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Antiaflatoxigenic property of food grade antioxidants under different conditions of water activity in peanut grains

María A. Passone [a], Silvia Resnik [b], Miriam G. Etcheverry [a,*]

[a] (CONICET) Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36, km 601, 5800, Córdoba, Argentina
[b] (CIC) Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires, Argentina

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Abstract

Analytical grade (AG) and industrial grade (IG) of three-food grade antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl paraben (PP) were analyzed to prove their fungitoxic effect on *Aspergillus* section *Flavi* strains. The effect of interactions among 10 antioxidant treatments at water activity levels (0.982, 0.955, 0.937 a_W) for 11 and 35 days of incubation and at 25 °C in peanut grains on mycelial growth (CFU g^{-1}) and aflatoxin B_1 (AFB_1) accumulation were evaluated. Both antioxidant grade treatments had a significant effect ($P<0.001$) on fungal count. All antioxidant treatments showed the highest effectiveness on control of growth of peanut aflatoxigenic strains at 0.937 a_W and at 11 days of incubation. Overall, AG and IG binary mixtures M3 (20+10 mM), M4 (20+20 mM) and ternary mixtures M5 (10+10+10 mM), M6 (10+20+10 mM), M7 (20+10+10 mM) and M8 (20+20+10 mM) were the treatments most effective at inhibiting growth of *Aspergillus* section *Flavi* strains. Industrial grade BHA 10 and 20 mM, binary mixtures M1 (10+10 mM), M2 (10+20 mM), M3 (20+10 mM), M4 (20+20 mM) and ternary mixtures M5 (10+10+10 mM), M6 (10+20+10 mM), M7 (20+10+10 mM) and M8 (20+20+10 mM) completely inhibited AFB_1 production. The studied results suggest that IG antioxidant mixtures have potential for controlling growth of these mycotoxigenic species and prevent aflatoxin accumulation at the peanut storage system.

Keywords: Industrial grade antioxidants; Analytical grade antioxidants; Aflatoxins; Peanut

1. Introduction

The best-known mycotoxins are the aflatoxins, which are produced by certain strains of *Aspergillus flavus* Link, *A. parasiticus* Speare, *A. nomius* Kurtzman, *A. pseudotamarii* and *A. bombycis* (Horn et al., 1996; Orth, 1997; Ito et al., 1999; Petterson, 2000). These fungi can infect almost any foodstuff and have been found on most agricultural products. There is thus a potential for the occurrence of aflatoxins in a wide variety of foods, mainly groundnuts and many other nuts (Bathnagar and García, 2001). This family of secondary metabolites had attracted considerable research because of their extreme toxicity, mutagenicity, carcinogenecity (IARC, 1993), significant reductions in crop yield and economic losses (Gourama and Bullerman, 1995; Gqaleni et al., 1996). Among all classes of aflatoxins, aflatoxin B_1 (AFB_1) is known to be most significant in terms of animal and human health risk (Coulombe, 1993; Salunkhe et al., 1987). In populations where regulations are not adequately enforced and have relatively high exposure, a role for aflatoxins as a risk factor for primary liver cancer in humans has repeatedly been suggested (Robens and Richard, 1992). Recent evidence indicates that aflatoxins are involved in the very high incidence of primary liver cancer in parts of Africa, Southeast Asia and China (Pitt, 2004). Optimum conditions for aflatoxin production by these species is at 33 °C and 0.99 a_W, while that for growth is 35 °C and 0.95 a_W (Hill et al., 1985). Peanut grains

* Corresponding author. Tel.: +54 358 4676113; fax: +54 358 4676231.
E-mail address: metcheverry@exa.unrc.edu.ar (M.G. Etcheverry).

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are usually into storage at relatively low moisture content (less than 9.5%). If the a_W (or equilibrium relative humidity; ERH) of the grains remains below 0.60, moulds are unable to grow and the stored grain will be stable. However, if temperature and moisture gradients develop in the store by the insects and rodents biological activity, localized pockets of higher moisture may develop, opening the way for mould germination, growth and consequently aflatoxin production (Hocking, 2003).

It is for this reason that much effort has been devoted to the search for new antifungal materials for use in food and grain (Filip et al., 2003; López García et al., 2003). However, disappointingly few of them have been developed for use in foods (Shelef, 1984). Synthetic antioxidants, namely food grade antioxidants (JECFA, 1996) are products widely used as preservatives especially in rich foods in oils or fats, because these antioxidants exhibit an exceptional stress oxidative protection. Furthermore, a report from studies conducted by Farnochi et al. (2005) on natural maize grains has shown that synthetic antioxidants, butylated hydroxyanisole (BHA) and propyl paraben (PP) exhibited antifungal activity. In previous studies it has been determined the effectiveness of the food grade antioxidants butylated hydroxytoluene (BHT), trihydroxybutyrophenone (THB), PP and BHA at concentrations of 1, 10 and 20 mmol g^{-1} on germination, growth and aflatoxin B_1 accumulation by *Aspergillus* section *Flavi*. The antioxidants BHA, PP and BHT were effective fungal inhibitors to *A. flavus* and *A. parasiticus* strains *in vitro* on peanut extract meal agar, but PP and BHT sprayed on irradiated peanut only showed a solid fungal control when applied in combined form (Passone et al., 2005, 2006).

The present study was undertaken to compare the antifungal activity of analytical and industrial grade antioxidants mixtures of BHA, PP and BHT against *Aspergillus* section *Flavi* in natural peanut grains under different water activity conditions.

2. Materials and methods

2.1. Fungal isolates

Two isolates belonging to the genus *Aspergillus*, *A. parasiticus* CHG24 and *A. flavus* CHG46, were used in these experiments. The strains were isolated from peanut soil (Barros et al., 2005). It had been demonstrated that these strains were aflatoxin producers in peanut meal extract agar (PMEA). In this medium, aflatoxin B_1 production by *A. parasiticus* CHG24 was 13.07 μg g^{-1} and the production by *A. flavus* CHG46 was 0.12 μg g^{-1} (Passone et al., 2005). The isolates were maintained at 4 °C on slants of malt extract agar (MEA) and in 15% glycerol at − 80 °C.

2.2. Substrate

Natural peanut grains with initial water content of 0.582 a_W and AFB_1 free were used throughout this study and kept at 4 °C. The peanuts were rehydrated to achieve the required a_W (0.982, 0.955, 0.937) by addition of sterile distilled water using a moisture absorption curve for the grain. The a_W of the grains was determined with a Thermoconstanter Novasina TH 200 (Novasina, Zurich, Switzerland).

2.3. Antioxidants

The following antioxidants were used: benzoic acid, 2(3)-*tert*-butyl-4 hydroxyanisole (BHA); *n*-propyl *p*-hydroxybenzoate (PP); 2,6-di (*tert*-butyl)-*p*-cresol (BHT). Analytical grade (AG) antioxidants (99.5 to 99.9% of purity) were obtained from Sigma Chemical (Dorset, U.K.) and industrial grade (IG) antioxidants were obtained from Eastman Chemical Company. Industrial grade antioxidants PP and BHT had a purity of 99%, containing as contaminants ash < 0.02%, arsenic < 3 μg g^{-1} and heavy metals < 10 μg g^{-1}. Butylated hydroxyanisole had a purity of 98.5% containing as trace elements sulfated ash < 0.01%, citric acid < 2500 μg g^{-1}, arsenic < 3 μg g^{-1} and heavy metals < 10 μg g^{-1}. These contaminating compounds did not exceed the levels allowed by JECFA (1996). Stock solutions of BHA, PP and BHT (10 and 20 mmol g^{-1}) were prepared in 950 ml l^{-1} ethyl alcohol/distilled water. The antioxidants mixtures used in this assay were: BHA (10 and 20 mM); BHA–PP mixtures M1 (10 + 10 mM), M2 (10 + 20 mM), M3 (20 + 10 mM) and M4 (20 + 20 mM) and BHA–PP–BHT mixtures M5 (10 + 10 + 10 mM), M6 (10 + 20 + 10 mM), M7 (20 + 10 + 10 mM) and M8 (20 + 20 + 10 mM).

2.4. Inoculation and incubation conditions

Seventy grams of natural peanut grains were dispensed as a monolayer into sterile Petri dishes. Peanut grains plates of different a_W values were amended with the appropriate antioxidant according to the treatment. The substrate was conditioned with the appropriate amount of water and kept at 4 °C for 48 h with periodic shaking to allow absorption and equilibrium. Fungi were grown on MEA for 5 days at 30 °C to obtain heavily sporulating cultures. Peanut grains were inoculated with 1 ml of spore suspensions (1×10^4 spores ml^{-1}). Petri

Table 1
Significance of incubation time (*I*), water activity (a_W), antioxidant grades (*G*), antioxidant treatments (*T*) and their interactions on growth of *Aspergillus* section *Flavi* on inoculated peanut grains

Factor	*df* [a]	MS [b]	*F* value [c]	*P*>*F*
T	10	367.45	79461.73	0.0001
G	1	124.26	26870.75	0.0001
I	1	109.59	23698.26	0.0001
a_W	2	54.66	11820.14	0.0001
$I \times a_W$	2	4.46	964.20	0.0001
$I \times G$	1	3.46	748.92	0.0001
$I \times T$	10	15.00	3244.23	0.0001
$a_W \times G$	2	62.60	13536.74	0.0001
$a_W \times T$	20	18.63	4029.05	0.0001
$G \times T$	10	25.31	5473.37	0.0001
$I \times a_W \times G$	2	9.08	1963.12	0.0001
$I \times a_W \times T$	20	7.94	1718.02	0.0001
$I \times G \times T$	10	1.55	335.72	0.0001
$a_W \times G \times T$	20	18.56	4013.13	0.0001
$I \times a_W \times G \times T$	20	5.04	1089.83	0.0001
Error	264	0.01		

[a] *df* = degrees of freedom.
[b] MS = mean squares.
[c] Significant at $P < 0.001$.