Food Control 87 (2018) 17–21

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Aflatoxin binding efficiency of *Saccharomyces cerevisiae* mannoprotein in contaminated pistachio nuts

Anna Abdolshahi [a], Farideh Tabatabaie yazdi [a], Ali Akbar Shabani [b, *], Seyed Ali Mortazavi [a], Leila Monjazeb Marvdashti [a]

[a] Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[b] Biotechnology Research Center, Department of Biotechnology, Semnan University of Medical Sciences, Semnan, Iran

ARTICLE INFO

Article history:
Available online 5 December 2017

Keywords:
Aflatoxin
Binding
Coating
Mannoprotein
Pistachio

ABSTRACT

Mannoprotein (mannan), an important cell wall component of *Saccharomyces cerevisiae*, is a bioactive glycoprotein that has aflatoxin binding effect. In this study, mannan from the cell wall of *Saccharomyces cerevisiae* was extracted and lyophilized. Aflatoxin binding assay of mannan in water/methanol were spiked with 0.5 μl. Aflatoxin standard solution showed that aflatoxin binding and mannan concentration had a positive correlation ($p < 0.05$). To evaluate the binding efficacy of mannan on aflatoxin content in pistachio, the contaminated pistachio kernels were soaked in mannan solution (25 and 50 mg/ml). Highest binding percent (84.40 ± 0.20% for aflatoxin B1) was seen in higher concentration of mannan. An increasing trend was observed by contact time (5, 15, 30, 60,120, 180 min); furthermore, there were 80% aflatoxin binding occurrence in the first 5 min contact time. The aflatoxin binding efficacy of mannan incorporated in gelatin -based coating on pistachio showed that the gelatin/mannan (3.5:1.5) coating can bind 83.49 ± 0.17% aflatoxin in contaminated pistachio. Since aflatoxin contamination of nuts such as pistachio is a critical defect in quality of control and safety guarantee, elimination/reduction of aflatoxin content attracted more attentions. According the results of this study, mannan alone or in corporation with gelatin -based coating has a potential application to reduce aflatoxin level in contaminated pistachio.

1. Introduction

Aflatoxins (AFs) are secondary metabolites produced mainly by *Aspergillus* species such as *A. flavuse*, *A. parasitcus*, *A. nominus*, *A. bombycus*, and *A. pseudo tamari*. Ingestion, absorption, and inhalation of AFs cause diseases in vertebrates (Bennett & Klich, 2003; Frisvad et al., 2007; Manso, Pezo, Gómez-Lus, & Nerín, 2014). The most common types of aflatoxin, namely B1 (AFB1), B2 (AFB2), G1 (AFG1) and, G2 (AFG2) are toxic to various species (Murphy, Hendrich, Landgren, & Bryant, 2006; Pittet, 1998). Because of the high potential effect on liver cancer, the International Agency for Research on Cancer has classified AFB1 as a class 1 carcinogen (Hontanaya, Meca, Luciano, Mañes, & Font, 2015; Luo et al., 2014; Organization & Cancer, 1993). Nuts are frequently exposed to AF contamination (Bedi & Agarwal, 2014; Molyneux, Mahoney, Kim, & Campbell, 2007; Pittet, 1998) which may start in the field then increase during harvest, drying, processing, or storage of pistachio nuts (Sauer, 1992). However, preventive strategies such as Hazard Analysis Critical Control Point, Good Agriculture Practice, and Good Manufacturing Practice, are effective at preventing AF contamination. Hereupon, any negligence could provide ambient conditions for *Aspergillus* growth and AF production in pistachio nuts (Abdolshahi, Tabatabaiee Yazdi, Shabani, Mortazavi, & Mohammadi Nafchi, 2016; Magan & Olsen, 2004). Problems and contemporary issues in prevention strategies, resistance, and stability of AFs under conventional food processing, as well as the exposure to AF contamination in all fields (from farm to fork) has resulted in considerable ongoing research on methods of AF detoxification or destroying toxic agents (Gonçalves, Rosim, de Oliveira, & Corassin, 2015; Liu & Wu, 2010; Luo et al., 2014). Different approaches are available to reduce the risk of AF contamination; some of the detoxification methods include addition of binder agents to feeds (contaminated diets) to bind and remove AFs, which subsequently prevents intestinal absorption of AFs in animals. The predominant aflatoxin binders are silicates,

* Corresponding author.
E-mail address: aashabani15@gmail.com (A.A. Shabani).

https://doi.org/10.1016/j.foodcont.2017.12.008
0956-7135/

activated carbons, complex carbohydrate polymers derived from cell walls of yeast and bacteria, and inorganic polymers such as cholestyramine and polyvinylpyrrolidone. *Saccharomyces cerevisiae* (*S. cerevisiae*) live yeast is classified on the GRAS (generally recognized as safe) list of food/feed additives able to reduce AF in diets; its AF protection efficacy was also confirmed in rats (Stanley, Ojo, Woldesenbet, Hutchinson, & KUBENA, 1993). It has been reported that the use of *S. cerevisiae* cell wall in a yeast based food product could efficiently remove AFB 1 (2.5%–49.3%) (Joannis-Cassan, Tozlovanu, Hadjeba-Medjdoub, Ballet, & Pfohl-Leszkowicz, 2011). Moreover, many studies have indicated that cell wall polysaccharides of *S. cerevisiae*, like mannan and glucan, are capable adsorbing or reducing AFs (Diaz et al., 2004; Raju & Devegowda, 2000).

In this study, the mannoprotein (mannan) was extracted from the cell wall of *S.cerevisiae*, to evaluate the possible effect of AF binding on AF-contaminated pistachio kernels. Hence, contaminated pistachios were soaked in mannan solution to determine the percent AF binding. The incorporation of mannan into gelatin-based edible coating as an AF binder was also studied in contaminated pistachio.

2. Materials and methods

2.1. Reagents and apparatus

All chemicals and reagents used for mannan extraction, coating preparation (bovine gelatin type B bloom 150 and glycerol), and preparation of an AF standard solution were purchased from Sigma St. Louis, MO, USA. *S. cerevisiae* (PTCC 5052) was obtained from the Microbial Collection of Iran. The solvents intended for AF analysis were HPLC (high performance liquid chromatography) grade and purchased from Merck (Darmstadt Germany).

2.2. Extraction of yeast mannoprotein (mannan)

S. cerevisiae (PTCC 5052) cultured in yeast mold (YM) agar (Difco ™) (0.1% glucose, 0.3% yeast extract, 0.5% peptone, 0.3% malt extract, and 2% agar) was incubated at 24 °C for 48 h then subcultured into YM broth (Difco™) and incubated at 30 °C for 24 h. The mannoproteins were extracted according Dikit, Maneerat, Musikasang, & H-kittikun, (2010). The suspension was centrifuged at 4500×*g* for 10min 5- 6 times until the supernatant was clear. Then the yeast cells were suspended in 100 ml potassium citrate (0.1 M) and potassium metabisulphite (0.02 M) buffer (pH 7) and autoclaved (121 °C) for 120min. The resulting suspension was centrifuged at 6000×*g* for 10 min at 4 °C. The supernatant was retained and mixed with five volumes of chilled ethanol (containing 1% acetic acid) and incubated overnight at 4 °C. The precipitate was recovered by centrifugation at 8000×*g* for 10 min at 4 °C then washed twice with chilled ethanol. To purify the mannoprotein, the final precipitate was dialyzed (8 kDa mw cut-off) against distilled water overnight at 4 °C. The whole mannoprotein extract was freeze-dried and used in this study.

2.3. AF binding assay

Evaluations of AF binding were performed according to Goncalves, Rosim, de Oliveira, & Corassin. (2015) with some modifications. Different quantities of mannan (1, 3, 25, 50,100, 150, and 200 mg) were transferred to microtubes containing 1.0 ml water/methanol (60:40) spiked with 0.5 μl AF standard solution (1000 ng/ml AFB1, AFG1 and 200by/ml AFB2, AFG2). The tubes were kept in a shaker with gentle rotation for 12 h at room temperature then centrifuged at 10,000×g for 10min. The supernatant was used for AF analysis.

2.4. AF contamination in pistachio

The aflatoxin binding efficacy of mannan was evaluated on naturally contaminated pistachio. In this regard, fresh whole pistachio nut (Akbari cultivar purchased from a pistachio research center in Damghan, Iran in August 2015) was placed in a plastic container. After 20 days, pistachios were peeled manually and dried at 40 °C. The AF contents of 10 pistachio kernel samples (100 g) were analyzed, and their AF mean values were considered as the initial AF content. Negative and positive control samples were non-contaminated pistachio kernels, and contaminated pistachio kernels were spiked with a pre-determined AF content (100 ppb).

2.5. AF binding and contact time assay in contaminated pistachio

Contaminated pistachios (10 g) were soaked in mannan solution (25 and 50 mg/ml mannan) for 5 min at room temperature then dried at 40 °C. After 5 days AF contents were analyzed. To determine the effect of contact time on AF binding of mannan, the contaminated pistachios (10 g) soaked in mannan solution (50 mg/ml) for different contact times of 5, 10, 15, 30, 60, 120, and 180 min and after each period pistachios were dried.

2.6. AF binding assay in coated pistachio

The AF binding efficacy of mannan incorporated into a gelatin-based coating on contaminated pistachio was evaluated. Gelatin was solubilized in water (55 °C) with stirring. After complete dissolution, glycerol as a plasticizer (2 g) and mannan were added in a water bath under soft agitation for 30min. Each solution was homogenized using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 1min. Gelatin/mannan solutions were prepared in four ratios (5:0, 4.5:0.5, 4:1, and 3.5:1.5). Pistachio kernels were immersed in coating solution for 10min then placed on sterile steel screen to remove the additional solution then dried at 45 °C. After 5 days AF contents were analyzed.

2.7. AF binding calculation

The percentage of AF binding in all assays was calculated by using the following equation:

$$\%\ \text{AF binding} = \frac{\boldsymbol{AF_i} - \boldsymbol{AF_f}}{\boldsymbol{AF_i}} \times 100 \quad (1)$$

where, $\boldsymbol{AF_i}$ and $\boldsymbol{AF_f}$ were the initial and final (after treatment) AF contents in pistachio, respectively.

2.8. AF analysis

Quantification of AF was performed by using a Waters e2695 (USA) HPLC, consisting of a chromolith C18, 100 mm × 4.6 mm, column (Phenomenex, USA) equipped with a fluorescence detector (Waters 2475, USA). The mobile phase was water/methanol/acetonitrile (60:20:20) with a flow rate of 2.5 ml/min. The excitation and emission wavelengths for detection were 365 nm and 435 nm respectively. The limit of detection (LOD) for AFs was 0.3 μg/ml. The chromatogram of AFB1, AFB2, AFG1 and AFG2 can be seen in Fig. 1.