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Effect of gamma irradiation and heat treatment on the artificial contamination of maize grains by *Aspergillus flavus* Link NRRL 5906

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A B S T R A C T

Maize (*Zea mays* L.) is one of the main crops, which is easily susceptible to *Aspergillus flavus* infection resulting in huge losses worldwide. This study was carried out to investigate the effect of combining heat and irradiation treatments in controlling the fungal growth in maize grains. Surface disinfected maize grains were artificially contaminated with spores of *Aspergillus flavus* Link NRRL 5906, and then exposed to gamma radiation with doses of 3.0, 4.0 and 5.0 kGy. The samples were additionally heat treated at 60 °C for 30 min. The heat and irradiation treatments showed a synergistic effect on controlling *Aspergillus flavus* growth. The heat treatment reduced the required radiation dose of about 0.5–1.0 kGy when 4.0 kGy or 5.0 kGy irradiation was used. The combined heat and irradiation treatment of moisture reduced the average CFU by 8 log cycles when 4 kGy or 5 kGy irradiation was used and by 7 log cycles when 3 kGy irradiation was used. The heat treatment of moisture alone reduced the average CFU by only by 0.8 log cycles. Combining irradiation with heat treatment to reduce the required radiation dose is very useful especially when there is a concern over biological side effects of irradiation.

1. Introduction

Molds are widely spread on agricultural commodities causing a considerable economical damage and risk for human and animal health (Bryden, 2007; De Lucca, 2007; Shephard, 2008). The mold contamination can occur in the field, during handling, and/or storage. Fungal mycotoxins occurring in food are of a great concern worldwide, and especially in the developing countries (Kabak et al., 2006; Bryden, 2007; Reddy et al., 2009; Remža et al., 2014). There are more than 100 countries who retain specific regulations or detailed guidelines for mycotoxins in food nowadays (Van Egmond et al., 2007). Despite of all of these regulations or guidelines, aflatoxins were found worldwide on average in 26% of various samples in 2009 (Rodrigues and Griessler, 2009). In fact, in that study 76% of the samples contained at least one of the major studied mycotoxins. Therefore, the impact of mycotoxins on the safety of agricultural products is significant.

Maize (*Zea mays* L.) is susceptible worldwide to contamination by different *Fusarium* and *Aspergillus* strains (Wood, 1992; Munkvold, 2003; Reddy et al., 2009). In particular, toxigenic strains of *Aspergillus flavus* grow on nuts, maize and other grains and produce highly toxic carcinogenic compounds known as aflatoxins. Aflatoxins represent a worldwide threat to public health (Strosnider et al., 2006). They transmit transplacentally and to the newborns through breast-feeding (Goldman and Shields, 2003). Normally, *Aspergillus flavus* produces aflatoxin B1 (AFB1) and B2 (AFB2). AFB1 is the most potent natural carcinogen known and is associated with human hepatocellular carcinomas (Bennett and Klich, 2003; Ferguson and Philpott, 2008). AFM1 and AFM2, which are the hydroxylated metabolites of AFB1 and AFB2, are usually found in milk or milk products obtained from livestock that have ingested contaminated feed (Dutton et al., 1985; Bennett and

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Klich, 2003). In all maize grains, the fungal population was above 10^4 CFU/mg after 30 days of incubation and the aflatoxin was over 23.7 μg/kg after 60 days of incubation (Franzolin et al., 1999). Large number of stored maize samples (storage time from 1 year to 11 years) were collected in Lianoing Province, China, where all these samples contained aflatoxins with an average content of 0.99 μg/kg (Liu et al., 2006).

Pre- and post-harvest strategies are necessary to prevent mold infestation and production of mycotoxins (Jouany, 2007). Adequate working rules and handling procedures are crucial aspects in reducing the risk of mycotoxin production in maize (Munkvold, 2003; Kabak et al., 2006; Strosnider et al., 2006). After harvest, low moisture content, low storage temperature and aeration are very critical factors espectially, when there is a possibilty of fungal growth. In that case, the grains should be dried quickly at high temperature (Munkvold, 2003). Usually, fungal growth is prevented by using physical, chemical and/or biological treatments (Davis et al., 1984). There are many factors that contribute to the fungi production and aflatoxin growth. The most important ones are humid climate, warm temperature and high moisture content. After harvesting, a drying process is required to reduce the moisture content to a safe level. In this study, heat is required to provide 80–90% relative humidity for at least one hour before gamma irradiation treatment takes place.

There is a number of different specific methods to prevent the mold contamination of crops (Suttajit, 1991; Kabak et al., 2006; Yin et al., 2008). Inhibitors are also developed over the years for aflatoxin production (Holmes et al., 2008). Many studies reported, 40 years ago, using irradiation to control *A. flavus* (Malla et al., 1967; Jemmali and Guilbot, 1970; Applegate and Chipley, 1973, 1974). Gamma irradiation has been in wide use in food preservation. However, there are many concerns regarding its safety due to reactive radiolytic products and other undesired effects (Sommer and Fortlage, 1966; Grolichova et al., 2004; Smith and Pillai, 2004). The current view is that irradiation is the safest and one of the most reliable methods for preservation of food and agricultural commodities (Shah et al., 2014). Moreover, it is also shown that doses below 10 kGy in controlling the contamination of food are safe (Smith and Pillai, 2004). According to International Atomic Energy Agency (IAEA, 1982), the lethal radiation dose for molds is in the range of 2.5–6.0 kGy. In most studies, a dose of 1–3 kGy can reduce significantly the amount of pathogens (Shah et al., 2014). However, a dose 3–20 kGy was required to sufficiently reduce the microbial populations in dry food ingredients (Farkas and Mohácsi-Farkas, 2011). There are many factors affecting gamma radiation process, such as initial mycotoxin concentration, absorbed dose and the amount of moisture content (Calado et al., 2014). As a rule, using a combination of two treatment methods, such as heat and chemical treatment or fermentation and steamimg, reduces AFs more than each individual respective method alone (Jalili, 2016).

The general efficiency of irradiation in reducing the growth of pathogens is well-documented (Shah et al., 2014), however, there is a concern that irradiation treatment of food commodities may enhance the mycotoxin production by the remaining fungi (O'Neill et al., 1996). Several studies found that gamma radiation in fact increased the aflatoxin production (Jemmali and Guilbot, 1970; Applegate and Chipley, 1973, 1974; Schindler et al., 1980; Odamtten et al., 1986, 1987; Aziz et al., 2002). In addition, heat and irradiation may eliminate the protecting natural strains (O'Neill et al., 1996).

As an example, the irradiation has to be strong enough (5–10 kGy) to prevent the mycotoxin production (Ferreira-Castro et al., 2007). Furthermore, when low dose, 2.0 kGy, was used, the production of mycotoxins was increased, and for *Fusarium* infection of maize, 10 kGy was required to fully eliminate the mycotoxin production (Ferreira-Castro et al., 2007). The dose of at least 10 kGy is generally necessary for total prevention of aflatoxin production in the sample (Van Dyck et al., 1982; Mutluer and Ergoc, 1987; Aziz and Youssef, 2002; Aquino et al., 2005). When following the fungal growth after irradiation for 100 storage days, it was observed that while 4.0 kGy dose was required to significantly reduce the fungal growth, the 6.0 kGy dose was required to completely inhibit the fungal growth (Aziz et al., 2006). Several studies have identified treatments or chemicals that sensitize the fungi to irradiation, thus, lowering the required irradiation dose (Mohyuddin and Skoropad, 1977; Shahin and Aziz, 1997; Patel et al., 1989). Combined treatment of heat and radiation has also been studied in the control of fungal growth (Farkas, 1990). When treating fungi, the heat treatment should precede the irradiation treatment, whereas with bacterial spores the reverse order of the treatments is necessary (Farkas, 1990). The double-strand breaks caused by ionizing radiation are lethal if not repaired or if misrepaired. High temperature can prevent DNA damage repair and therefore exposure to high temperature enhances greatly the cell killing (El-Awady et al., 2001).

The combination of heat and irradiation was more effective than either treatment alone to reduce the amount of aflatoxins in *Aspergillus parasiticus* grown on rice (Narvaiz et al., 1988). The treatment of the spores by gamma radiation of 4.0 kGy in combination with moist heat of 60 °C for 30 min stopped the *A. flavus* growth and aflatoxin production in flask cultivations (Odamtten et al., 1986, 1987). However, those authors did not study the effect in conditions mimicking the handling of maize crop.

Low radiation doses tend to increase the aflatoxin production, since dilution of spores stimulates aflatoxin production (Odamtten et al., 1986, 1987; Ferreira-Castro et al., 2007). Thus, the size of inoculum affects the toxin production. Accordingly, the maize grains inoculated with a high number of spores develop less aflatoxin than the grains inoculated with a lower number of spores (Picco et al., 1999). Aflatoxin production occurs during the period of intense sporulation and dilution of the spores induces sporulation, hence, the aflatoxin production. Therefore, an inadequate treatment may lead to an increase in the aflatoxin production.

Aspergillus is one of the major seed-borne fungi contaminating maize in Libya (Baraka et al., 1999). Maize grains are seasonally cultivated for consumption as fresh or dried. Maize is important as animal feed, and imported animal feeds containing maize grains are usually stored for a long time (Baraka et al., 1999). Food irradiation research started in Libya at the beginning of 1980s. According to the IAEA Food Irradiation Clearances Database (FICDB), the Libyan clearance irradiated food list contains potatoes, onions, garlic, dates, dried spices and poultry since January 1989. The objective of this study is to evaluate the effect of gamma radiation, heat treatment and their combinations in controlling *A. flavus* growth on maize grains produced in Tripoli, Libya.

2. Materials and methods

2.1. Sample materials

Samples of maize grains cultivated in Tripoli were collected from the local market. *A.flavus* Link NRRL 5906 spores were obtained from Tripoli University, Faculty of Agriculture, Libya, originally from the International Mycological Institute (IMT).

2.2. Growing of A. flavus on maize agar and cracked maize

To prepare maize agar, two hundred grams of maize flour were mixed well with about 400 ml of distilled water. Then the filtrate was filled upto 1 liter with distilled water. Using a hot plate with