Microbial Decontamination of Stored Cinnamon (*Cinnamomum cassia*) by Gamma Irradiation

Gamal El-Deen, M. R. El-Shamery[(1)], Mukhtar, A.M. Ali[(2)], Fahd, A.A. Al-Sharjabi[(1)], M.O. AbdoElmonem[(3)].

[(1)]Dept. of Microbiology, Faculty of Sci. Taiz Univ. Yemen.

[(2)]Dept. of Food Sci. Faculty of Agriculture & Veterinary Ibb Univ. Yemen.

[(3)] Dept. of Botany, Faculty of Sci. Benha Univ. Egypt.

Abstract

This investigation was carried out in an attempt to study the possibility of using gamma irradiation for microbial decontamination of stored Cinnamon taken from Taiz city markets. Cinnamon samples were subjected to irradiating by gamma radiation of 3.0 and 6.0 KGY doses and the samples were stored at room temperature for 7 months. The microbiological changes were determined during storage at zero time and monthly. The results indicated that the applied doses of gamma irradiation caused significant reduction in microbial population (pathogenic and non pathogenic) and increased the shelf-life. Gamma irradiation (3.0 KGY) completely destroyed Enterobacteriaceae *Staphylococcus* spp, *Sterptococcus* spp, *Enterococcus* spp, Coliform group, *Salmonelle* spp, *Clostridium* spp. Yeast and Moulds. Also reduction the microbial contamination density in aerobic, anaerobic bacteria, and *Bacillus* spp were noted with percentages 73.9%, 29.4%, and 17.0%, respectively. Whether for, at higher dose (6.0 KGY) the population of aerobic, anaerobic bacteria, and *Bacillus spp*. were reduced by the following percentages 80.5%, 45.27%, and 29.3% respectively as compared with the control samples. Irradiation was sufficient to complete elimination of the microbial for above mentioned does (3.0KGY).

Introduction

Irradiation is a very effective decontamination method **(Murano 1995)**. Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process **(Spoto *et al.,* 2000)**. The treatment doses used to control microorganisms are much higher than those required for pest control. Herbs, spices and dry ingredients are irradiated to control pests and microorganisms such as bacteria, yeasts and molds **(Murano 1995)**. It can be used to reduce insect infestation of grain, dried spices, and dried or fresh fruits and vegetables; inhibit sprouting in tubers and bulbs; retard postharvest ripening of fruits; inactivate parasites in meats and fish; eliminate spoilage microbes from fresh fruits and vegetables; extend shelf life in poultry, meats, fish, and shellfish; decontaminate poultry and beef; and sterilize foods and feeds **(Thayer 1990)**. Cinnamon are produced in rural areas and the traditional methods of cultivation, harvesting, packing and processing such as cleaning and drying are often under taken under unsanitary conditions. Consequently, Cinnamon contain a large number of micro-organisms capable of causing spoilage or disease. There are microorganisms mainly indigenous to soil and surrounding in which the cinnamon tree are grown. Fungal growth may occur prior to drying or during drying , storage and shipping. Many of cinnamon are traditionally packed in jute sacks, which offer little protection against insect and microbial contamination food-born diseases are extremely costly to society and cinnamon is among the sources of these food-born diseases because it represent a suitable substance for the growth or long survival of pathogenic organisms. Cinnamon is often used in foods that consumed raw or they are added to processed food or food after cooking**.** The use of gamma radiation in food preservation has now become legally accepted in many countries of the world. Moreover, the irradiation of food was approved for improving the hygienic quality in many countries (**IAEA, 1992)**. Irradiation is a safe technology and has been recognized as such by the FAO/WHO codex Alimentarius commission 40 countries have approved its use for pathogen control in dry food like dry cinnamon **(Molins and Matarjemi, 1999)**. Food is generally irradiated at levels from 50 KGY to 10 KGY, depending on the goals of the process. Low-dose irradiation (up to and including 1 KGY) is used primarily to delay ripening of produce or kill or render sterile insects and other higher organisms that may infest fresh food.

Medium-dose irradiation (1–10 KGY) pasteurizes food and prolongs shelf life. High-dose irradiation (10 KGY) sterilizes food **(Murano, 1995)**. The FDA limit for culinary herbs, seeds, spices, vegetable seasonings, and blends of these aromatic vegetable substances is up to exceed 30 KGY **(Code of Federal Regulation, 2004)**.

Materials and Methods

Samples:

The samples were collected from different local markets in Taiz city in Yemen. They were packed in polyethylene bags (each bag contained 100g) and the bags were sealed. These bags were divided into three groups. Two groups were transported to National Center for Radiation Research and Technology (N.C.R.R.T- Egypt) for irradiation at 3.0 and 6.0 KGY doses using gamma chamber 4000A (Dose rate 9.33 KGY/hr) while the third group was the control sample. Both irradiated and un irradiated samples were stored at room temperature for 7 months. The microbiological changes were followed by examination of packages during storage at zero time and monthly.

Sample preparation:

Ten grams of each sample were homogenized with 90 ml peptone water (under sterile conditions) in mortar. From this suspension, decimal dilutions in peptone water were prepared and spread on the appropriate plates, according to **Harrigan and McCance (1976).**

Microbiological examination:

The microbiological examination of all samples comprised the determination of standard bacterial counts (colony forming unit per gram C.F.U/g). Both aerobic and anaerobic bacteria were detected using the methods described by **APHA, 1992).** Counts of *Enterobacteriacease* were determined using Violet red bile glucose agar medium at 37^{o}C for 48 hrs **(Oxoid, 1985)**. Counts of Coliform group were estimated according to M P N method **(IAEA, 1992)** using MacConkey broth **(Oxoid, 1985)** and incubation at 44^{o}C for 24 – 48 hrs. *Salmonella spp*. counts were estimated using Brilliant green phenol red agar medium **(ISO, 1982)** whereas *Staphylococcus spp*.

counts were determined using Baired Barker agar method (**Oxoid, 1985** and **Mossel and Tamminge, 1982)**. Dried kanamycin aesculin azdi agar medium **(Mossel and Tamminge, 1982)** was applied to determine the counts of *Enterococcus spp*., whereas Trypton soya agar, manitol-egg-yolk- polymyxin agar medium **(Holobrook and Anderson, 1980)** was used to estimate the counts of *Bacillus spp*. The cooked meat agar medium incubated at 37^{o}C for 24 hrs. In anaerobic system using gas generating Kit **(Oxoid , 1975)** was used to determine the total counts of *Clostridium spp*. **(Craven *et al.*, 1979)**. Counts of *Streptococcus spp.* were estimated using Dried brain heard fusion agar and MacConkey agar medium **(Oxoid, 1985)** and incubation at 37^{o}C for 24 – 48 hrs, then the blue colonies surrounded by clear zone were counted. The dilution plate method **(Johnson and Curl, 1972)** and Malt extract agar medium **(Oxoid, 1985)** were used for the estimation of Yeast and Moulds, also were determined as described by **Pitt and Hocking, 1985.**

Results and Discussions

Microorganisms in food may cause loss of food value, leading to eventual food loss to such or extent as to render unfit for human consumption and deterioration in quality which may be affected by the loss of aroma and flavor. Moreover, many microorganisms are known to naturally produce toxic substances in food which if consumed, could be a potential hazard to human nutrition. Since cinnamon may be contaminated with microorganisms during preparation, processing, operation and partly owing to the neglect of the basic principles of sound storage. Therefore, any attempt to decontaminate this important food in order to increase the shelf-life is valuable. Treatment of cinnamon samples with doses 3.0 and 6.0 KGY of gamma radiation reduced the bacterial load to 2.0 x 10 and 9.5 C.F.U/g from control sample 9.9x10^{3} C.F.U/g, respectively. In other hand the reduction percentages were 73.95% and 80.5% for the above mentioned doses comparing with their control samples reduction of total bacterial counts may be attributed to cold sterilization effect of irradiation on the microorganisms **(Singh *et al.*, 1998 ; Shamshed *et al.*, 1999 and El-Shamery, 2001).** During subsequent room temperature storage slight increase in total

bacterial count, it reached to 4.5 x10^4, 5.6x10 and 2.4x10 C.F.U/g after 210 days of un-irradiated and irradiated samples with 0.0, 3.0 and 6.0 KGY respectively (Table,1). The total plate count of viable aerobic bacteria were taken as index of microbial changes that food contain during storage. From the aforementioned data it is clear that the 6.0 KGY treatment is the best for keeping the total aerobic bacterial counts of Cinnamon at lower level during storage at room temperature and hence gives the longest shelf-life. Non-irradiated Cinnamon sample was found to contain 3.5x10 C.F.U/g anaerobic bacteria. The number of recovered anaerobic bacteria was reduced to 1.2x10 and 7.0 C.F.U/g on exposing the samples to radiation of 3.0 and 6.0 KGY doses, respectively. In other hand the reduction percentages were 29.4 and 45.27% for the above mentioned doses comparing with their control samples. The reduction in the anaerobic bacterial counts is mainly due to the effect of gamma radiation on these micro-organisms **(U.S.D.A, 1997 ; Shady, 1999 ; Mahmod, 1999 ; Bennett, 2001 ; El-Feky, 2002 and Aycicek *et al*., 2004)**. However, a slight increase in the total anaerobic bacterial counts of radiated and non radiated samples was recorded during the storages period. It reached to 9.4x10, 3.0x10 and 9.9 C.F.U/g after 210 days of storage for irradiation and un irradiation samples with (0.0, 3.0 and 6.0 KGY). Results in Table (1) also showed that the initial *Bacillus* counts of the control samples at the beginning (zero time) of storage was 1.6x10 C.F.U/g and slightly increased during storage reaching 5.5x10 C.F.U/g after 210 days. Application of gamma radiation led to a slight reduction in the *Bacillus* recovered from the Cinnamon samples reflecting the resistance of these microorganism to the used doses of radiation. It was 9.8 and 7.1 C.F.U/g and reached to 3.5x10 and 9.9 C.F.U/g after 210 days of storage with 3.0 and 6.0 KGY respectively. In other hand the reduction percentages were 17.0 and 29.31% for the above mentioned doses comparing with their control samples. However it could be notice that application of gamma-radiation with 2.5 and 5.0 KGY doses led to increase the shelf-life of Cinnamon. The total count of *Bacillus* species recovered from their radiated samples at the end of the storage period was still lower than that recovered from the non-irradiated ones. In other hand the reduction percentages were 16.95 and 24.584% for the above mentioned doses comparing with their control samples. Irradiation is a safe technology for dry food like Cinnamon that has been recognized as

such by FAO / WHO and Codex Alimentarius Commission. The effect of gamma irradiation doses of 2.5, 5.0 KGY on a number of known pathogenic microorganism contaminated Cinnamon mainly. Yeast, Mould, *Clostridium* spp, Enterobacteriaceae; *Enterococcius* spp., Coliform group, *Salmonella* spp, *Staphylococcus* spp and *Streptococcus* spp has been studied. Results in Table (2) showed that members of all the abovementioned pathogenic microorganisms were recovered from the non-irradiated control samples but in relatively small numbers ranging from 2.0 to 4.0 C.F.U/g. Treatment of Cinnamon with gamma radiation of doses 3.0 and 6.0 KGY were found to be sufficient for the destruction of Yeast, Moulds and all the pathogenic bacteria under investigation, These finding are in agreement with those obtained by **Crawford, (1999) ; Owezarezk *et al*., (1999) ; Afifi and El-Nashaby, (2001) and Lacroix *et al*., (2004).** They reported that gamma irradiation doses (2 to 10 KGY) had a great effect to kill the pathogenic bacteria i.e *Salmonella* spp, *Listeria, Staphylococcus* spp, *Streptococcus* spp, *E. coli*, *Closteridium* spp and others that cause food borne illness. Generally, it could be concluded that treatment of Cinnamon with gamma-radiation at a dose of 3.0 KGY was almost enough and sufficient for decontamination of pathogen microorganisms from this widely used dry food, either through complete elimination of the present microorganisms or reduction of their numbers to non-harmful limit , that lead to increase the shelf-life Cinnamon This is agree with the results of **Kotzekidou, (1996) ; Duran *et al*., (1998) ; Demaro, (1999) ; Abd-Eltaife, (2001) and Ali, (2004).**

Table (1) Effect of gamma irradiation on microbial count/g (C.F.U/g) of Cinnamon during storage at room temperature.

Microbes	Aerobic Bacteria			Anaerobic Bacteria			Yeast and Mould		
Days of storage	0.0 KGY	3.0 KGY	6.0 KGY	0.0 KGY	3.0 KGY	6.0 KGY	0.0 KGY	3.0 KGY	6.0 KGY
0	$9.9X10_3$	2.0X10	9.5	3.5X10	1.2X10	7.0	1.2x10	---	---
30	$9.9X10_3$	2.0X10	1.0 x10	3.9X10	1.5X10	7.8	1.3x10	---	---
60	$1.0X10_4$	2.9X10	1.2X10	4.7X10	1.5X10	7.8	2.9x10	---	---
90	$1.1X10_4$	3.5X10	1.5X10	5.8X10	2.0X10	9.0	3.8x10	---	---
120	$2.3X10_4$	4.4X10	1.8X10	6.6X10	2.0X10	9.4	3.8x10	---	---
150	$2.8X10_4$	4.8X10	2.1X10	7.2X10	2.2X10	9.7	4.2x10	---	---
180	$3.3X10_4$	5.4X10	2.3x10	8.2X10	2.9X10	9.9	5.8x10	---	---
210	$4.5 X10^4$	5.6X10	2.4X10	9.4X10	3.0X10	9.9	6.7x10	---	---

--- = Not detected

Table (2) Effect Of Gamma Irradiation On Some Bacterial Group Count/G (C.F.U/G) Of Cinnamon During Storage At Room Temperature.

microbes	*Bacillus* spp.			*Clostridium* spp.			Enterobacteriaceae			*Enterococcus* spp.		
Days of storage	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*
0	1.6X10	9.8	7.1	2.0	---	---	2.0	---	---	1.0	---	---
30	1.6X10	1.0x10	7.1	2.2	---	---	2.2	---	---	2.8	---	---
60	1.8X10	2.2X10	7..5	2.5	---	---	2.4	---	---	3.5	---	---
90	2.2X10	2..3X10	8.4	2.5	---	---	3.7	---	---	4.2	---	---
120	2.8X10	2.6X10	9.2	2.6	---	---	4.1	---	---	4.9	---	---
150	3.4X10	2.8 X10	9.4	2.9	---	---	5.3	---	---	5.5	---	---
180	4.7X10	2.8X10	9.8	2.9	---	---	6.2	---	---	6.7	---	---
210	5. 5x10	3.5X10	9.9	3.2	---	---	7.7	---	---	7.9	---	---

* = KGY

--- = Not detected

(follow) Table (2) Effect Of Gamma Irradiation On Some Bacterial Group Count/G (C.F.U/G) Of Cinnamon During Storage At Room Temperature.

microbes / Days of storage	Coliform group			*Salmonella* spp.			*Staphylococcus* spp.			*Streptococcus* spp.		
	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*	0.0*	3.0*	6.0*
0	4.0	---	---	2.0	---	---	3.0	---	---	3.1	---	---
30	5.5	---	---	2.5	---	---	3.8	---	---	3.7	---	---
60	6.1	---	---	3.0	---	---	4.4	---	---	4.3	---	---
90	7.4	---	---	3.3	---	---	5.7	---	---	5.1	---	---
120	7.9	---	---	3.9	---	---	6.6	---	---	5.9	---	---
150	7.9	---	---	4.4	---	---	7.8	---	---	6.6	---	---
180	8.5	---	---	5.1	---	---	8.9	---	---	8.0	---	---
210	9.8	---	---	6.4	---	---	8.9	---	---	8.7	---	---

* = KGY

--- = Not detected

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ازالة التلوث الميكروبيولوجى للقرفة المخزنة بواسطة اشعة جاما

جمال الدين محمد رسام الشميري[1]. مختارعبدة محمد على[2]. فهد عبد الحميد الشرجبي[1] & محمد عثمان عبدالمنعم[3]

[1]الجمهورية اليمنية- جامعة تعز- كلية العلوم- قسم الميكروبيولوجي.

[2]الجمهورية اليمنية-جامعة أب-كلية الزراعة والطب البيطرى-قسم علوم الاغذية.

[3]جمهورية مصر العربية– جامعة بنها– كلية العلوم– قسم النبات.

"الملخص العربي"

يهدف هذا البحث إلى دراسة إمكانية إزالة التلوث والفساد الميكروبيولوجي للقرفة المخزنة تحت ظروف الجو العادي والتي جمعت عيناتها من السوق المحلي لمدينة تعز في الجمهورية اليمنية وعرضت لأشعة جاما بجرعات (3.0- 6.0 كيلو جراي) باستخدام مصدر إشعاعي كوبلت60 عدا عينات الكنترول ثم خزنت في الجو العادي. وأجريت للعينات المعالجة والغير معالجة بالإشعاع الاختبارات الميكروبيولوجية المختلفة عند نقطة الصفر (في بداية التحليلات) وشهرياً لمدة 7 أشهر خلال فترة التخزين. ودلت النتائج أن المعالجة بالإشعاع أدى إلى إزالة معظم الملوثات الميكروبيولوجية المرضية والغير مرضية كما أدى إلى زيادة فترة الحفظ أكثر من 7 أشهر. المعالجة بالجرعة الإشعاعية (3.0 كيلو جراي) أدت للقضاء على الفطريات- الخمائر- المجموعة القولونية- عائلة البكتيريا المعوية وأجناس (الأسيتافيلوكوكس - الستربتوكوكاس- أنتروكوكاس- السالمونيلا- كلوستريديم) وإلى الإقلال من أجناس الباسلس بنسبة (17.0%) والميكروبات الهوائية بنسبة (73.95%) واللأهوائية بنسبة (29.4%). في حين أن المعالجة بالجرعة الإشعاعية الكبيرة (6.0 كيلو جراي) أدت أيضاً إلى الإقلال من أعداد الميكروبات الهوائية بنسبة (% 80.5) واللاهوائية بنسبة (45.27%) وأجناس الباسلس بنسبة (29.3%) مقارنة بعينات الكنترول. كما أدت إلى القضاء التام على أجناس الكلوستريديم بالإضافة إلى القضاء على الميكروبات التي تم القضاء عليها بالمعالجة الإشعاعية السابقة (3.0 كيلو جراي).