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EFFECT OF GAMMA RADIATION(^{60}CO) ON THE GROWTH *Aspergillus ochraceus* COFFEE (*Coffea arabica* L.)

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ABSTRACT

Coffee is a major product on the world market. Its acceptance depends strongly on the sensory characteristics of the beverage, that is its aroma and flavor. One of the most important aspects of coffee culture today is the cup quality as such all segments of coffee production network are concentrating efforts to improve this trait. Foods that have been contaminated with fungi may contain may produce many mycotoxins including ochratoxin A (OTA). To prevent OTA in foodstuffs, it is necessary detect the producing fungi early. Ionizing radiation is a safe, environmentally clean, energy efficient process that can be used to increase the quality and reducing the microbiological contamination of the coffee. The aim of the present study was to evaluate the effects of different gamma radiation doses (0.0, 6.0, 12.0 and 18.0 kGy) on the growth of *Aspergillus ochraceus* in coffee (*Coffee arabica* L.). The analysis were performed to determine the fungi contamination the results were expressed as the viable counts per gram of sample (CFU/g) coffee samples irradiated and unirradiated. The results shows that microbiological contamination of coffee disagrees when increase doses of irradiation. The radiation doses 6.0, 12.0 and 18.0 kGy used resulted in a elimination of the number of *Aspergillus ochraceus* CFU/g when compared to the nonirradiated control group. Under the present conditions, gamma radiation was found to be an alternative for the control of *Aspergillus ochraceus*.

1. INTRODUCTION

Microbial action detrimental to the quality and safety of the final product will depend on environmental conditions as well as crop and product management [1-2].

Ochratoxin A (OTA) is a nephrotoxic and nephrocarcinogenic mycotoxin [3] that has been detected in a variety of food products, including green coffee beans, nuts, cocoa beans, cereals, dried fruits, spices [4], beer and wine [5-6-7].

OTA was originally described as a metabolite of *Aspergillus ochraceus* Wilhelm [8]. However, it is produced by several related Aspergillus species: *A. alliaceus* (in section Flavi), *A. albertensis* (in section Circumdati), *A. niger*, and *A. carbonarius* (in section Nigri) [9-10-11] and by *Penicillium verrucosum* [12].

OTA production by *A. ochraceus* was discovered by Van der Merwe et al. (1965) [8], but this species has been regarded as an uncommon fungus except in long-stored food commodities [13].

The presence of OTA in green coffee beans has been reported by several authors in concentrations between 0.2 and 360 Ag/kg [14- 15- 16], the source of OTA in coffee remains poorly understood.

Temperature and water activity (Aw) are important factors limiting the fungal colonization of substrates and consequently for the OTA production capacity [17]. The importance of these abiotic factors in spoilage of barley grains [18] and coffee beans [19].

The knowledge of optimal and marginal temperature and aw levels for mould proliferation and OTA formation can be useful to optimize post-harvest handling and storage of food products [19].

The control of all stages of food processing is important for the producers to realize that good agricultural practices (GAP) represent the primary line of defence against contamination of cereals with mycotoxins, which should be complemented by good manufacturing practices (GMP) during the handling, storage, processing, and distribution of cereals for human food and animal feed [5].

Irradiation of food is the use of ionizing radiations from radioactive isotopes of cobalt or cesium or from accelerators to reduce microbial contamination on food, resulting in improved microbial safety, as well as extended shelf life of food [20]. Ionizing radiation are applied to foods to improve their keeping quality [21].

Efficiency and safety of food irradiation has been approved by several authorities such as World Heath Organization (WHO), International Atomic Energy Agency (IAEA) and Food Agriculture Organization (FAO) [22- 23].

2. MATERIAL AND METHODS

2.1. Samples

Samples of green coffee grains (Figure 1.) were purchased from local retail market in São Paulo, Brazil.

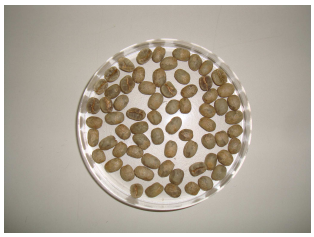

Figure 1. Samples of green coffee grains kept in dishes

2.2. Irradiation

Twelve samples, with 10g each, , were kept in dishes and irradiated with 20 kGy irradiated at Nuclear and Energy Research Institute - IPEN/CNEN (São Paulo, Brazil) using a Gammacell

220 cobalt-60 (MDS Nordion Ottawa, Canada Ltd) to eliminate the natural microbial contamination.

The water activity was adjusted and inoculation of spore suspension of *Aspergillus ochraceus* in dishes. Then was applied doses 0.0, 6.0, 12.0 and 18.0 kGy to evaluate the fungi resistance.

2.3. Temperature control and Humidity

The samples were kept in sterile dishes inside a plastic container with a relative humidity (RH) of 98.1%. This relative humidity was produced by 200 mL of a 30% solution of potassium sulfate (K_2SO_4) for high level of water activity (Aw) [24].

2.4. Measurement of water activity (Aw)

The measurement of water activity of the green coffee grains at ± 20,7°C was conducted after the samples were irradiated in a AQUALAB CX-2 equipment from DECAGON Devices Inc. Moisture content determination was carried out with a digital thermo-hygrometer (Digital Thermo- Hygro Clock).

2.5. Inoculation of spore suspension in the green coffee samples

The spore suspension was prepared by diluting *Aspergillus ochraceus* strain (Figure 2) in 100mL of sterile distilled water was mixed with two drops of Tween 20. The spores were counted in a Neubauer chamber. The suspension concentration was adjusted to $0{,}7\ 10^7$ spores/mL. In the samples were inoculated by spraying with 2 mL of the fungal spore suspension (using a pipette). The samples were kept in plastic containers, while humidity and temperature were controlled with a thermo-hygrometer. The containers were sealed and incubated at 25ºC for 7 days.

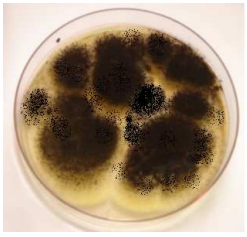

Figure 2. Colonies of *Aspergillus ochraceus*

2.6. Plate counting

Samples were test immediately after irradiation. The analysis were performed to determine *Aspergillus ochraceus* presence in green coffee samples irradiated and unirradiated were measured according to the method described by Pitt and Hocking (1997) [25].

The analysis of fungi were performed by counting the colony forming units per gram of green coffee grains (CFU/g) by plating on the surface, using the dilution of 10 g of product thoroughly mixed with 90 mL of sterile distilled water and shaken in sterile tubes for 30 min. Spore counting was performed by the plate-count technique in the Potato Dextrose Agar (PDA) using each suspension in a serial dilution from 10^{-1} to 10^{-5}. After incubation at 25°C for 7 days, the counting was performed according to Pitt et al. (1983) [26].

3. RESULTS AND DISCUSSION

Green coffee presents a real problem related to microbiological load due to the fact that it is extremely sensitive to contamination. Figure 3 shows the microbiological contamination of coffee (*Coffea arabica* L.) by *Aspergillus ochraceus*. It was observed from that green coffee samples that the fungi count no microorganisms present after irradiation with 6.0 kGy. It was shown, that irradiation effectively reduces the number of colony forming units in the green coffee beans samples under investigation at all doses used (Table 1).

Figure 3. Contamination of coffee (*Coffea arabica* L.) by *Aspergillus ochraceus*

Braghini et al. (2009), studied the Sunflower seed samples inoculated with pore suspensions irradiated with 2, 5 and 7 kGy presented a significant reduction in the umber of CFU/g, with this decrease being proportional to the radiation dose used. The level of contamination detected in control (nonirradiated) samples was higher than that observed in the other samples [26].

Table 1. *Aspergillus ochraceus* in green coffee samples irradiated and unirradiated

Samples	Control (CFU/g)	6.0kGy (CFU/g)	12.0kGy (CFU/g)	18.0kGy(CFU/g)
1	$2,7\ 10^{-1}$	Absence	Absence	Absence
2	$3,1\ 10^{-1}$	Absence	Absence	Absence
3	$3,5\ 10^{-1}$	Absence	Absence	Absence
Average	$3,1\ 10^{-1}$	-	-	-
Standard Deviation	$\pm\ 0,4\ 10^{-1}$	-	-	-

The measurement of water activity is important considering the development of a product, can be used for the determination of shelf-life and it is an analysis of quality control [27].

The initial water activity of the coffee beans were 0,614 that were increased after adjusting the temperature and humidity. A high level of water activity (Aw) is necessary for fungal growth. The water activities for the analyzed samples ranged between 0.938 and 0.944. The table 2 shows the water activity of samples irradiated with their respective doses.

Table 2. Levels of water activity (Aw) of coffee samples

Samples	Control	6.0kGy	12.0kGy	18.0kGy
1	0,939	0,942	0,943	0,940
2	0,941	0,940	0,941	0,942
3	0,938	0,939	0.942	0,939
Average	0,9393	0,9403	0,9420	0,9407
Standard Deviation	$\pm 1{,}247\ 10^{-3}$	$\pm 1{,}528\ 10^{-3}$	$\pm 1{,}000\ 10^{-3}$	$\pm 1{,}155\ 10^{-3}$

E. Pardo et al (2004) demonstrated that the minimum water activity and temperature levels at which germination of *Aspergillus ochraceus* in Green coffee beans occurred were 0.90 aw at 10ºC and 0.80 aw at 20–30ºC, which showed a minimum level for germination of 0.85 aw [28].

4. CONCLUSION

Therefore, the results shows that gama radiation doses 6.0, 12.0 and 18.0 kGy are effectively inhibit growth of *Aspergillus ochraceus* in green coffee. Under the condicions of the present search, gamma radiation was found to be a alternative for the control of *Aspergillus ochraceus*.

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