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Starch and thermal treatment, important factors in changing detectable fumonisins in maize post-harvest

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ABSTRACT

The drying process is commonly applied to maize grain to guarantee a safe storage. Two different conditions were tested (70 °C × 24 h and 95°C × 9 h) on 6 maize hybrids collected in 2010 in Italy. Maize samples were analysed for fungal incidence, kernel chemical composition (proteins, fat, starch) and free and hidden forms of fumonisins (FBs) pre- and post-drying treatments. Drying treatments reduced fungal incidence; in particular, both temperature × time combinations were able to reduce more than 85% *Fusarium* spp. incidence. In general, both free and total FBs increased after drying, especially at 70 °C for 24 h exposure. Regarding chemical composition, proteins and starch resulted unvaried, while fat content had a mean decrement of 26% and 14% after treatment at 70 °C × 24 h and 95 °C × 9 h, respectively. A possible retrogradation of starch, and in particular amylose, after heating has been proposed in this study; these changes seemed to be strongly related to the modifications in detectable fumonisin. This study confirms the variation in detectable FBs occurring after the drying treatment. Therefore, the quantification of fumonisin after the post-harvest treatments is strongly suggested to avoid maize contaminated above the legal limit to enter the food/feed chain.

1. Introduction

Maize is an important crop grown worldwide, destined for food, feed, industry and energy production (www.fao.org). Independently of its use, maize grain is commonly stored post-harvest for long periods before its final usage. Many ecological parameters are known to affect storage safety but, among them, water activity (a_w) plays the main role (Gregori et al., 2013). Usually, maize harvesting is carried out when kernel humidity is too high for a safe storage (Battilani et al., 2011). Harvest after physiological maturity, with kernel humidity around 24%, is currently suggested to limit cumulative mycotoxin contamination, especially aflatoxins (Battilani et al., 2011). Moisture content below 14%, corresponding to about 0.70 a_w (Battilani et al., 2011), is however mandatory for safe storage (Yilmaz and Tuncel, 2010).

Fungal pathogens associated with maize, mainly potential toxigenic strains of *Aspergillus flavus* and *Fusarium verticillioides* (Battilani et al., 2008; Giorni et al., 2009), can continue to develop and produce mycotoxins, aflatoxins and fumonisins respectively, in conducive conditions post-harvest. Therefore, maize drying is required, before storage, to prevent further synthesis of mycotoxins. A prompt reduction of grain humidity is suggested especially when *A. flavus* is present, this fungus being very efficient in aflatoxin production till to 0.78 a_w (Astoreca et al., 2014).

Many different technologies can be applied for cereal drying, but dryers using heated air, with the possibility to change air temperature and flux speed according to the grain batch, are the most commonly used for drying maize in Europe (Tuncel et al., 2010).

Some authors have considered the effect of drying temperature on fungi and related mycotoxins. Hawkins et al. (2005) found that the incidence of *F. verticillioides* was significantly reduced by the use of drying temperatures above 60 °C, while 70 °C was needed to reduce *A. flavus* incidence. However, these results were obtained with quite long lasting treatments (4 days), not comparable with those applied in commercial maize driers. Drying at high temperatures (80–100 °C) could result in the complete inhibition of all the spoilage agents present in the grain (moulds, insects, bacteria and yeasts), but it also results in the loss of grain quality, causing kernel

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cracking (Davidson et al., 2000) and structural damage of maize components, primarily starch (Bhuiyan et al., 2010; Izli and Isik, 2013).

Regarding mycotoxins, controversial data are reported in literature. The use of very high temperatures (≥190 °C), like those reached during roasting and frying, seems to reduce mycotoxin content, leading to the formation of N-acyl derivatives such as N-deoxyfructosyl-fumonisins (NDF) or hydrolysed forms like hydrolysed fumonisin B1 (HFB1) (Humpf and Voss, 2004; Mohanlall et al., 2013). On the other hand, it has also been proven that fumonisins can strongly interact with matrix macroconstituents, such as proteins and starch (Dall'Asta et al., 2010). The supramolecular structures formed by fumonisins and macromolecules may lead to poor recovery during analytical detection with a consequent underestimation of the total fumonisin content. These hidden forms can be affected by thermal treatments, on account of a different physico-chemical organisation of the matrix. In fact, in a recent study on fumonisin fate along the maize production chain, an increasing trend during drying was observed for the free forms of fumonisins (Falavigna et al., 2014). This was previously found also by Ono et al. (2002) and is frequently reported by farmers (Battilani, personal communication).

This study was therefore designed with the aim of investigating the possible effects of drying, taking into account the possible role of maize hybrid characteristics, on i) the quantifiable fumonisin content in grains, hidden forms included and ii) fungi survival.

2. Materials and methods

2.1. Chemicals

Fumonisin B1, B2 and B3 (5 mg, powder) were from Romer Labs (Tulln, Austria).

All salts and solvents (LC grade) were purchased by Sigma–Aldrich (Saint Louis, MO, USA). Fumonisins B_1, FB_2 and FB_3 standard solutions (a mixture of FB_1, FB_2 and FB_3, 50 μg/ml each, in acetonitrile/water, 1:1 v/v) were purchased from Romerlabs (Tulln, Austria). Hydrolysed fumonisins (HFB_1, HFB_2, HFB_3) were prepared in our laboratory (Dall'Asta et al., 2010).

Carrez I + II were part of the Carrez Clarification reagent Kit (BioVision, Milpitas, CA, USA). Formic acid (99%) was obtained from Acros Organics (Morris Plains, NJ, United States). Ultra pure water was produced in our laboratory by utilising an Alpha-Q system (Millipore, Marlborough, MA, USA).

2.2. Sample collection and preparation

Samples (300 g each) of 6 maize hybrids (2 of class FAO 600: H3 and H8; 4 of class FAO 700: H13, H14, H15 and H16) were all collected in commercial fields close to each other in Cremona (Northern Italy) at harvest in 2010. They were grown with a comparable cropping system. Each field was divided into 3 sectors, from where ten ears were collected in an X-shape design; a total of thirty ears were sampled from each field and considered as a representative sample for the field. The hybrid names were not reported, both because only a limited number of hybrids was studied and because the aim is to find out relevant factors not related to the specific commercial hybrid, but to kernel composition and therefore of more general value, having in mind the short commercial life of hybrids. Therefore, sample codification was given according to Dall'Asta et al. (2012); the hybrids already considered in the previous study are named with the same code (H3, H8), other hybrids are reported with increasing numbers.

Husked cobs were shelled, the kernels of each sample were mixed and 3 sub-samples of about 500 g were collected; one sub-sample was used as test (not treated) and the other 2 sub-samples were used for drying treatments.

2.3. Drying test

Maize kernels sub-samples were put in an oven (NSC9180, So.Ge.Sa. srl, Potenza, Italy) working with forced air flow. Two different temperature × time combinations were considered in addition to the untreated test: (i) 70 °C × 24 h; (ii) 95 °C × 9 h. Water activity was measured both before and after the drying process using AquaLab LITE (version 1.3 © Decagon devices Inc., WA, USA).

2.4. Incidence of infected kernels

Fifty kernels were randomly selected from each sample, surface disinfected in 1% sodium hypochlorite solution for 2 min, then in 90% ethyl alcohol solution for 2 min, rinsed with sterile distilled water and dried under a sterile hood. Then, kernels were plated in Petri dishes with Potato Dextrose Agar (Oxoid LTD., Basingstoke, Hampshire, England) and incubated at 25 °C for 7 days.

After incubation, fungal strains developed from the kernels were counted and identified at genera/section level based on their phenotypic aspects. In particular, *Fusarium*, *Aspergillus* and *Penicillium* spp. were taken into account. Incidence of infected kernels was reported as the number of kernels with growing moulds rated on the total number of plated kernels.

2.5. Starch and determination of its components

Total starch was determined by polarimetry as the difference between total hydrolysed sugars and reducing sugars (EN 1972L0199, 1999). Briefly, ground kernels (2.5 g) were added to hydrochloric acid (50 ml), and vigorously shaken. The flask was boiled in a water bath for 15 min, then the mixture was cooled at 20 °C after the addition of bi-distilled water (30 ml). By shaking, Carrez I (5 ml) and Carrez II (5 ml) solutions were added. The optical rotation was then measured in a 200 mm tube using a Perkin Elmer 341Polarimeter (Perkin–Elmer, Waltham, MA, USA).

For reducing sugars, the sample (5 g) was added to ethanol 80 ml and shaken at room temperature for 1 h. After filtration, hydrochloric acid (2.1 ml) was added to 50 ml of the filtrate; the solution was boiled in a water bath for 15 min and then cooled at 20 °C. After clarification using Carrez I + II reagents as above, the optical rotation of the solution was polarimetrically measured. A specific optical rotation of 184.6°, typical for maize starch, was applied at 589 nm (D-line of the sodium lamp).

For determining the amylose amount, maize flour samples were analysed using a Megazyme amylose/amylopectin assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland). The amylose determination is obtained after the selective quantitative precipitation reaction of amylopectin with concanavalin A (ConA) (Gibson et al., 1997). The percentage of starch components is obtained through the determination of amylose and total starch concentration based on the absorbance read with a spectrophotometer UV 1601 at 510 nm (Shimadzu Italia, Milano, Italia). Data were corrected for the humidity content before comparison.

Final data are reported on dry matter basis.

2.6. Fat content determination and fatty acid analysis

Fat content was determined using a Soxhlet extractor (Velp Scientifica, Monza-Brianza, Italy). In particular, 5 g of ground sample was extracted using diethyl ether as solvent and evaporated by rotavapor to yield the fat fraction. Then, in order to determine