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Aflatoxigenic fungi and aflatoxins occurrence in sultanas and dried figs commercialized in Brazil

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Abstract

The presence of aflatoxins, *Aspergillus flavus* and *Aspergillus parasiticus* in dried fruits was investigated. A total of 62 dried fruit samples were analyzed (24 black sultanas, 19 white sultanas and 19 dried figs). A total of 10 *A. flavus* isolates were found, nine in one white sultana sample (corresponding to 18% infection) and one isolate in dried figs (2%), and all of them were aflatoxin B_1 and B_2 producers. *A. parasiticus* was not found. Aflatoxins were detected in 3 of 19 (16%) white sultana samples analyzed and, the limits were not higher than 2.0 μg/kg. In dried figs 11 of 19 (58%) samples were contaminated with aflatoxins and, with exception of one sample that was contaminated with 1500 μg/kg of B_1 aflatoxin, the others had less than 2.0 μg/kg. Neither aflatoxigenic or aflatoxins contaminated black sultanas.

Keywords: Aflatoxin; Dried fruits; *Aspergillus flavus*

1. Introduction

Aflatoxins consist of a group of approximately 20 related secondary fungal metabolites although only aflatoxins B_1, B_2, G_1 and G_2 are normally found in foods. They are produced by three species of *Aspergillus: Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* and can occur in a wide range of important raw food commodities including cereals, nuts, spices, figs and dried fruits. These fungi are capable of growing when the conditions of temperature, relative humidity and product moisture are favourable.

Aflatoxins are considered the most toxigenic metabolites from mycotoxin classes and become a potential risk to human and animal health. Aflatoxin B_1 is known to be a potent hepatocarcinogen for humans (IARC, 1993).

Current legislation limits 4 μg/kg for total aflatoxins in dried fruit for direct human consumption and 10 μg/kg to be subjected to sorting or other physical treatment before consumption or use as an ingredient in foodstuffs (Commision Regulation, 2003).

The occurrence of aflatoxins in dried fruits has been studied by some authors in different countries. In the United Kingdom, 140 dried fruit samples (currants, raisins, sultanas, apricots and dates) were analyzed and aflatoxins were not detected (Mac Donald et al., 1999; MAFF, 1997). Studies carried out with dried figs from Turkey detected the presence of aflatoxin B_1 (30 μg/kg) in one of 32 samples analyzed (Özay, Aran, & Pala, 1995). In a second study 4%, 2% and 2% of 284 of samples were contaminated with B_1, B_2 and G_2 aflatoxins respectively (Boyacioglu & Gonul, 1990). Stenier, Rieker, and Battaglia (1988) studied the aflatoxin distribution in 5 kg of dried fig lots sold in Turkey. A total of 11 lots were analyzed and the maximum limits of

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B_1 and G_1 aflatoxins found were 0.7 and 0.8 μg/kg, respectively. However, fruits with blue and green fluorescence under ultraviolet light, taken from the same lots, were analyzed separately, levels of 100–1400 μg/kg B_1 and 0.2–1900 μg/kg B_2 aflatoxins were found. The correlation between the presence of aflatoxins and toxigenic fungi was not verified in these studies.

Due to the significant health risks associated with aflatoxins in foods and also because dried fruits are normally consumed directly, it is important to verify the quality of these products, mainly if they are imported. The objectives of this work were to investigate the presence of toxigenic fungi and aflatoxins in sultanas and dried figs on sale in Brazil.

2. Materials and methods

2.1. Sampling

Samples from worldwide origin (Argentina, Chile, Iran, and Turkey) were purchased from different markets in Campinas and São Paulo in the period of 2002–2003. Black sultanas (24 samples), white sultanas (19) and figs (19), were analyzed. Until the beginning of the analyses, the samples were maintained at refrigeration temperature (7 °C) and stored in a freezer (−18 °C).

Approximately 500 g of each sample were used. For mycological analysis, whole black and white sultanas were plated, while dried figs were cut into small pieces. For aflatoxin determination, the remaining samples were homogeneized by thoroughly blending in a Waring blender.

2.2. Water activity

The water activity was obtained using Decagon T3 equipment (Aqualab, USA), at a constant temperature of 25 °C. The samples were analyzed in triplicate.

2.3. Mycological analysis

For mycological analysis, a sample of 150 g from a total of 500 g was externally disinfected with sodium hypochloride solution 0.4% for 1 min and 50 fruits or pieces of fruit were plated aseptically in dichloran 18% glycerol agar (DG18), according to Pitt and Hocking (1997). The plates were incubated at 25 °C for 7–15 days. The suspected colonies of *A. flavus* and *A. parasiticus* were isolated in agar malt extract (MEA) for identification according to Klich and Pitt (1988). The percentage infection was calculated.

2.4. Aflatoxins production

The production of aflatoxins from each isolate was verified using the agar plug (Filtenborg, Frisvald, & Svendensen, 1983) and chloroform (Taniwaki, 1995) techniques. The isolates were inoculated onto yeast extract 15% sucrose agar and incubated at 25 °C for 7 days. For the agar plug, a small piece of colony was tested using thin layer chromatography (TLC). If isolates were negative for aflatoxin A production, extraction with chloroform was used. In this technique, the whole colony from the Petri dish was extracted with chloroform in a Stomacher for 3 min, filtered and concentrated in a water bath at 60 °C to near dryness and dried under a stream of N_2. The residue was re-suspended in toluene:acetonitrile (98:2) and spotted on TLC plates which were developed in toluene:ethyl acetate:formic acid (5:4:1) and visualized under UV light at 365 nm. Aflatoxin B_1, B_2, G_1 and G_2 standards (Sigma Chemical, St Louis, USA) were used for comparison.

2.5. Aflatoxins analysis

2.5.1. Clean-up

The dried fruit samples were analyzed according to the Stroka, Anklam, Jorissen, and Gilbert (2000) method for aflatoxin analyses in dried fruits but some modifications were carried out. The post-column derivatization was substituted by pre-derivatization with trifluoreacetic acid. The remainder of the sample, approximately 350 g, was totally homogeneized in a Waring blender and 50 g was taken for aflatoxin extraction using 300 ml of methanol:water (8:2) and 5 g of NaCl. The mixture was homogeneized in Polytron, (Sweden) for 3 min and filtered in qualitative filter paper. An aliquot of 10 ml was diluted with 60 ml of phosphate buffer saline (0.20 g potassium dihydrogen phosphate, 1.10 g anhydrous disodium hydrogen phosphate, 8.0 g NaCl, 0.20 g KCl) pH7.0 and passed through an Aflatest™ (Vicam) immunoaffinity column (1–2 drops/s), followed by washing with distilled water. The aflatoxins were eluted with 1.25 ml of methanol using a 1–2 drops/s flow. The total volume was collected in a dark flask, dried under a stream of N_2, derivatized with 300 μl of trifluoreacetic acid at 75 °C for 8.5 min. At room temperature, the extract was re-suspended with 1 ml of mobile phase and injected into high liquid pressure chromatography (HPLC).

2.5.2. HPLC parameters

The HPLC equipment was a Shimadzu LC-10VP system (Shimadzu, Japan) set at 360 nm excitation and 460 nm emission. The HPLC was fitted with a Shimadzu CLC G-ODS (4 × 10 mm) guard column and Shimadzu Shimpack CLC-ODS (4.6 × 250 mm) column. The mobile phase was water:methanol:acetonitrile (4:1:1) and the flow rate was 1.0 ml/min. Aflatoxin standards ($B_1 = 1.21$ μg/ml, $B_2 = 0.77$ μg/ml, $G_1 = 1.08$ μg/ml and $G_2 = 0.66$ μg/ml) (Sigma Chemical, St Louis, USA) were used for construction of the five point calibration curve, peak areas versus mass (ng). The aflatoxin concentrations in the sample extract were determined by interpolation of resulting peak areas from the calibration graph.

2.5.3. Methodology validation

The recovery assays were carried out in black sultanas (five repetitions) only for aflatoxins B_1 and B_2 because the

other toxins (G_1 and G_2) were not produced by any *A. flavus* isolates. The samples were spiked with aflatoxins B_1 and B_2 standards (Sigma Chemical, USA) and maintained in the darkness at room temperature for 12 h. Three contamination levels were used: 20.8; 6.5 and 1.1 μg/kg for aflatoxin B_1 and 23.8; 6.2 and 0.8 μg/kg for aflatoxin B_2. The detection limit of the method was obtained analyzing ten repetitions of black sultana samples with 0.4 and 0.2 μg/kg of aflatoxins B_1 and B_2, respectively.

3. Results

Table 1 shows the incidence of *A. flavus* in the dried fruit samples. From the aflatoxin producer species investigated only *A. flavus* was isolated. There was no presence of *A. flavus* in black sultana samples. In both white sultanas and dried figs, only one sample was contaminated, with nine isolates (18% percentage infection) and one isolate (2%), respectively. All species were aflatoxin B_1 and B_2 producers.

The results of validation tests are shown in Table 2. The highest variation (CV) was verified in the lowest contamination levels coinciding with the lower recovery values. The results were satisfactory and the methodology was shown to be efficient with a coefficient variation lower than 20%; the limit reported by Horwitz (1982) for within-laboratory studies.

Fig. 1 shows the aflatoxin standard peaks ($B_1 = 1.21$ ng/ml, $B_2 = 0.77$ ng/ml, $G_1 = 1.08$ ng/ml and $G_2 = 0.66$ ng/ml, with total of 3.72 ng/ml) and their retention times values ($B_1 = 11.8$ min, $B_2 = 26.5$ min, $G_1 = 8.8$ min and $G_2 = 17.5$ min).

The calibration curves of aflatoxins B_1 and B_2 are shown in Fig. 2.

Table 3 shows the results of aflatoxin determinations in dried fruits analyzed. Aflatoxins were not detected in black sultanas. In white sultanas 16% of samples presented detectable aflatoxin levels with a maximum of 2.0 μg/kg. The highest contamination by aflatoxins was obtained in dried figs; 10 of 19 (53%) samples were contaminated with 0.3–2.0 μg/kg and one sample presented 1500 μg/kg of aflatoxin B_1. In this same sample, fungal growth was observed but it was not possible to isolate and

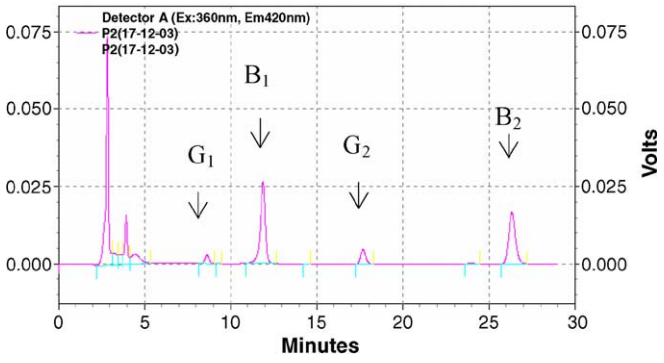

Fig. 1. The aflatoxin standards B_1, B_2, G_1 and G_2.

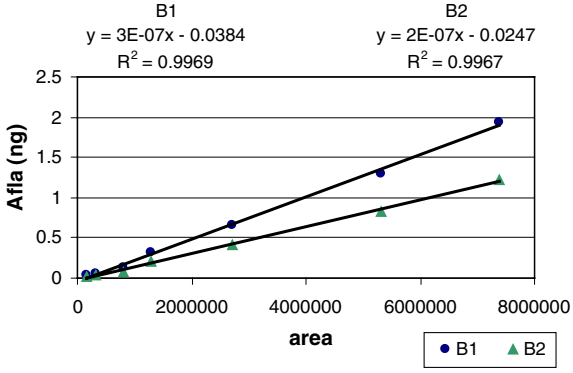

Fig. 2. Calibration curves of aflatoxins B_1 and B_2.

Table 1
The contamination with *A. flavus* in the dried fruit samples analyzed and percentage of toxigenic species

Dried fruits	No. samples analyzed	No. samples infected with *A. flavus*	No. *A. flavus* isolated	% Aflatoxin producers (B_1 and B_2)
Black sultanas	24	–	–	–
White sultanas	19	1	9	100
Dried figs	19	1	1	100

Table 2
Recovery and detection limits of aflatoxins (B_1 and B_2) in black sultanas

Aflatoxins	Contamination levels (μg/kg)	Recovery[a] (%)	CV (%)	Detection limit (μg/kg)
B_1	1.1	67.9	14.4	0.2
	6.5	70.5	15.1	
	20.8	91.9	10.1	
B_2	0.8	73.0	13.4	0.1
	6.2	69.0	7.9	
	23.2	95.7	5.9	

[a] Average of five repetitions.

Table 3
The aflatoxin levels (B_1 and B_2) in black sultana, white sultana and dried fig samples

Aflatoxins B_1 and B_2 (μg/kg)	Black sultanas	White sultanas	Dried figs
<0.3	24	16	8
0.3–2.0	–	3	10
2.1–1000	–	–	–
>1000	–	–	1

identify the species because the fungi could no longer be grown or recovered.

4. Discussion

Aflatoxins were not detected in black sultana samples. This product does not seem to be a satisfactory substrate for *A. flavus* growth and aflatoxin production. Other authors analyzing black sultanas reported the same results (Mac Donald et al., 1999; MAFF, 1997). Besides that, black sultanas seem to be a more adequate substrate for black aspergilli such as *A. niger* and *A. carbonarius* and for ochratoxin A production (Abarca, Accensi, Bragulat, Castellá, & Cabanes, 2003; Iamanaka, Taniwaki, Menezes, Vicente, & Fungaro, 2005).

A. flavus and aflatoxin presence has been related mainly to dried figs. According to Pitt and Hocking (1997) *A. flavus* and *A. niger* were reported as being the most common species in dried figs which was explained by their high sugar content, making them more susceptible than apricots and sultanas (Morton, Eadie, & Llewellyn, 1979). In this study, in spite of *A. flavus* incidence in dried figs being almost absent, aflatoxins were detected, indicating the production of these metabolites before the death of the fungi. If the fruits were dried by heat treatment or forced air the toxigenic species may have died because the temperature usually used is 50–70 °C (Karathanos & Belessiotis, 1997) and the correlation between fungal presence and aflatoxin contamination is not always evident.

In the present study, one sample of figs showed a high contamination of aflatoxin B_1, 1500 μg/kg, Stenier et al. (1988) evaluating dried figs from Turkey, also detected isolated fruits with 100–1400 μg/kg of aflatoxin B_1 and 0.2–1900 μg/kg of aflatoxin G_1. These fruits had blue and green fluorescence when ultraviolet light was used. However, the averages of aflatoxins B_1 and G_1 were 0.7 and 0.8 μg/kg respectively, showing that there is no uniformity of toxin distribution in the samples.

Özay et al. (1995) analyzing 32 samples of dried fig samples reported the low incidence of aflatoxin B_1; only one sample had 30 μg/kg. These authors used manual harvest and tunnel drying which were effective methods to reduce fungal contamination. The incidence of aflatoxins in dried figs could be avoided or at least decreased if good agricultural and manufacturing practices from harvesting to processing were used.

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References

Abarca, M. L., Accensi, F., Bragulat, M. R., Castellá, G., & Cabanes, F. J. (2003). *Aspergillus carbonarius* as the main source of ochratoxin *A* contamination indried vine fruits from Spanish market. *Journal of Food Protection, 66*, 504–506.

Boyacioglu, D., & Gonul, M. (1990). Survey of aflatoxin contamination of dried figs grown in Turkey in 1986. *Food Additives and Contaminants, 7*, 235–237.

Commision Regulation no. 2174 (13/12/2003). Maximum levels for aflatoxin B1 and aflatoxin total in certain foodstuffs. *Official Journal of the European Union*, L326, pp. 12–15.

Filtenborg, O., Frisvald, J. C., & Svendensen, J. A. (1983). Simple screening method for molds producing intracellular mycotoxins in pure cultures. *Applied and Environmental Microbiology, 45*, 581–585.

Horwitz, W. (1982). Evaluation of analytical methods used for regulation of foods and drugs. *Analytical Chemistry, 54*, 67–75.

Iamanaka, B. T., Taniwaki, M. H., Menezes, H. C., Vicente, E., & Fungaro, M. H. P. (2005). Incidence of toxigenic fungi and ochratoxin *A* in dried fruits sold in Brazil. *Food Additives and Contaminants, 22*, 1258–1263.

International Agency for Research on Cancer—IARC. (1993). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. In *IARC Monographs on the evaluation of carcinogenic risks to humans* (Vol. 56, pp. 489–521). Lyon, France: International Agency for Research on Cancer.

Karathanos, V. T., & Belessiotis, V. G. (1997). Sun and artificial air drying kinetics of some agricultural products. *Journal of Food Engineering, 31*, 35–46.

Klich, M. A., Pitt, J. I. (1988). A laboratory guide to common *Aspergillus* species and their Teleomorphs. Commonwealth Scientific and Industrial Research Organization, Division of Food Science and Technology, North Ryde, New South Wales, Australia, pp. 116.

Mac Donald, S., Wilson, P., Barnes, K., Damant, A., Massey, R., Mortby, E., et al. (1999). Ochratoxin *A* in dried vine fruit: method development and survey. *Food Additives and Contaminants, 16*, 253–260.

Ministry of Agriculture, Fisheries and Food-Maff (1997). Survey of aflatoxins and ochratoxin *A* in cereals and retail products. Food Safety Information Bulletin no. 130, United Kingdom.

Morton, S. G., Eadie, T., & Llewellyn, G. C. (1979). Aflatoxigenic potential of dried figs, apricots, pineapples and raisins. *Journal of the Association of Official Analytical Chemists, 69*, 958–962.

Özay, G., Aran, N., & Pala, M. (1995). Influence of harvesting and drying techniques on microflora and mycotoxin contamination of figs. *Die Nahrung, 39*, 156–165.

Pitt, J. I., & Hocking, A. D. (1997). *Fungi and food spoilage*. London, United Kingdom: Blackie Academic and Professional.

Stenier, W., Rieker, R. H., & Battaglia, R. (1988). Aflatoxin contamination in dried figs: distribution and association with fluorescence. *Journal of Agricultural and Food Chemistry, 36*, 88–91.

Stroka, J., Anklam, E., Jorissen, U., & Gilbert, J. (2000). Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanuts butter, pistachio paste, fig paste and paprika powder: collaborative study. *Journal of the Association of Official Analytical Chemists International, 83*, 320–340.

Taniwaki, M. H. (1995). Growth and mycotoxin production by fungi under modified atmospheres. PhD (Thesis) (Department of Food Science and Technology)—University of New South Wales, Kensington, Australia, pp. 243.