Food Chemistry 214 (2017) 293–299

Contents lists available at ScienceDirect

Food Chemistry

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

Phenolic compound in beans as protection against mycotoxins

Annie Campello Telles *, Larine Kupski, Eliana Badiale Furlong

Laboratório de Micotoxinas e Ciência de Alimentos, Escola de Química e Alimentos, Universidade Federal do Rio Grande – FURG, Brazil

ARTICLE INFO

Article history:
Received 23 January 2016
Received in revised form 6 June 2016
Accepted 11 July 2016
Available online 12 July 2016

Keywords:
Aflatoxins
α amylase
Antifungal effect
Chlorogenic acid
Ferulic acid
Legumes

ABSTRACT

Phenolic compounds, their inhibitory activity against fungal amylase and the occurrence of aflatoxins were determined in edible beans. The free, conjugated and bounded phenolic compounds and their phenolic acid profiles were determined in ten bean varieties. A method for aflatoxin B_1, B_2, G_1 and G_2 determination and confirmation by LC–MS/MS was validated. The red and carioca beans presented the highest total phenolic content (1.8 and 1.2 $mg.g^{-1}$, respectively); the fradinho and white beans the lowest (0.18 and 0.19 $mg.g^{-1}$, respectively). In the free and conjugated forms, chlorogenic acid was present in 60% of the samples, while in the bounded phenolic, ferulic acid was in 90% of the samples. The phenolic extracts were able to inhibit fungal amylase, and the PCA analysis confirmed that the relation between the chlorogenic and gallic acids is important to this effect. The absence of aflatoxins in samples confirm the protector effects of these phenolic compounds.

1. Introduction

Several factors including climate, growing conditions, harvesting methods, storage conditions and starch-rich fraction presence of these grains can favour fungal contamination. If the contaminant species are toxigenic and the environment is favourable, they can produce mycotoxins that remain on the substrate, representing a risk to human health (Heidtmann-Bemvenuti, Mendes, Scaglioni, Badiale-Furlong, & Souza-Moraes, 2011; Sweeney & Dobson, 1998).

Mycotoxins are produced under fungal stress conditions, and their presence in a matrix demonstrates that the fungi were present before (Sweeney & Dobson, 1998). A concern about their presence in food arises from their acute or chronic effects to human or animal health. The most studied mycotoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, and the aflatoxins B_1 ($AFLAB_1$), B_2 ($AFLAB_2$), G_1 ($AFLAG_1$), G_2 ($AFLAG_2$), M_1 ($AFLAM_1$) and M_2 ($AFLAM_2$), are associated with declines in the immune system as well as mutagenicity and carcinogenicity (Heidtmann-Bemvenuti et al., 2011; Sweeney & Dobson, 1998). $AFLAB_1$, generally found in the highest concentrations in contaminated feed, is considered to be the most potent liver carcinogen in animal and human species (Dors et al., 2011; Sweeney & Dobson, 1998).

Plant defence against fungal infection and the manifestation of its toxigenic potential is determined by many variable biotics or abiotics. The chemical composition represents an important role, especially with regard to the presence of inhibitors that prevent against the invader microorganism (Mendes, Alves, Cavalheiro, & Badiale-Furlong, 2013).

Phenolic compounds possess one or more aromatic rings with one or more attached —OH groups, which may occur in free, combined, or bound forms that are considered to be part of the defence mechanisms in plants (Richardson, 1980; Wang et al., 2015). Their role against oxidative processes in plant and microbial tissues has been widely explored, and the demonstration of their presence has been used to claim functionality from various food sources (Guajardo-Flores, Serna-Saldívar, & Gutiérrez-Uribe, 2013; Ramírez-Jiménez, Reynoso-Camacho, Mendonza-Díaz, & Loarca-Piño, 2014).

Vegetables, such as alfalfa, lentils, chickpeas, peanuts, soy, lupine, broad beans, and common beans, have been widely exploited in the nutritional field to obtain macro nutrients for human and animal consumption. However, few reports on the contamination of these grains with mycotoxins produced by field fungi (*Fusarium*) or storage fungi (*Aspergillus* and *Penicillium*) are found in the literature (Costa & Scussel, 2002; Domijan et al., 2005). Among the vegetable seeds, the exception is the peanut that is frequently contaminated by aflatoxins and has its own legislation about the acceptable levels of these contaminants in different countries (ANVISA – Agência Nacional de Vigilância Sanitária. Resolução RE n° 7 & de 18 de fevereiro de, 2011).

* Corresponding author at: Laboratório de Micotoxinas e Ciência de Alimentos, Escola de Química e Alimentos, Universidade Federal do Rio Grande – FURG, Av. Itália, Km 8, Rio Grande, RS CEP 96203-900, Brazil.

E-mail addresses: annie.ctelles@gmail.com (A.C. Telles), larinekupski@yahoo.com.br (L. Kupski), dqmebf@furg.br (E.B. Furlong).

http://dx.doi.org/10.1016/j.foodchem.2016.07.079
0308-8146/

In the specific case of genus *Vigna* and *Phaseolus*, exploited by its protein supply, little has been studied about the phenolic profile and its relationship with the occurrence of mycotoxins in grains (Jager, Tedesco, Souto, & Oliveira, 2013; Ruadrew, Craft, & Aidoo, 2013). Therefore, studies on the presence of compounds with a defence function against fungi contamination should be explored as subsidiary breeding strategies for other grains or even for a more effective use of vegetables as a potential source of antifungal agents.

This study aimed to assess the profile of phenolic compounds and their relationship with the defence mechanism of beans from genus *Phaseolus* and *Vigna* against fungal contamination indicated by the occurrence of aflatoxins and inhibitory activity against fungal amylase.

2. Materials and methods

2.1. Samples

Ten bean varieties were selected for the study belonging to genus *Phaseolus* and *Vigna*. They were collected and sorted according to their use for human consumption, as follows. *Phaseolus vulgaris* varieties, black, white, carioca, peanuts, jalo, red, and black beans; *Vigna unguiculata:* fradinho and black-eyed beans and *Vigna angularis:* azuki beans.

Sampling was performed randomly at different time intervals (2013–2014) from supermarkets and street markets. Beans of the same species were homogenized to constitute a sample representing each variety that was collected. The sample of black beans imported from China were donated by EMATER (Empresa de Assistência Técnica e Extensão Rural do Governo do Distrito Federal); which was not homogenized with the similar beans from Brazilian crops. The samples were grounded and sieved in order to standardize the particle size to 0.5 mm for the physicochemical determinations.

2.2. Physicochemical characterization of the samples

2.2.1. Centesimal composition

The centesimal composition was determined according to the procedures described by Association of Official Analytical Chemists. AOAC (2000). The moisture content was determined by oven drying at 105 °C (935.29), lipids were determined by extraction with petroleum ether (920.85), and nitrogen was determined by the micro-Kjeldahl method (920.87); the results were converted to protein using a factor of 6.25, and ash was determined by incineration in a muffle at 550 °C (923.03). Carbohydrates were calculated by difference.

2.2.2. Free phenolic compounds

Three grams of the sample and 10 ml of methanol were homogenized in an orbital shaker at 200 rpm for 1 h at room temperature (25 °C). The agitation was stopped for 15 min, 10 ml of methanol were added and the mixture was homogenized for an additional 90 min. The extract was centrifuged at 3220*g* and was filtered, clarified with 5 ml 0.1 M barium hydroxide and 5 ml 5% zinc sulfate, left to stand for 30 min, centrifuged at 3220*g* for 5 min, and subjected to the clarification step again. After 30 min of rest, the mixture was filtered into a 25 ml volumetric flask that was filled with methanol. Quantification of the phenolic compounds was performed by the spectrophotometric method using a ferulic acid standard curve (1.8–12.5 $\mu g.ml^{-1}$) (Souza, Recart, Rocha, Cipolatti, & Badiale-Furlong, 2009).

2.2.3. Conjugated phenolic compounds

Three grams of the sample and 10 ml of ethanol were homogenized in an orbital shaker at 200 rpm for 10 min at room temperature (25 °C) and centrifuged at 3220*g* for 10 min at 10 °C. The supernatant was filtered and placed in a 25 ml volumetric flask. The extraction procedure was carried out twice, and the volume was raised to 25 ml with 80% ethanol (Mira, Massaretto, Pascual, & Marquez, 2009). Quantification of the phenolic compounds was performed in a spectrophotometer using a ferulic acid standard curve (1.8–12.5 $\mu g.ml^{-1}$) (Souza et al., 2009).

2.2.4. Bound phenolic compounds

Ten millilitres of hexane were added to the residue from the ethanolic extraction, homogenized on an orbital shaker at 200 rpm for 10 min at room temperature (25 °C), centrifuged at 3220*g* for 20 min at 10 °C, and the supernatant was discarded. Then, 60 ml of 4 M NaOH was added to the precipitate following agitation at 200 rpm for 4 h at room temperature. The pH was adjusted to 1.0 with 6 M HCl. The solution was centrifuged at 3220*g* for 40 min at 10 °C, transferred to a separatory funnel, and 30 ml of ethyl acetate were added. The partition was repeated two more times, the acetalic fraction was collected in an Erlenmeyer flask, and 10 cm^3 of anhydrous sodium sulfate were added to allow separation of the organic and aqueous phases. The aqueous layer was removed and the acetalic fraction containing the phenolic compounds was evaporated on a rotary evaporator at 55 °C (Mira et al., 2009). The residue was resuspended in 10 ml of 80% ethanol, and the quantification was performed according to the methods described by Souza et al. (2009) using a ferulic acid standard curve (1.8–12.5 $\mu g.ml^{-1}$).

2.2.5. Profile assessment of phenolic acids (PA)

The phenolic acid profiles of the samples were determined by high-performance liquid chromatography coupled with a UV detector (Shimadzu – CTO-20AC) equipped with a Supelco C18 column (4.6 × 250 mm), particle size of 10 μm and an ultraviolet detector. Aliquots of 5 ml of each extract were dried under a nitrogen atmosphere and were dissolved in the mobile phase (methanol:water acidified with 1% acetic acid, 20:80). The flow rate of the mobile phase was 0.7 $ml.min^{-1}$ with a run time of 25 min at 35 °C. The detection was carried out at 280 nm in the first 15 min for detection of gallic acid, protocatechuic acid, chlorogenic acid, hydroxybenzoic acid, caffeic acid, syringic acid, and vanillin. A wavelength of 350 nm was used for the remaining 10 min to detect coumaric and ferulic acids (Scaglioni, Souza, Schmidt, & Badiale-Furlong, 2014).

2.3. Inhibition of fungal α-amylase activity by the phenolic extracts

Initially, the activity of the commercial Fungamyl enzyme (0.176 μg $starch.mg^{-1}_{protein}.min^{-1}$) was determined using 1 ml of enzyme extract, 1 ml of starch solution (5 $\mu g.ml^{-1}$) and 1 ml of sodium acetate buffer at pH 7; these were kept at 30 °C for 30 min. The reaction was stopped by adding 1 ml of 0.1 M hydrochloric acid. The residual starch was quantified in spectrophotometer (Bioespectro – SP-220 spectrophotometer) at 620 nm using a starch standard curve (2–15 $\mu g.ml^{-1}$) (Baraj-Aceves, Hassan, Tinoco, & Vazquez-Duhalt, 2002).

The maximum reaction rate (V_{max}), expressed as $\mu g\ starch.ml^{-1}.min^{-1}$, was estimated in the absence of the inhibitor extract, and the specific enzyme activity (AU) was estimated by Eq. (1).

$$\text{UA } \alpha\text{-amylase} = \frac{\textit{initial startch conc.} - \textit{final startch conc.}}{\text{mg protein.min}} \quad (1)$$