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DISINFECTION OF *Aspergillus ochraceus* AND INACTIVATION OF OCHRATOXIN A IN CREAL – BASED BABY FOOD USING GAMMA RADIATION

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ABSTRACT

Ochratoxin A is a typical cereal contaminant with strong nephrotoxic activity. Gamma irradiation was used as a technique of food preservation to disinfect the cereal – based baby food of *Aspergillus ochraceus,* as well as inactivation of ochratoxin A. Unirradiated samples of cereal-based baby food were spiked with *A.ochraceus* and incubated for 30 days, the fungus could grown reaching to mean counts of 2.3×10^7 (CFU/g) and produced ochratoxin A in mean amounts of 86 (ppb) through the incubation period. At a radiation dose of 5 (K Gy), the growth of *A.ochraceus* and the subsequent ochratoxin A production in cereal – babsed baby food were completely inhibited. The results of detoxification trial revealed that destruction of ochratoxin A by gamma irradiation doses had already begun at 5 (KGy). A radiation dose of 10 (KGy), controlled the occurrence of ochratoxin A in cereal – based baby food only by 23%.

INTRODUCTION

The consumption of cereal – based baby food is increasing rapidly by the young children in the weaning period. However, cereal – based baby food could be a source of toxic substances such as ochratoxin A (OTA). Ochratoxin A is a typical cereal contaminant with strong nephrotoxic activity. This mycotoxin is produced mainly by molds such as *Aspergillus ochraceus*. The permitted level for ochratoxin A in cereals is 5 µg Kg^{-1} (5 ppb) according to the European commission (D'Mello, 2003). Irradiation as a method of food preservation has excellent potential to improve food safety and extend shelf-life. The use of irradiation was approved by the FAO/IAEA/WHO joint committee (WHO, 1981) and the FDA (1997) on the wholesomeness of irradiated food. Gamma irradiation is known to cause injury to microorganisms and has been widely reported to prevent or delay food spoilage and the effect of radiation on mycotoxins was studied (Farkas, 1989; Erhart, 1990; Refai et al., 1996; and Aziz and Mahrous, 2003). The stability of mycotoxins with respect to different physical and chemical agents is well-known (WHO, 1979; Arafa, 1995 and Ibrahim and Arafa,1995).

The present study was designed to evaluate the effect of gamma radiation on the growth rate, ochratoxin A production by *Aspergillus ochraceus* and to ascertain the effect of gamma irradiation on ochratoxin A stability in cereal – based baby food.

MATERIALS AND METHODS

Samples:

Samples of cereal-based baby food were purchased from a pharmacy.

Mold:

A local strain of Ochratoxin A producing *Aspergillus ochraceus* of our stock cultures was used through this study.

Mycotoxin:

Ochratoxin A used in the experiments of detoxification was of the product of Sigma, chemical company, St. Louis, US.

Preparation of spores suspension:

Aspergillus ochraceus was grown on slants of potato dextrose agar (PDA) supplemented with 0.1% yeast extract and 2% sodium chloride (Refai *et al*., 1996), for 7 days at 25°C.

Spores were harvested in sterile 0.1% Tween 80, filtered through several layers of sterilized cheese cloth, pelleted by centrifugation at 12,000 rpm for 5 min at 5°C, washed with sterilized distilled water and resuspended in sterilized 0.1% Tween 80 solution.

Ability of *Aspergillus ochraceus* to grow and produce Ochratoxin A on cereal-based baby food:

A local strain of *A. ochraceus,* known to be ochratoxin A producing on synthetic medium was assayed for growing and producing of ochratoxin A on cereal-based baby food.

Appropriate concentration of spores in sterile 0.1% Tween 80 in water was added to 100g of ochratoxin A-free sterilized cereal-based baby food samples in a 1000 ml Erlenmeyer flask then the cultures adjusted to have a final moisture content of 25% and maintained at 28°C in humidified incubator for 10,20 and 30 days.

The numbers of fungal colony forming units and the ochratoxin A levels were determined at interval incubation periods.

Extraction and analysis of ochratoxin A:

Ochratoxin A was extracted from cereal-based baby food with 70% methanol by the procedure of (AOAC, 1995). Ochratoxin A was quantified by the enzyme immunoassay for the quantitative analysis of ochratoxin A using RIDASCREEN® FAST Ochratoxin A Test Kit (Art. No.: R5402).

All reagents required for the enzyme immunoassay – including standards – are contained in the test kit. The measurement of the absorbance is made photometrically at wavelength 450 nm against blank using computerized spectrophotometer (Jen Way 6105 uv / vis). The absorbance is inversely proportional to the ochratoxin A concentration in the sample. The ochratoxin A concentration in µg / Kg corresponding to the extinction of each sample can be read from the calibration curve. The values were calculated with provided software. The mean lower detection limit of the RIDASCREEN® FAST Ochratoxin A test kit is 5 µg / Kg (5 ppb).

Radiation effects on *Aspergillus ochraceus* growth and ochratoxin A production:

Samples (50g) of cereal-based baby food were irradiated with various doses ranging from 0 to 5 (KGy) by using ^{60}Co gamma rays (Gamma cell, National Centre for Radiation Research and Technology, Cairo, Egypt).

The cereal-based baby food samples were inoculated with *A.ochraceus* spores suspension (10^5 spores per g) before irradiation. Prior to irradiation the samples were adjusted to have a final moisture content of 25%. Following irradiation the samples were maintained at 28°C in a humidified incubators for 45 days. The ochratoxin A levels and numbers of fungal colony forming units were determined at the end of each incubation period in the course of 45 days.

Detoxification of ochratoxin A-contaminated cereal-based baby food by gamma radiation:

The cereal-based baby food samples were mixed with 100 µg / Kg (100ppb) ochratoxin A and mechanically shaken to be ready for detoxification. 25 g of spiked samples were packed into polyethylene pouches, sealed and irradiated with various doses ranging from 0 to 10 (KGy) using ^{60}Co gamma rays. The ochratoxin A residues in cereal-based baby food samples were determined as described above.

Statistical analysis:

The statistical analysis of the present results was calculated according to Bailey (1994).

RESULTS AND DISCUSSION

Ability of *Aspergillus ochraceus* to grow and produce ochratoxin A in cereal-based baby food:

Data of the numbers of fungal colony forming units and ochratoxin A levels are presented in Table (1). The results show that the cereal-based baby food was a favorable medium for both growth of *A. ochraceus* and production of ochrotoxin A. The data in Table (1) revealed that *A.ochraceus* had the ability to grow and produce ochratoxin A during the incubation period of 30 days, whereas the mean counts of *A.ochraceus* increased from 1.5×10^5 (CFU/g) at start of the experiment reaching to overall means of 7.4×10^5, 4.8×10^6 and 2.3×10^7 (CFU/g) after 10, 20 and 30 days of incubation respectively. On the other hand, the amounts of ochratoxin A produced by *A.ochraceus* were directly proportional to the *A.ochraceus* growth, the overall mean levels were 17, 52 and 86 (ppb) after incubation periods of 10, 20 and 30 days respectively.

Aspergillus ochraceus has been reported as the most common mycotoxigenic mould of grain and some food commodities which can produce ochratoxin A (Krogh, 1983; Abramson *et al*., 1990; Refai *et al*., 1996; and Aziz and Moussa, 1997). Beretta *et al*. (2002) reported that the analyses of 119 batches of baby foods considered indicated that, 20 batches contained

detectable quantities of ochratoxin A and 4 of these contained ochratoxin A above the Italian permitted value. Several surveys have revealed the incidence of ochratoxin A in cereal grains and food products, ochratoxin A was detected in 5 and 6 out of 47 and 40 organic and conventional cow's milk samples with range of 15-28 ng / l and 11-58 ng / l respectively (Skaug, 1999). The occurrence of ochratoxin A in wheat grown under organic farming conditions was investigated by Birzele *et al*. (2000), They reported that ochratoxin A contamination was found in 24.1% of wheat samples. Another study of Rafai *et al*. (2000), reported that the incidence rate and / or concentration of ochratoxin A in Hungarian – grown cereals is occasionally considerable, also Vrabcheva *et al*. (2000) recorded the occurrence of ochratoxin A in cereals from Bulgarian villages. Krysinska – Traczyk *et al*. (2001), stated that ochratoxin A was detected in 60% of grain samples (0.5 ppb).

Table 1. Ability of *A.ochraceus* to grow and produce ochratoxin A in cereal-based baby food through 30 days incubation

Incubation periods (days)	Counts of *A.ochraceus* (CFU/g)*	Amounts of ochratoxin A produced (ppb)*
0	$1.5 \times 10^5 \pm (1.0 \times 10^4)$	ND
10	$7.4 \times 10^5 \pm (6.5 \times 10^4)$	17 ± (3.5)
20	$4.8 \times 10^6 \pm (3.5 \times 10^5)$	52 ± (3.1)
30	$2.3 \times 10^7 \pm (2.2 \times 10^6)$	86 ± (2.8)

*** Values are mean of five replicates ± (standard deviation)**
ND, not detected
CFU, colomy forming units

Effect of gamma irradiation on *A. Ochraceus* growth and ochratoxin A production in baby food:

The influence of gamma irradiation on *A.ochraceus* growth and ochratoxin A production in the cereal-based baby food is reported in Table (2). It is clear that, by increasing radiation doses, the *A.ochraceus* counts mean is greatly decreased as well the fungus is completely inhibited at 5 (KGy). Regarding this, as shown in Table (2), the initial mean counts of *A.ochraceus* in unirradiated baby food increased from 5.2×10^5 (CFU / g) to 7.5×10^8 (CFU / g) through the incubation period of 45 days. Whereas a radiation dose of 2.5 (KGy) was required to reduce the fungal mean counts from 5.2×10^5 (CFU / g) to 2.1×10^2 (CFU / g) immediately after irradiation and to 5.7×10^3 ,5.2×10^4 and 9.5×10^4 (CFU / g) after 15, 30 and 45 days of incubation at 28°C. From the tabulated data in Table (2), it could be stated that the irradiation dose of 2.5 (KGy) made difficult or slow the progress of the *A.ochraceus* growth in the baby food samples throughout the incubation period of 45 days compared with the unirradiated baby food samples. A radiation dose of 4-6 (KGy) was recorded to completely inhibit the growth of the fungal flora contaminated food and feed products (Refai *et al*., 1996; Aziz *et al*., 1999; El. Bazza *et al*., 2001; and Aziz and Mahrous,2003). As shown in Table (2), the obtained results indicate that the amounts of excreted

ochratoxin A by *A.ochraceus* in the baby food samples after exposure to radiation dose of 2.5 (KGy) were 14,22 and 28 (ppb) after 15,30 and 45 days of incubation respectively. Level of ochratoxin A reduced to 28 (ppb) in the cereal-based baby food samples by 2.5 (KGy) after 45 days of incubation as compared with 142 (ppb) produced by the non irradiated baby food control. There was an increasing interest in the use of ionizing radiation for inhibition or killing the fungal flora and reducing the production of mycotoxins in stored foods, mainly cereals. In previous study, Aziz *et al*. (1989) reported that gamma irradiation delayed the mycelial growth of *A.ochraceus* and ochratoxin A production, and no growth and ochratoxin A had occurred for fungal conidia exposed to 3.0 (KGy). Also, Refai et al. (1996), reported that the gamma irradiation dose required to inhibit *A.ochraceus* growth and ochratoxin A production was 4 and 3 (KGy) respectively. In study of inactivation of naturally occurring of mycotoxins in some Egyptian food by gamma irradiation, Aziz and Youssef (2002) reported that at a radiation dose of 5 (KGy), the growth of moulds was completely inhibited and a dose of 10 (KGy) was detoxified ochratoxin A by 44-48%. Furthermore, there are numerous studies suggested that the production of mycotoxins is decreased after irradiation of grains or fungal spores under various laboratory conditions (Farkas, 1989; Sharma *et al*., 1990; Badshah *et al*., 1992).

Detoxification of ochratoxin A-contaminated cereal-based baby food by gamma radiation:

In respect of detoxification by gamma irradiation, tabulated data in Table (3) indicated that ochratoxin A was stable against irradiation dose of 2.5 (KGy), whereas by increasing the radiation dose, the percentage of detoxification was increased gradually to reach 23% with irradiation dose of 10 (KGy). These results of the present study reveal that gamma radiation for up to 10 (KGy) does not destroy this mycotoxin, while on the contrary a radiation dose of 5 (KGy) was sufficient for killing of *A.ochraceus* in the samples of cereal-based baby food. Regarding this, the published literatures are conflicting for the influence of irradiation on detoxification of mycotoxins in food. Paster *et al*. (1985) indicated that pure ochratoxin A is stable even at 75 (KGy), in that no reduction in its fluorescence intensity under UV light is recorded following that dose. Also, Kume *et al*. (1987), in a study of the radiation influence on aflatoxins in polished rice, suggested that aflatoxins are very stable to radiation and the dose required for destruction is found to be greater than 50 (KGy). On the other hand, Van Dyck *et al*. (1982) showed that complete destruction occurred at doses exceeding 10 (KGy) when aflatoxin B, was exposed to different doses of gamma rays. As well Refai *et al*. (1996) reported that a dose of 15 or 20 (KGy) was sufficient for complete destruction of ochratoxin in yellow corn and soybean. Although further information regarding the ability of gamma radiation to destroy other mycotoxins is needed, in the light of the available data that the use of irradiation to decompose mycotoxins in foodstuffs is not proven. It is therefore concluded, for the present, that the decontamination of moulds by irradiation, prior to their production of mycotoxins, is the preferred method (Refai, *et al*., 1996).

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Table 3. Detoxification of spiked cereal-based baby food samples with ochratoxin A using gamma irradiation

Radiation doses (KGy)	Ochratoxin A (ppb)	Detoxification (%)
0.0	100 ± 0.0	0
2.5	100 ± 1.6	0
5.0	95 ± 2.3	5
7.5	88 ± 3.0	12
10.0	77 ± 4.2	23

Values are mean of five replicates ± standard deviation

The world health organization (WHO) reviewed 500 studies and concluded that food irradiation poses no toxicological, microbiological, or nutritional problems. Therefore, gamma irradiation may be more attractive for mycotoxins decontamination of foods (Steele, 2001).

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التأثير المطهر والمثبط لأشعة جاما على الفطر أسبيرجيلس أوكراشيس والتوكسين الفطرى أوكرا توكسين فى أغذية الأطفال من الحبوب

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يعتبر التوكسين الفطرى أوكرا توكسين ملوث أصيل وتقليدى للحبوب وله تأثير سام قوى على الكلى. تم إستخدام أشعة جاما – كطريقة لحفظ الأغذية – لتطهير غذاء الأطفال (من الحبوب) – المستخدم فى هذه الدراسة – من الفطر أسبير جيلس أوكراشيس، كذلك لتثبيط التوكسين الفطرى أوكرا توكسين. فى هذه الدراسة تم تلويث عينات من غذاء الأطفال من الحبوب بمعلق جراثيم الفطر وتم التحضين لمدة 30 يوم.

أثبتت النتائج المتحصل عليها قدرة الفطر على النمو وإنتاج التوكسين فى غذاء الأطفال. تم تقدير أعداد خلايا الفطر فى نهاية فترة التحضين حيث وصل متوسط أعدادها إلى 2.3×10^{7} خلية/جرام، كذلك وصل إنتاج الفطر من الأوكرا توكسين إلى 86 جزء فى البليون.

عند دراسة تأثير أشعة جاما على نمو الفطر وإنتاجه للأوكرا توكسين، وجد أن بزيادة الجرعة الإشعاعية تنخفض درجة التلوث الفطرى، كما أن الجرعة الإشعاعية 5 كيلو جراى كافية لتثبيط نمو الفطر أسبيرجيلس أوكراشيس وإنتاج الأوكراتوكسين تماماً فى عينات غذاء الأطفال من الحبوب.

ولدراسة تأثير المعاملة بالإشعاع على تكسير الأوكراتوكسين والتخلص منه، تم تعريض عينات من غذاء الأطفال لجرعات من أشعة جاما وهى صفر، 2.5، 5.0، 7.5، 10 كيلو جراى، ثم تقدير المتبقى من التوكسين. وقد إتضح أن الجرعة الإشعاعية 10 كيلو جراى قد أدى إلى التخلص من الأوكراتوكسين بنسبة 23% .

Table 2: Gamma irradiation effects on *Aspergillus ochraceus* growth and ochratoxin A production in cereal-based baby food during different incubation periods.

Radiation doses (KGy)	Incubation periods (days)							
	0		15		30		45	
	Population (CFU/g)*	Ochratoxin A (ppb)*	Population (CFU/g)*	Ochratoxin A (ppb)*	Population (CFU/g)*	Ochratoxin A (ppb)*	Population (CFU/g)*	Ochratoxin A (ppb)*
0.0	5.2×10^5 ± (1.7×10^4)	ND	4.5×10^6 ± (4.1×10^5)	49 ± (3.7)	4.4×10^7 ± (3.0×10^6)	110 ± (2.8)	7.5×10^8 ± (3.7×10^7)	142 ± (11.7)
2.5	2.1×10^2 ± (1.0×10^1)	ND	5.7×10^3 ± (2.2×10^2)	14 ± (0.9)	5.2×10^4 ± (2.8×10^3)	22 ± (2.0)	9.5×10^4 ± (4.0×10^3)	28 ± (2.3)
5.0	NG	ND	NG	ND	NG	ND	NG	ND

*** Values are mean counts of five replicates ± (standard deviation).**
CFU, colony forming units.
ND, not detected.
NG, No growth of *A. ochraceus*