feed or oil production contained higher levels (Bennett and Richard, 1996; Saunders et al., 2001; Broggi et al., 2002; Brera et al., 2004; Bullerman and Bianchini, 2007; Scudamore, 2008; Pietri et al., 2009; Castells et al., 2008; Scudamore and Patel, 2009).

The main purpose of the present study was to investigate the effect of dry milling on the distribution of FB, DON and ZEA mycotoxins between the different milling fractions utilising specific designed composite maize samples containing these mycotoxins. Samples were milled under controlled laboratory conditions and the results were compared to those obtained from South African commercial dry milled maize fractions. The sampling of whole maize and the milling fractions for mycotoxin analysis, even with well-defined legislated sampling protocols in industrial maize milling settings, is still subjected to error and other confounding factors (Blanc, 2006; Scudamore, 2008). The current study included novel and special strategies to overcome some of these errors and ensure homogenous and representative samples for mycotoxin analyses.

2. Materials and methods

The present study was a collaborative effort between a reputable and prominent South African grain-based manufacturing company, Southern African Grain Laboratory (SAGL) and the South African Medical Research Council (MRC).

2.1. Chemicals

Water was obtained from a Milli-Q system (Millipore, Bedford, Massachusetts, United States). Methanol and acetonitrile were HPLC-grade and formic acid was analytical reagent grade, all obtained from MERCK, South Africa. All analytical standards were sourced from Industrial Analytica and Tega (South Africa) as well as the PROMEC Unit, South African Medical Research Council (MRC).

2.2. Experimental composite maize samples

To *effectively* monitor the influence of the dry milling process on *various* mycotoxin levels, *five* experimental composite maize samples were constructed, representing specific contamination levels of the different mycotoxins. Different grades 1 and 2 (according to South African regulations) uncleaned white maize consignments were selected from various maize growing localities within South Africa during the 2010 harvest season. Multiple-mycotoxin analyses were conducted to determine the presence of AFs (B_1, B_2, G_1 and G_2), FB_1, FB_2, OTA, DON, ZEA and T-2 toxin. Following analyses appropriate samples were selected and composite samples ($n = 5$) of approximately 4 kg each, were prepared for the study (Table 1). As no aflatoxins (B_1, B_2, G_1 and G_2), OTA, and T-2 toxin were detected, the selection of samples was based on the levels of FB_1, FB_2, DON and ZEA. The *five* composite maize samples were screened and cleaned by the *removal* of non-kernel impurities using a Carter Day Dockage Tester (Carter Day, United States) but were not re-analised for mycotoxin levels prior to the experimental maize processing. All visible foreign material was *removed* by hand and the cleaned samples were stored in marked and sealed containers at 4 °C. As the composite samples were prepared based on maize grading parameters for the current experiment, certain samples would not *have* been suitable for human consumption. Composite sample 2 contained high levels of DON and ZEA, whereas the remainder of the samples lack the presence of ZEA whereas samples 3 and 5 contained *relative* high levels of FB and sample 4 a relative high level of DON.

2.3. Maize conditioning, degerming and dry milling

Conditioning or tempering of each composite sample was conducted in batches to ensure a more consistent sample for degerming and to prevent the pericarp and germ from drying out resulting in insufficient degerming. Prior to the conditioning of the composite samples, each sample was divided into two halves (batch A and B) and treated separately. Conditioning was conducted in two stages; 1) softening of the maize and 2) toughening and loosening of the pericarp for easy removal. For the first stage, the following formula was used to calculate the addition of water to obtain a moisture content of 16.5%.

$$\text{Mass} \times (\text{Target moisture } \% - \text{Actual moisture } \%)/(100 - \text{Target moisture } \%)$$

Each batch was transferred to a sealed bucket and rolled horizontally on an in-house rolling device for an hour and left standing for an additional 2 h and 45 min. For the second conditioning stage, each batch was again subdivided into halves (batches a and b), followed by the addition of water (20 mL/1.0 kg maize, 2%) and rolled horizontally for 5 min. Sample division was necessary due to the limited capacity of the degerming procedure and the experimental milling plant.

Degerming of the sample (300 g) was conducted using a modified Grainman rice polisher (Grainman, USA) and produced two products, 1) the germ (a mixture of the germ, hull, pericarp and tip cap), 2) the degermed maize consisting mainly of the “endosperm” and remnants of the germ. Finally, for each composite sample the resultant germ and