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Effect of alkaline cooking of maize on the content of fumonisins B_1 and B_2 and their hydrolysed forms

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ABSTRACT

The effect of nixtamalization on the content of fumonisins (FBs), hydrolysed (HFBs) and partially hydrolysed (PHFBs) fumonisins in maize was investigated at laboratory-scale. Maize naturally contaminated with FBs and PHFBs was cooked with lime. Starting raw maize, steeping and washing waters and final masa fractions were analysed for toxin content. Control-cooking experiments without lime were also carried out. The nixtamalization reduced the amount of FBs and PHFBs in masa and converted them to HFBs. However, the three forms of fumonisins collected in all fractions amounted to 183%, indicating that nixtamalization made available forms of matrix-associated fumonisins that were then converted to their hydrolysed forms. Control-cooking enhanced FBs and PHFBs reduction, due to the solubility of fumonisins in water during the steeping process, but did not form HFBs. These findings indicate that benefits associated with enhancing the nutritional value of nixtamalized maize are also associated with a safer product in terms of fumonisin contamination.

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal grain for human consumption in the world after wheat and rice, and is a staple food in many regions. It is estimated that in 2012, the total world production of maize was about 900 million tons, with the United States, China, Brazil and Argentina being the top maize-producing countries (FAO, Food & Agriculture Organization of the United Nations Statistics Division, 2013). Maize is mainly used for animal feeding and for human consumption or is processed to make food and feed ingredients using physical or chemical processing methods.

Fumonisins are mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum*, which frequently contaminate maize and maize-based products worldwide. Fumonisins B_1, B_2 and B_3 are the major fumonisins found in food. Fumonisin B_1 (FB_1) is chemically described as a diester of propane-1,2,3-tricarboxylic acid (TCA) and 2-amino-12,16-dimethyl-3,5,10,-14,15-pentahydroxyleicosane. The C-14 and C-15 hydroxyl groups are involved in ester formation with the terminal carboxyl group of TCA. Fumonisins B_2 (FB_2) and B_3 (FB_3) are the C-10 and C-5 dehydroxy analogues of FB_1 (Shephard, 1998). Fumonisins have been implicated in several animal diseases and are suspected to be involved in some human diseases (Scott, 2012). The International Agency for Research on Cancer (IARC) classified FB_1 as a Group 2B carcinogen (possibly carcinogenic to humans) (IARC, 2002). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee for Food of the European Commission (SCF) established a group tolerable daily intake (TDI) of 2 μg/kg body weight for FB_1, FB_2, and FB_3, alone or in combination (Bolger et al., 2001; SCF, 2003). In the European Union, the combined maximum levels for fumonisins (sum of FB_1 and FB_2) in maize and derived products range from 0.2 to 4 mg/kg (European Commission, 2007) while US guidelines for total FB_1, FB_2 and FB_3 are 2 or 4 mg/kg in maize products used for human foods and 3 mg/kg for popcorn grain (FAO, Food & Agriculture Organization, 2004).

Although fumonisins are relatively heat-stable and persist through most of the conditions used in food manufacturing, they may undergo reactions in food systems that alter their chemical structure and toxicity. The fate of fumonisins during various processing stages has been the subject of various research papers showing that large reductions in contamination levels can be achieved (Bullerman, Ryu, & Jackson, 2002; Castells, Ramos, Sanchis, & Marín, 2009; De Girolamo, Solfrizzo, & Visconti, 2001; Humpf & Voss, 2004). However, the degree of reduction is variable and depends on cooking conditions and food matrix composition.

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The mechanisms underlying these reductions are not well understood and could involve dissolution in cooking liquids, thermal decomposition or binding to food matrix components (Humpf & Voss, 2004).

Nixtamalization is an ancient cooking process involving cooking of the whole maize in a lime solution (calcium hydroxide); cooked maize is then grounded to produce a dough called "masa" that is used in many countries for the manufacture of tortillas, tamales, atole and snacks. The alkali-temperature treatment enhances the nutritional value of maize, for example by increasing its calcium content, by making niacin available and by reducing phytic acid levels (Rosentrater, 2006). In an epidemiological study of the Texas border region, consumption of tortillas has been implicated as a risk factor for neural tube defects (Gong et al., 2008; Voss, Riley, Snook, & Waes, 2009). Mexico has one of the highest per capita consumptions (120 kg/year) of maize in the world and maize is primarily consumed as tortillas. Human exposure to fumonisins is therefore to be expected, especially in areas where nixtamalized maize is a diet staple. Surveys conducted on nixtamalized products originating from Mexico indicated that fumonisin levels were up to 1.8 mg/kg (De Girolamo, Pascale, & Visconti, 2011; Dombrink-Kurtzman & Dvorak, 1999; Scott & Lawrence, 1996).

The few studies on the combined effect of nixtamalization and cooking on fumonisin levels have shown that nixtamalization, conducted at small-scale facilities or in the laboratory, reduced the concentration of fumonisins by up to about 80% in the final nixtamalized products as compared to the starting raw maize (Cortez-Rocha et al., 2005; De La Campa, Miller, & Hendricks, 2004; Dombrink-Kurtzman, Dvorak, Barron, & Rooney, 2000; Palencia et al., 2003; Sydenham et al., 1995; Voss, Poling, Meredith, Bacon, & Saunders, 2001). The alkaline hydrolysis process occurring during nixtamalization causes the removal of one or both TCA side chains, yielding partially hydrolysed fumonisins ($PHFB_1$ and $PHFB_2$) or hydrolysed fumonisins (HFB_1 and HFB_2), respectively (De Girolamo, Lattanzio, Schena, Visconti, & Pascale, 2014; Poling & Plattner, 1999; Sydenham et al., 1995; Voss et al., 2001). Fumonisin concentrations in the cooked product are reduced during this step by at least two means: (i) extraction into the cooking/steeping liquid and (ii) hydrolysis of one or both of the two TCA groups. The monitoring of partially (PHFBs) and fully hydrolysed fumonisin (HFBs) levels in these food matrices, together with the native forms of fumonisins (FBs), becomes an important contributing factor in evaluating the human risk exposure to these mycotoxins through the consumption of nixtamalized products. Surveys carried out on the fate of hydrolysed and partially hydrolysed forms during nixtamalization only focused on HFB_1 and/or $PHFB_1$, whereas no data were available on the other hydrolysed forms (Cortez-Rocha et al., 2005; De La Campa et al., 2004; Dombrink-Kurtzman et al., 2000; Palencia et al., 2003; Sydenham et al., 1995; Voss et al., 2001). This was because no standards were commercially available and only few procedures have been described for the quantitative production of $PHFB_1$ standard (Poling & Plattner, 1999) or HFB_1 and/or HFB_2 standards alone (Dall'Asta et al., 2009; Hartl & Humpf, 1999). We have recently reported for the first time a suitable procedure for the simultaneous preparation of $PHFB_1$, $PHFB_2$, HFB_1 and HFB_2 to be used as reference standards (De Girolamo et al., 2014). From a small survey on maize and maize-based products, it emerged that fumonisins co-occurred with both HFBs and PHFBs in commercial nixtamalized products (masa flour and tortilla chips) (De Girolamo et al., 2014). In view of the growing consumption in several countries of nixtamalized snacks such as tortilla chips, nachos and tacos, it is of relevant importance to see whether the benefits associated with enhancing the nutritional value of maize after nixtamalization are also associated with safer products in terms of mycotoxin contamination. It could also be commercially interesting to compare different cooking conditions to evaluate how they affect the safety of the final masa.

The aim of the present work was to evaluate the effect of alkaline cooking of maize carried out at laboratory scale on the content of FB_1, FB_2, $PHFB_1$, $PHFB_2$, HFB_1 and HFB_2 in the masa by using different lime concentrations and cooking times. A mass balance calculation was applied to quantitatively estimate the fate of toxins during the processing. To the best of our knowledge, this is the first time that the fate of these 6 toxins during alkaline-cooking of maize has been simultaneously investigated.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN), methanol (MeOH) (both for HPLC purposes), glacial acetic acid and citric acid ($C_6H_8O_7{\cdot}H_2O$) were purchased from Mallinckrodt Baker (Milan, Italy). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). Di-sodium hydrogen phosphate (Na_2HPO_4), phosphate buffered saline (PBS) tablets and calcium hydroxide were from Sigma–Aldrich (Milan, Italy). Paper filters were from Whatman (Maidstone, UK); micro-spin filter tubes (0.20 μm, regenerated cellulose, GRACE) were from Alltech (Deerfield, IL, USA).

2.2. Analytical standards

Pure standards of FB_1 and FB_2 were from Sigma–Aldrich (Milan, Italy), whereas $PHFB_1$ and HFB_1 and $PHFB_2$ and HFB_2 standards were prepared by mild alkaline hydrolysis of FB_1 and FB_2, respectively, according to the procedure described by De Girolamo et al. (2014).

Mycotoxin stock solutions were prepared by dissolving separately FB_1, $PHFB_1$, HFB_1, FB_2, $PHFB_2$ and HFB_2 in ACN/H_2O (1:1, v/v) at a concentration of 1000 μg/mL for each toxin. A multi-toxin spiking solution was prepared by combining aliquots of each individual standard stock solution and by diluting them with appropriate volumes of ACN/H_2O (1:1, v/v). The final mycotoxin concentration was 200 μg/mL for FB_1, 40 μg/mL for both $PHFB_1$ and HFB_2, 50 μg/mL for FB_2 and 10 μg/mL for both $PHFB_2$ and HFB_2. A multi-toxin calibration solution was prepared by diluting 1:10 with ACN/H_2O (1:1, v/v) the multi-toxin spiking solution. All solutions were stored at 4 °C before their use.

2.3. Maize samples and alkaline-cooking experiments

A batch (13.5 kg) of maize kernels obtained from field inoculation experiments with a fumonisin-producing strain of *F. verticillioides* was provided by Cereal Research Non-Profit Company (CRC, Hungary). The lot was first cleaned by sieving (size of sieve was 0.4 cm), mixed by a laboratory rotary mixer for about 15 min for homogeneity purposes and split into 9 sub-lots (1.5 kg each). A total of 9 small aliquots (about 0.06 kg each) were taken from the whole sub-lot, combined to form a single representative sample (about 0.50 kg) of each sub-lot and stored at 4 °C till LC–HRMS analysis to evaluate FB_1, $PHFB_1$, HFB_1, FB_2, $PHFB_2$ and HFB_2 content. The remaining 1 kg-sub-lot was used for alkaline-cooking experiments carried out at laboratory scale according to the flow-diagram shown in Fig. 1. Maize kernels were placed in a pan containing 3 L of distilled water and 10 g or 50 g (i.e. 1% or 5%, respectively) of $Ca(OH)_2$ and then cooked at 90 °C using a heater ceramic plate (IKA Werke RCT Basic) provided with a digital contact temperature control (ETS-D4 fuzzy, IKA Works GmbH & Co. KG, Staufen, Germany). Time to reach 90 °C was about 55 min, then