The Effect of Ionizing Radiation on Microorganism in some spices

khalid K. AL.Bayatti

College of Pharmacy - AL-Mustansiriya University - Baghdad-Iraq

Summery

The present study was undertaken to evaluate the use of ionizing radiation in decontaminating the total microbial burden of Capsicum peppers (*Capsium annuum*), Caraway (*Carum carvi*), and Clove (*Dianthus caryphyllus*). The spices were irradiated in polyethylene bags with 5KGy and 10KGy of gamma radiation from Co-60 irradiator. Total number of bacteria and molds survived per gram of spices after irradiation were calculated. Results showed that 5KGy gamma radiation reduced the total number of bacteria by 95-98% and the number of molds and yeast by 62.8-98.2%, while 10KGy reduced the total number of bacteria by 95.5-99.9% and the number of the molds and yeast by 67-99.7%. These percentages are accepted within the maximum permissible microbial counts for human consumption except mold and yeast counts in caraway which its number was still more than the maximum permissible count for human consumption, and the doses used in these experiments have no significant changes on the sensory quality of the spices.

تأثير الأشعة المؤينة على الأحياء الدقيقة في بعض التوابل خالد خورشيد كاظم كلية الصيدلة – الجامعة المستتصرية– بغداد–العراق تاريخ التسليم 11/05/2008 تاريخ القبول 19/04/2009 الخلاصة

أجريت هذه الدراسة لمعرفة مدى الاستفادة من استخدام الأشعة المؤينة في تعقيم التوابل وتقليل المحتوى الميكروبي . تمت الدراسة باستخدام ثلاثة أنواع من التوابل شائعة الاستعمال , وهي الفلفل الأحمر والكروية والقرنفل , عبئت التوابل في اكياس من البولي اثيلين وشععت بالجرعتين 5 كيلوكري و 10 كيلوكري من اشعة كاما في جهاز Co-60 irradiator . وقد تبين بان الجرعة 5KGy قللت العدد الكلي للبكتريا بنسبة 94-98% والفطريات والخمائر بنسبة 2.50-98.9% . أما الجرعة 10KGy فقد قللت نسبة البكتريا بحدود 5.59-99.9% ونسبة الفطريات والخمائر بحدود 67-99.9% , وهذه النسبة هي ضمن الحدود المسموح بها للتناول البشري بالنسبة للحمل الميكروبي . عدا الاعفان والخمائر في الكروية إذ لا يزال عددها بعد التشعيع بالجرعتين 5 كيلو كري و 10 كيلو كري اكبر من الحدود المسموح بها للتناول البشري و 10 كيلو كري أن علي مؤثرة على التوابل التي درست من ناحية اللون والطعم والرائحة

Introduction

Spices are used in virtually all categories of the food industry, meat, fish, vegetable products and bakery products. There is an increasing demand around the world for spices in connection with increasing amount of processed food, which must be flavored (1).

With regard to spices, both producers and food industries face the problem of the high microbial count of spices, especially contamination by molds (2) and heat-resistant bacterial spores (3). In general, black peppers, caraway, coriander, capsicum peppers, ginger, marjoram and turmeric are the most highly contaminated spices. The total viable count may reach the 80-100 million per one gram(4), the majority of the microbial flora of spices consists of aerobic and anaerobic spore-forming bacteria, including genera of *Bacillus*, *Salmonella* and *Clostridium* (5). Powers *et.al* (6) tested 110 samples of various spices for incidence and level of *Bacillus cereus*, the organism was found in 53% of the spices and counts ranged from 50 to 8500 per gram. Of the isolates, 89% were enterotoxigenic. The frequent occurrence of *Bacillus cereus* in spices is significant with respect to food safety and sanitation.

Spices play a major etiological part in mold contamination of meat products (2,3). Spices examined for mold counts have revealed that *Penicillium species*, *Aspergillus glaucus*, *Aspergillus niger*, and <u>Aspergillus flavus</u> are frequently found to varying extent (7,8).

Relative high incidence of toxigenic molds has been found in spices (8, 9, 10) and several authors have detected aflatoxins in a range of spices (11, 12) although the levels of aflatoxin recorded are generally low.

Chemical treatment of spices with ethylene oxide (EO) or propylene oxide (PO) is used commercially in several countries to reduce the viable cell counts, but what is more serious disadvantage, such as toxic chemical residue, inducing mutagenic effects, propylene oxide is causing carcinogenic effects in animal tests(13,14).

Heat sterilization, Microwave treatment and UV radiation had also been applied in an attempt to control microorganisms in selected spices, but all of them were considered to be unsuitable to bring about decontamination (15).

Electron bombardment technique, X-rays, and gamma rays have been employed for the sterilization of spices, however because of the limited penetration of the electron been, more penetrating radiation such as gamma rays, can be applied. It was established to achieve nearly total sterilization (i.e. a reduction of total viable counts to less than 10 microorganisms per gram) a reduction dose of 8 to 20 KGy is required, depending on the initial level of contamination (5).

The purpose of the present study is to reduce the viable number of bacteria and molds in some selected spices, capsicum pepper, caraway and clove to lower allowable number for human consumption using as low dose of gamma radiation as possible without altering or destroying the spice sensor quality as color, odor and flavor and chemical structure.

Materials and Methods

Spices: All spices were purchased from local markets.

Irradiation of spices: Samples of 10 gram of spice (capsicum peppers, caraways and cloves) were placed in polyethylene containers and irradiated with total doses of 5KGy and 10 KGy gamma rays from Co-60 irradiator.

Total viable counts of Bacteria and Molds:

One gram of irradiated spices were suspended in 100 ml. of sterilized saline solution (0.9 % Nacl), agitated vigorously and serial decimal dilutions were made. Then 3ml of the same dilution were added to 3 plates (one ml. on each plate to give about 100-300 colonies per plate) containing 25 ml per plate of sterilized nutrient agar, and incubated at 37° c for 48hrs.

For total mold counts, the same dilution procedure was followed, and 3ml of the specified dilutions were plated on 3 potato-dextrose agar plates and incubated at 25°c for 5-7 days. At the end of the incubation period, total number of bacterial or mold colonies were counted on each plate and the mean number of 3 plates of the same dilution was calculated per gram of the irradiated spices.

For control samples, the same pervious procedure was followed using unirradiated spices.

Results and Discussion

Table 1 represents the effect of 5KGy and 10KGy of gamma radiation on capsicum pepper. Total number of viable counts of bacteria after 5KGy irradiation was reduced by two log cycle (98% killing) and after 10KGy irradiation was reduced by three log cycle (99% killing). The number of bacteria remained per gram of capsicum pepper after 10KGy irradiation was within the maximum allowable number for human consumption according to the International Standards (4).

For molds and yeast , total number of viable counts have also been reduced by one log cycle (94% killing) and two log cycle (99% killing) after exposure of capsicum pepper to 5KGy and 10KGy respectively .

Table (2) shows the effects of the same gamma radiation doses (5KGy and 10 KGy) on the number of viable counts of bacteria and molds on caraway, the number reduced by one log cycle for both doses or the killing rates were 94% and 95% for bacteria and 63% and 67% for molds and yeast for dose of 5KGy and 10KGy respectively. The number of viable counts of molds and yeast after irradiation with 10KGy was still more than the maximum allowable counts per gram of the caraway, this may due to some technical errors in experimentation or may need more doses of radiation to reduce the number to the allowable counts for human consumption, although the experiment repeated two times but ends with the same results. The mold contamination of spices is highly important because of the microbial flora of the spices, only molds are capable of growth in storage at an ambient temperature and a minimum relative humidity of 72-75% (17), this may illustrate the reason why the number of viable counts of molds and yeast in the present study on the caraway was still more than the maximum allowable counts after irradiation with 5KGy and 10KGy. Thus the ability of surviving microorganisms in irradiated spices after irradiation for several months was high.

Table (table 3) shows the effects of irradiating cloves with 5KGy and 10KGy gamma radiation. In turn the number of bacteria reduced by one log cycle for both doses (about 95% and 98% killing rates respectively). For molds and yeast their number were reduced by one log cycle also (98% killing rate) after 5KGy irradiation and three log cycles (99.9% killing rate) after 10KGy irradiation, the remaining number of bacteria and molds per gram of irradiated cloves are within the maximum allowable number for human consumption according to the International Standard (4).

The results obtained in this study are in accordance with the results obtained by (18) and Vajdi and Pereira (15) and the level of the viable counts are generally accepted as maximal counts of the decontaminated spices desired in the spice trade (17).

Irradiation of food at less than 10KGy significantly reduces the number of many spoilage pathogenic bacteria; it is less effective in reducing the number of relatively radiation-resistant spores of certain pathogenic bacteria such as *Clostridium botulinum* (19).

It is noteworthy that no significant changes were observed in the sensory quality like flavor, odor and color of the capsicum pepper, caraway and cloves respectively, after exposure to 5KGy and 10KGy doses levels, this agree with the finding of (15, 16, 18) where they found less damage to the color and volatile oil content of various spices decontaminated by gamma irradiation than in those treated with ethylene oxide.

Researcher in India found that chili powder capsicum, common spice, black pepper and turmeric can actually prevent bacteria from being destroyed by irradiation, this finding may have practical medicinal uses. Extracts from these spices potentially can protect other organisms (not only bacteria) from the damaging effects of gamma radiation. These researchers believe that certain spices extracts have the potential to protect healing tissues in people undergoing radiation therapy (20). Two antioxidants in black pepper and turmeric (piperine and curcumin) were found to help protect the bacterial DNA from damage by irradiation, the researcher evaluate the spices against two microorganisms during the study *E.coli* and *B.megaterium* (21).

We recommend that capsicum pepper and cloves ought to be irradiated in a well-sealed containers with doses not to exceed 10KGy gamma radiation, while caraway might need more than 10KGy irradiation to reduce the microbial counts to permissible human consumption levels and have no damaging effects on the sensory quality of the spices, like color, odor and flavor before releasing to the markets for general public consumption.

_	Bacteria		Fungi and Yeast	
Dose KGy	Total count cell/gm	% Killing	Total count	% Killing cell/gm
0	3.6×10^{8}	0	$9.5 imes 10^7$	0
5	$4.7 imes 10^6$	98.7	$5.6 imes 10^6$	94.2
10	$3.6 imes 10^5$	99.9	$2.0 imes 10^5$	99.8

Table (1): Effects of 5KGy and 10KGy gamma radiation on total microbial counts of capsicum pepper

Table (2): Effects of 5KGy and 10KGy gamma radiation on total microbial counts of caraway

Dose	Bacteria		Fungi and Yeast	
KGy	Total count cell/gm	%Killing	Total count cell/gm	%Killing
0	$5.7 imes 10^4$	0	$1.4 imes 10^7$	0
5	3.3×10^3	94.2	$5.2 imes 10^6$	62.8
10	$2.5 imes 10^3$	95.5	$4.5 imes 10^6$	67

Table (3): Effects of 5KGy and 10KGy	gamma radiation on total microbial counts of
	clove

Dose KGy	Bacteria		Fungi and Yeast	
	Total count cell/gm	%Killing	Total count cell/gm	%Killing
0	$8.4 imes 10^7$	0	$5.4 imes 10^8$	0
5	4×10^{6}	95.2	1×10^7	98.2
10	1.2×10^6	98.5	$6 imes 10^5$	99.9

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