TOXIGENIC FUNGUS IN COWPEA BEAN GRAINS SUBMITTED TO DIFFERENT GAMMA IRRADIATION DOSES

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RESUMO: LIMA, K.S.C.; COELHO, M.J.; LIMA, A.L.S.; FREITAS SILVA, O.; GODOY, R.L.O. Fungos Toxigênicos em Grãos de Feijão Caupi Submetidos à Diferentes Doses de Irradiação Gama. O processo de irradiação vem sendo indicado como uma alternativa para controlar pragas e aumentar o *shelf life* dos grãos de feijão caupi, mantendo suas características químicas, nutricionais e sensoriais. O presente trabalho tem o objetivo de avaliar o efeito da irradiação sobre a microbiota fúngica dos grãos de feijão caupi, após a irradiação e durante o tempo de armazenamento de 30 e 60 dias. Foram realizadas análises da percentagem fúngica, do potencial de toxidez de fungos isolados e de micotoxinas nos grãos controle (sem irradiação) e irradiados. O processo de irradiação reduziu significativamente a presença dos fungos do gênero *Aspergillus* a partir da dose de 2,5kGy e do gênero *Penicilium, Rhizopus e Fusarium* a partir da dose de 5,0kGy. Aflatoxinas e ocratoxina A não foram detectadas nas amostras controle e irradiadas. A irradiação do feijão caupi é uma alternativa segura e eficiente para combater perdas causadas pela presença de fungos toxigênicos.
Keywords: cowpea bean, irradiation, toxigenic fungus.

INTRODUCTION

Cowpea bean (*Vigna unguiculata* (L.) *Walp*) is originated from Africa and is known in Brazil as "*fradinho*", "*corda*" or "*macassar*". It is grown in the middle of the Northeast of Brazil (a semi-arid region) and can be found in parts of the North of the country, being one of the most important components of those people's diet. Leguminous plants are very important sources of proteins, vitamins, carbohydrates and minerals (RIBEIRO et al., 2007). Food irradiation process in Brazil has shown to be very promissory trough its contribution to conservation, reducing post-harvesting losses, and with the possibility to improve food availability. Among food irradiation benefits are: disinfestation of cereal grains and leguminous, control of pathogenic microorganisms, increase in time life of meats, fruits, vegetables, roots and tubercles, fruit maturation delay, fruit bugs elimination, sprouting inhibition of tubercles and bulbs and others (DIEHL, 2002; LIMA et al., 2003). According to Lima et al. (2007), the irradiation of cowpea bean is a safe and effective alternative to combat losses, helps in the reduction of antinutritional substances, not changing significantly the nutritional value and sensory characteristics required by consumers. This study has the objective to evaluate the effect of different gamma irradiation doses (0.5; 1.0; 2.5; 5.0 and 10.0 kGy) on microbiological characteristics of the crude grains of cowpea bean just after irradiation process and

during storage time of 30 and 60 days. For that, fungical percentage analysis were performed in order to evaluate the presence of *Aspergillus ochraceus, Aspergillus niger* and *Aspergillus flavus*, the toxicological potential of the fungus suspected to produce some type of aflatoxins B_1, B_2, G_1 and G_2 or ocratoxin A.

METHODOLOGY

Cowpea beans were acquired at a Rio de Janeiro local grocery store, a total of 60 units of 500g each, packed in plastic bags, all from the same lot. **2.1. Irradiation Process-** The irradiation process was performed with Cs-137 radiation source and maximum dose rate of 1.8 kGy/h. The irradiation doses applied to the grains of cowpea bean in this study were 0.5; 1.0; 2.5; 5.0 and 10.0 kGy. **2.2. Fungical Percentage Analysis-** *Petri* plates of mean size (90 x 15mm) with selective culture median AFPA (Base Oxoyd, with 0,1g of Chloronphenicol Sigma) for *Aspergillus flavus* and plates with culture median DG 18 (*Dichloran* 18% *Glycerol Agar*) were prepared, according to technique described by Samson et al. (1996). Plating of cowpea bean grains was performed according to direct plating technique (PITT; HOCKING, 1999), using tweezers of sterile metal, under laminar flow. Each Petri plate received 10 grains and six replicates of each treatment (control; 0.5; 1.0; 2.5; 5.0 and 10.0 kGy) were performed. After, the plates were incubated in a BOD (Biological Oxygen Demand) stove kept at 25ºC ± 1ºC, 85% ± 5% of humidity, for 7 days. The plates were supervised in a daily bases. Passed this time, after micelial growth and using hand lens for observation, the fungus were identified and quantified through the confirmation of some morphological characteristics of *Aspergillus ochraceus, Aspergillus niger* and *Aspergillus flavus* fungus colonies, besides others fungus such as *Penicilium, Fusarium* and *Rhizopus*. The same procedure was performed for time "0" (just after irradiation) and for the storage time of 30 and 60 days (BRASIL, 2000). **2.3. Toxicological potential of Isolated Fungus-** *Agar Plug* technique was employed, as described by Filtenborg And Frisvad (1980). **2.4. Aflatoxins and Ocratoxin A-** For aflatoxins B_1, B_2, G_1 and G_2.or ocratoxin A identification, Thin Layer Chromatography (TLC) technique was used. The plate was developed using chlorophorm acetate (90:10) and it was left in a cabinet (with exhausting air) for complete dry. Using a UV/Vis Spectrophotometer with wave length of 366 nm, fluorescent stains were verified, indicating the presence of aflatoxins B_1, B_2, G_1 and G_2.or ocratoxin A. The stains had the colors blue and green located in similar positions of the standards applied as reference (IAL, 2005).

RESULTS AND DISCUSSION

Fungus that are potentially producers of micotoxins, such as *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous,* were found, after 7 days of incubation, in control and irradiated (0.5; 1.0 and 2.5 kGy) cowpea bean samples, for the times t_0, t_1 e t_2 (just after irradiation and 30 and 60 days elapsed, respectively). Fungus of the classes *Penicilium, Fusarium* and *Rhizopus,* were also found in the samples. This was confirmed in the experiment using AFPA and DG 18 culture medians. Gamma irradiation showed to be efficient for destruction of fungical genus usually found in cowpea bean grains. The results suggest that irradiation process is also an important tool for public health area, helping to decrease the incidence of illnesses caused by fungus, some of them producers of micotoxins. It should be pointed out that, as for any other food conservation

process, the microbiological quality improvement obtained by irradiation should come along with previous and posterior process special cares (AZIZ; YOUSSEF, 2002).
The toxicological potential of isolated fungus showed positive result for fungus from genus *flavus* and time t_0 in AFPA culture median, for control and irradiated grains with the dose of 0.5 kGy, and also in DG 18 culture median, for control and irradiated grains with the dose of 0.5 and 1.0 kGy. “Positive” means that the isolated fungus in fungical percentage analysis could produce some type of aflatoxin, B_1, B_2, G_1 and G_2. or ocratoxin A. Using TLC method, aflatoxins and ocratoxin A were not found in the grains of cowpea bean control and irradiated with the doses of 0.5; 1.0; 2.5; 5.0 and 10.0 kGy, for the three times analyzed, zero, 30 and 60 days (t_0, t_1 and t_2, respectively). The detection limit of the method is 1,5µg/Kg for aflatoxins B_1 and G_1 and 1,0µg/Kg for aflatoxins B_2 and G_2 (BRASIL, 2000).

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