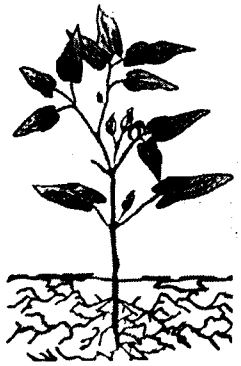

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Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 54(1), 99 - 108, 2009

IMPROVING QUALITY OF SPICES DURING STORAGE USING PHYSICAL TREATMENTS

[8]

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Keywords: γ-Irradiation, Spices, Fungi, Aflatoxine, Physical Treatments

ABSTRACT

Samples of spices (black pepper and cumin) collected from local markets were examined for total fungal contamination (T.F). Different fungal genera and species were isolated and identified. The selected spices were treated separately with physical treatments (γ rays, thermal and washing).The efficiency of these treatments on TF and aflatoxins (AFs) content were investigated. The obtained results revealed that raw marketed spices were contaminated naturally by different species of fungi i.e. *Aspergillus parasiticus, Aspergillus flavus, Aspergillus niger, Aspergillus ochraseus, Aspergillus terreus, Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp. and *Cladosporium* sp. at different levels. γ-irradiation at dose level 10 kGy completely sterilized the spices samples during the storage period (10 weeks), whereas some fungal growth started again after few weeks of storage on samples exposed to 4.0 and 5.0 kGy. In the same time, thermal and washing treatments failed to control TF during storage .The detection of AFs proved presence of B_1, B_2, G_1 in black pepper, B_1, B_2 in cumin with different concentrations. Generally, high γ-irradiation doses (10, 20 kGy) slightly reduced the AFs in spices samples. Thermal treatments and washing also slightly decreased the AFs contaminated in the tested spices. Slight reduction was clear in some AFs which can be express as linear regression analysis with high significant values (R^2) were tabulated in details. Finally, the obtained results proved the preferability of using high dose level of 10kGy for decontamination of TF exist in spices on the condition they are AFs free for the safe human consumption.

INTRODUCTION

Food irradiation by using γ- irradiation has been recommended to be used on a commercial scale in more than 40 countries. Egypt, now has three licenses for irradiated herbs, spices, dried onion and powdered garlic **(E.S. 1997)**. All these commercial applications were done according to the international agreement from World Health Organization **(WHO, 1981)** to use irradiation up to 10 kGy as safe treatment for food human consumption. Also, the same organization raised that level to 75 kGy **(WHO, 1997)**. Decontamination of spices by irradiation occupied the majority of irradiated food as safe alternative for hazard chemicals besides its advantages as applicable method for irradiation the package spices besides the suitability for using on large scale **(Farag *et al* 2005)**.

Black pepper has represented about 80% of the trade mark of all spices besides it is considered to be heavily contaminated with moulds **(Emam *et al* (1995)**. A survey from France implicated pepper as being the source of the toxigenic *Aspergillus flavus* responsible for the high levels of aflatoxin (AF) found in sausages and pepper cheese **(Farkas, 1988)**. Recently, studies showed that 14 spe-

(Received January 24, 2009)
(Accepted March 14, 2009)

cies, were recovered and identified from dried and ground spice samples on several media using standard dilution plate method (Mandeel, 2005). The same author showed that most heavily contaminated spice samples examined were observed in red chili and black pepper in order of magnitude of 1580 and 1120 cfu/g, respectively. The most predominant fungal genera encountered were *Aspergillus, Penicillium, Rhizopus, Cladosporium and Trichoderma*. Also, cumin seems to be most heavily contaminated with different molds as *A. glaucus groups, A. niger and Penicillium* spp. which are usually most prevalent (Mandeel, 2005). AFs are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus. *A. flavus* and *A. parasiticus* on variety of food products (Kelly *et al* 2002). Among of 18 different types of AF identified, major members are B_1, B_2, G_1 and G_2. AFB_1 is normally predominant in food products. In the same time inhalation exposure to the carcinogen AFB_1 in certain occupations is considerable. (Wang *et al* 2001, Henry *et al* 2002; Williams *et al* 2004 and Zinedine *et al* 2005).

The present work was carried out to reduce the fungal count and AFs contamination of most important spices (black pepper, cumin) using safe physical methods as γ-irradiation, gentle thermal treatments and water washing.

MATERIALS AND METHODS

Materials

Twenty samples of spices – ten per each black pepper (*Piper nigrum* L.) and cumin (*Cuminum cyminum* L.) were collected from various retailers in Cairo city (Egypt). Each fifty gram of spice was labeled in clean containing polyethylene packets. The samples were taken to the laboratory of the National Centre for Radiation Research & Technology (NCRRT), Nasr City as soon as they were collected and treated with different treatments. In the same time the test for isolation and identification of different pathogenic fungal genera were done at National Centre for Research, Dokki.

Methods

1- Samples preparation and treatments

Samples from black pepper and cumin were treated separately with the following treatments.

- Gamma irradiation

Pepper and cumin samples were irradiated with γ-irradiation at different doses of 0.0, 3.0, 4.0, 5.0, 10.0 and 20.0 kGy at NCRRT using Cobalt-60 γ-ray source manufactured in Russia at dose rate 0.09 kGy min^{-1}. The source had been calibrated by the National Physical Laboratory (NPL, Teddington, UK) using the dichromate dosimetry system. Three replicates were used per each dose.

- Thermal treatments

Samples of spices were spread on trays in electrical oven with thermometer then heated at 55°C/30 minutes and 55°C/1 hour. Samples were collected separately, packed and stored at room temperature (25-30°C, 70-75 RH%) until starting of analyses.

- Washing process

Whole seeds were washed by stream of pure tap-water for 10 minutes, dried by ventilation and fans. The dried seeds were packed after treatments, stored for 10 weeks at room temperature.

2- Microbiological analysis

The microbiological tests were carried out at Food Industries and Nutrition Division, Toxicology and Food Contaminants Department, National Research Center, Dokki. These analysises were TF and AFs in treated samples. *Potato Dextrose Agar* (PDA) *medium* (Difco) was used for isolation and identification of the different fungal genera. Whereas, AF. standard as B_1, B_2, G_1 and G_2 were obtained from Sigma Chemical Company, U.S.A.

-Isolation and identification of fungi

A survey of fungi and the fungal counts were carried out according to Koburger & Marth (1984). Ten grams of each ground sample were transferred to sterilized flask containing 90 ml of sterilized saline solution. Serial dilutions i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared then 1 ml was transferred on a Petri dish containing Potato Dextrose agar medium and Incubated at 25°C ± 2 for 7 days (Ejechi *et al* 1997). Fungi colonies were counted, then picked, purified on potato dextrose agar (PDA) slants and incubated for 5 days at 25°C ± 2 for identification. The identification were carried out on the purified fungi colonies according to Nelson *et al* (1983).

- Determination of aflatoxins (AFs)

The aflatoxins content extracted from randomly selected samples of spices using chloroform, then filtered through glass wool. The filtrates were transferred to a separating funnel and the lower chloroform layer was passed through anhydrous sodium sulphate. The extracts were finely dried under nitrogen and stored in vials at-20°C until AFs determination. The determination was carried out as follow: -

Spots of extracted samples and aflatoxin standards were applied on pre-coated and reactivated Thin Layer Chromatography (TLC) at 105°C for two hours. Silica gel (Gf_{254}) with thin layer (0.25 mm) was used as stationary phase. TLC plates were then developed in Toluene: ethyl acetate: 88% formic acid (60:30:10 v/v/v). After developments TLC were dried and exposed to long wave length ultra violet light for visual estimation. A Spectrodensitometer was used in assay with a reflectance mode at excitation wave length 360 nm. This was carried out by Micro Analytical Centre, Faculty of Science, Cairo University. AF content in the positive samples were determined, and calculated as µg/kg by the following equation according to AOAC (2000).

$$AFs\ (\mu g/kg) = (B\ X\ Y \times S \times V) / (Zx\ X \times W).$$

Where: B = area of AFs peak in sample, Y = concentration of AFs standard µg/ml, S = AF standard spotted µl, V = final dilution of sample extracts µl, Z = area of AF peak in standard, X = sample extract spotted µl, W = g sample represented by final extract.

AF Reduction (%) of AF can be calculate as following;

$$AF = \frac{conc.\ AF\ of\ treated\ sample}{conc.\ AF\ of\ untreated\ sample} X\ 100$$

3- Statistical analysis

The regression analysis of obtained results was carried out according tc **Snedecor and Cochran (1967)** by using Excel program with computer.

RESULTS AND DISCUSSION

- Fungal contamination of spices

Untreated samples of raw materials from Egyptian local markets either black pepper or cumin samples were heavy contaminated with fungi. Thirty seven and thirty one fungal isolates from pepper and cumin respectively were randomly collected from Petri dishes for identification tests. Five genera were identified in both spices samples as *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Fusarium* sp. Whereas genus *Cladosporium* sp. was only detected in black pepper as shown in **Table (1).** *Aspergillus* sp. occupied the highest percentage of isolates, it is recorded 54.03% and 51.58% for pepper and cumin respectively. Both of *A. niger* and *A. flavus* occupied the majority percentage of isolates in black pepper and cumin as 29.72% and 32.24% respectively. These results proved presence of fungi as heavy contamination naturally in raw spices-as found before treatment in the local market due to soil or air dust contamination. Also, the same things may be due to poor health and hygienic facilities during pre-, post-harvest as handling or packing process under tropical regions whereas its production area with high humidity, heavy rains and temperature which activate growth of fungi. The same results were obtained by different workers (**Farkas 1988; Farag *et al* 1996; Shaban *et al* 2003; Fazekaz *et al* 2005; Mandeel 2005 and Ardric *et al* 2008**).

- Decontamination of spices

As shown in **Tables (2 & 3)** Total fungal counts(TFC) were **$6.5x10^6$**, - **$5.8x10^4$** $CFU.g^{-1}$ in unirradiated black pepper and cumin, respectively. These numbers increased slightly during storage at room storage to record after ten weeks **$8.2x10^6$**, **$8.0x10^6$** $CFU.g^{-1}$ for black pepper and cumin, respectively. Most of the used treatments decreased TFC in spices either with low doses of irradiation (3,4 kGy), thermal or washing treatments. High doses of irradiation 5.0 kGy inactivated TFC but they grow again at 8 and 10 weeks for black pepper and cumin. In the same time, high doses 10.0 and 20 kGy were the best treatments which succeeded for decontamination the tested spices. Similar to these findings were obtained by some workers (**Farkas, 1988; Farag *et al* 1996; Shabana, 2003 and Mandeel, 2005**).

Effect of physical treatments on aflatoxins (AFs) content

As shown in **Tables (4 & 5)** the concentrations of AFs were higher in raw samples this is due to presence of toxigenic fungi with high contamination levels as mentioned above. The detection of AFs proved presence of B_1, B_2, G_1 in black pepper

Table 1. Fungal species isolated from Black pepper and Cumin

Types of fungi screened	Black pepper		Cumin	
	NPS	%	NPS	%
Aspergillus parasiticus	3	8.10	2	6.45
Aspergillus flavus	6	16.21	5	16.12
Aspergillus niger	5	13.51	5	16.12
Aspergillus ochraseus	4	10.81	3	9.67
Aspergillus terreus	2	5.40	1	3.22
Penicillium sp.	5	13.51	4	12.90
Mucor sp.	2	5.40	4	12.90
Rhizopus sp.	5	13.51	3	9.67
Fusarium sp.	3	8.10	4	12.90
Cladosporium sp.	2	5.40	ND	ND
TNI	37	100	31	100

NPS: Number of positive samples. TNI: Total number of fungal isolates.
N.D: Not detected. (total number of samples per each spice was ten samples.)

Table 2. Effect of physical treatments on Total Fungal Counts of black pepper during storage at room temperature (25-30^{0}C, 70-75 RH %)

Storage period (week)	Un-treated	Irradiation doses (kGy)					Thermal		Washing
		3.0	4.0	5.0	10	20	A	B	
Zero time	$6.5x10^5$	$6.3x10^1$	ND	ND	ND	ND	$5.4x10^3$	$8.4x10^1$	$5.5x10^2$
2	$6.8x10^5$	$6.5x10^1$	ND	ND	ND	ND	$5.9x10^3$	$6.8x10^1$	$5.7x10^2$
4	$6.9x10^5$	$6.7x10^1$	ND	ND	ND	ND	$6.3x10^3$	$8.8x10^1$	$6.0x10^2$
6	$7.0 x10^5$	$6.9 x10^1$	$5.00x10^1$	ND	ND	ND	$6.2 x10^3$	$9.1 x10^1$	$6.4 x10^2$
8	$7.1 x10^6$	$7.0 x10^1$	$5.4x10^1$	$3.2x10^1$	ND	ND	$6.9 x10^3$	$9.3 x10^1$	$6.7 x10^2$
10	$8.2 x10^6$	$7.4 x10^1$	$5.8x10^1$	$3.9x10^1$	ND	ND	$7.1 x10^3$	$9.6 x10^1$	$7.1 x10^2$

A,B = thermal treatments at 55°C/0.5hr and 55°C/1hr.

Table 3. Effect of physical treatments on Total Fungal Counts in cumin during storage at room temperature (25-30°C, 70-75 RH %)

Storage period (week)	Un-treated	Irradiation doses(kGy)					Thermal		Washing
		3.0	4.0	5.0	10	20	A	B	
Zero time	$5.8x10^4$	$5.6x10^1$	ND	ND	ND	ND	$5.0x10^3$	$6.6x10^1$	$4.2x10^2$
2	$5.9x10^4$	$7.5x10^1$	ND	ND	ND	ND	$5.6x10^3$	$6.9x10^1$	$5.2x10^2$
4	$6.1x10^4$	$5.9x10^1$	ND	ND	ND	ND	$5.8x10^3$	$7.0x10^1$	$5.3x10^2$
6	$6.9 x10^4$	$6.0 x10^1$	$4.7x10^1$	ND	ND	ND	$6.0x10^3$	$7.3 x10^1$	$6.0 x10^2$
8	$7.5 x10^5$	$6.4 x10^1$	$5.0x10^1$	$2.9x10^1$	ND	ND	$6.3x10^3$	$7.5 x10^1$	$6.4 x10^2$
10	$8.0 x10^5$	$6.7 x10^1$	$5.8x10^1$	$3.0x10^1$	ND	ND	$6.5 x10^3$	$7.8 x10^1$	$6.5 x10^2$

A, B = thermal treatments at55°C/0.5hr and 55°C /1hr

Table 4. Effect of different treatments of AF concentration* (ppb) in black pepper

Treatments	B_1	B_2	G_1
1- Untreated samples.	9.53	2.50	2.99
2- Irradiation doses (kGy):			
- 3.0	9.29	2.41	2.33
- 4.0	8.24	2.34	2.10
- 5.0			
- 10.0	8.14	1.97	1.80
- 20.0	8.11	1.95	1.86
	7.76	1.65	1.81
3- Thermal treatments			
- 55C/0.5 hr	7.71	2.16	2.12
-55C/1.0 hr	7.19	2.04	2.0
4- Washing treatments :	7.20	2.28	1.94

Each value express mean of three replicates.*

Table 5. Effect of different treatments on AF concentration* (ppb) in cumin

Treatments	B_1	B_2
1- Untreated samples.	6.58	1.33
2- Irradiation doses (kGy):		
- 3.0		
- 4.0		
- 5.0	6.52	1.25
-10.0	6.44	1.23
-20.0	6.32	1.22
	5.80	1.20
	5.64	1.19
3- Thermal treatments		
- 55C/0.5 hr	5.18	1.05
- 55C/1.0 hr	5.04	1.01
4- Washing treatments:		

Each value express mean of three replicates.*

and B_1, B_2 in cumin seeds. Used treatments slightly decreased the exist of AFs. A noticeable reduction was clear at high of doses 10 and 20kGy. By calculations (as percentage) from the initial AF (G_1) concentration in black pepper (2.99ppb), irradiation decreased the concentration to 1.86 and 1.81 (ppb), as 62.2% and 60.5%, respectively. Whereas, the same doses reduced B_1 (85.1%, 81.4%) and B_2 (78%, 66%) for 10.0 and 20.0 kGy, respectively. The same things were in cumin for B_1 which decreased to (88.15%, 85.4%), B_2 to (90.2%, 89.5%) from the initial AFs concentration of untreated samples by using 10.0kGy and 20.0kGy, respectively. The regression analysis proved a linear relationship between irradiation doses and rate of degradation of AFs. As shown in **Table (6)** these results were high significant R^2. The rate of degradation proved that irradiation reduced linearly AFs.

Many investigators proved presence of AFs in black pepper with high levels **(Farkas, 1988)**. Concerning effect of irradiation on AFs ,some workers showed that no effect can occur in raw irradiated food **(Ito *et al* 1994)** whereas, another studies proved the possibility of elimination of B_1 by irradiation at 5.0 kGy **(Aziz and Mahrous, 2004)**. Although the effectiveness of irradiation varied with different RH and media during, post irradiation incubation as proved by some workers **(Odamtten *et al* 1986 and Hilmy *et al* 1995).**

Regarding with thermal and washing effects on AF concentration as shown in **Tables (4 & 5)**. The calculations as reduction effect (%), proved that used gentle thermal (55°C) either at 0.5 and 1.0 hr reduced(80.9% and 75.4%) of B_1, (78%,86.4%) of B_2 and (70.9%, 66.9%) of G_1 in black pepper respectively. Whereas, washing by tap-water then drying decreased the same AFs to (75.5%, 91.2% and 64.9%) for B_1, B_2 and G_1 respectively. The same results were clear in cumin samples after thermal treatments for half and one hour had reduction for B_1 (76.1%, 76.5%), B_2 (78.9%, 75.9%), respectively. Also, washing reduced same AFs to B_1 (76.1%), and B_2 (66.2%), respectively.

It is worth to mention that AFs found exist in tested samples are less than the recommended levels as 20 ppb **(FAO 2002 and FDA, 2002)**. But, many countries have established very low maximum permitted levels of AFs in foods, usually in the range of 1 to 25 µg/kg (ppb) total AFs **(Van Egmond, 1989)**. The Codex Committee on Food Additives and Contaminants of the Codex Alimentarius has the limit for foods in international trade be set at 15 µg/kg total.

Table 6. Linear regression analysis of treatments effects on AFs concentrations in selected spices

Treatments	AF	Linear equation	R^{2*}
Irradiation:			
1- Black pepper	B_1	y=-0.36x+9.80	0.90
	B_2	y=-0.16x+2.70	0.91
	G_2	Y=-0.23x+2.90	0.80
2- Cumin.	B_1	Y=-0.20x+7.00	0.90
	B_2	Y=-0.02x+1.30	0.80
Thermal treatments: Thermal treatments:			
	B_1	Y=-2.30x+11.1	0.80
1- Black pepper	B_2	Y=-0.30x+2.80	0.91
	G_1	Y=-0.50x+3.40	0.84
2- Cumin	B_1	Y=-2.30x+11.1	0.80
	B_2	Y=-0.77x+3.08	0.90

*R^2 = Correlation coefficient

The obtained results proved the preferability of using high doses as 10.0kGy for decontamination of spices besides the possibility of decreasing the concentration of AF to safe levels for human consumption. Also, these results as alarm for take an adequate processing during post-harvesting as drying, storage under low moisture content, quality control by using traceability system to avoid AFs production.

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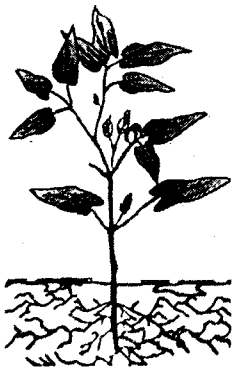
حوليـات العلـوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٤)، عدد (١)، ٩٩-١٠٨، ٢٠٠٩



تحسين جودة التوابل أثناء التخزين بإستخدام الطرق الطبيعية

[٨]

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الموجـــز

يتناول هذا البحث استخدام طرق طبيعية للتغلـب على التلوث بالفطريات الموجود بصورة منتشرة فـي التوابل والمفرزة للسموم الفطرية والمسببة للأمراض السرطانية كما ثبت بالمراجع العلميه.

حيث تم تجميع عشرين عينه من أسـواق مدينـة القاهرة خاصة المعروضة عشوائياً من الفلفل الأسود، الكمون وذلك لكثرة الاستخدام فى الاغذية .وقـد تـم إجراء الاختبارات الميكروبولوجيـة للعـدد الكلـي للفطريات الملوثة ، عمل عزلات من هذه الفطريـات والتعرف عليها مع تقدير السموم الفطريـة بالتوابـل المختبرة مع استخدام عدة طرق طبيعية مثل استخدام اشعه جاما بجرعات (٣،٤، ٥،١٠، ٢٠) كيلـوجراي كذلك استخدام الحرارة على ٥٥ درجه مئويـه لمـدة نصف ساعة وساعة، وأخيرا استخدم الغسيل بالمـاء الجارى من الصنبور ثم التجفيف على درجة حـراره الغرفة وقد تم دراسة تأثير هـذه المعـاملات علـى التلوث الفطرى وسمومه فكانت أهم النتائج التاليه:

١- تم عزل ٣٧ عزله فطرية من الفلفل الأسود ، ٣١ عزله من الكمون حيث وجد أنها تنتمى لخمـسة أجنـاس فطريـة هـى الاسـبرجلس (بأنواعـه باراستيكس - فلافس - نيجر - اكراتوكـسين - نير بوس) والجنس بنسليوم ، الجـنس ميكـور ، ريزوبيوم - فيوزاريوم - كلاد سبورم) وذلـك على كل من الفلفل الأسود والكمون .

٢- السموم الفطرية الموجودة فـى عـزلات الفلفـل الأسود هـى B_1 ، B_2 ، G_1 وغـاب G_2 وكانـت التركيزات فى المدى مـن ٢,٣٣ - ٩,٥ جـزء/ بليون.

أما الكمون فوجد B_1 ، B_2 تركيزات فـى المـدي ١,٣٣- ٦,٥٨ جزء/ بليون وغاب G_1 ، G_2.

أما تأثير المعاملات المستخدمة فكانت نتائجها هى

١- تقليل العدد الكلي للفطريات حيث انخفض بزيادة الجرعة الاشعاعية المستخدمة وتم التعقـيم التـام والأزالة العامة للفطريات الملوثة علـى مـستوى الجرعة ١٠,٠ كيلو جراى مـع تقليـل تركيـز السموم الفطرية بالعينات المعاملة حيث وجـدت علاقة خطية بين زيادة الجرعة ودرجـة تقليـل السموم الفطرية بالتوابل المعاملـة وذلك بدرجـة معنوية إحصائياً.

تحكيـم: أ.د محمد عبد الرازق النواوى
أ.د حسين عبد الكريـم

٢-وجد أن تأثير المعاملة الحرارية والغسيل بالماء العادي أقل تأثيراً عن استخدام الإشعاع وعليه فإنه يمكن التوصية بضرورة اتباع نظم الجودة الغذائية خلال معاملات ما بعد الحصاد خاصة أثناء الجمع والتجفيف للتوابل لتلافي التلوث بالفطريات مع إجراء التجفيف و النقل والتعبئة والتخزين تحت الظروف العلميه المناسبة لتلافي التلوث الفطري وبالتالي تزايد الفطريات وما يصاحبها من سموم فطرية مع اعتبار استخدام الاشعاع عند جرعة ١٠,٠ كيلو جراي كافيه للقضاء التام على الفطريات لتلافي السموم المدمرة لصحة الإنسان.