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MICROBIAL DECONTAMINATION AND IMPROVING THE QUALITY OF FRANKFURTER BY GAMMA IRRADIATION AND COLD STORAGE

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ABSTRACT: ***This present investigation was carried out to study the possibility of using gamma irradiation for microbial decontamination and improving the quality of (Chicken) Frankfurter during cold storage. The samples were taken from different market in Sanna , Adin and Taiz City-Republic of Yemen , and subjected to treatment by gamma radiation dosages (2.5-4.5-6.5 and 8.5 KGY) both irradiated and un-irradiated samples were stored at cold storage (4±1°C)for(91) days .Microbiological changes were studied by examination of fresh samples and at weekly during storage, until signs of spoilage appeared . The result showed that doses 2.5, 4.5, 6.5 and 8.5 KGY: (A) - Increasing the shelf-life to 7, 21, 35 and 49 days proportionally to the applied doses respectively , (B)- Complete destruction of Enterobacteriaceac , Coli form group , Staphylococcus spp, Enterococcus spp, Salmonella spp, Clostridium spp , Yeast and Moulds , (C)- Reduction the count of Aerobic, and Anaerobic, Spore-formers, Proteolysis, Psychrophilic bacteria and Bacillus spp the reducing had proportionate with the irradiation dose increasing as the following percentage : 89.01 , 82.72, 89.47, 85.45, 84.39, and 68.00 % for a dose 2.5 KGY , 98.68, 90.00, 97.10, 92.18, 89.51, and 88.00 % for a dose 4.5 KGY , 99.08 , 95.81, 99.30 , 98.63, 98.04 and 95.30 % for a dose 6.5 KGY , 99.97 , 99.54 , 99.89 , 99.09 , 99.52 and 98.20 % for a dose 8.5 KGY, respectively as compared with the control samples , (D) - Isolation and classification ten species were indentified of Bacillus spp, they were [B. subtilus, B. pumilus, B. licheniformis, B. megaterium, B. lentus, B. macerans, B. thuringiensis, B. cereus, B. stearothermophilus and B. coagulans].***

Key word: ***Gamma Irradiation, Cold Storage, Frankfurter***

INTRODUCTION

Poultry meat can be sold alive or ready–to-cook . practically all chicken are now sold in the ready-to- cook form , This maybe as quartered or disjointed . Further - processing of chicken products has grow Substantially in recent years . Products such as chicken Frankfurters are now easily found on the Supermarket Shelves . The development of these new , Further – Processed poultry item has been nurtured by the demand for convenience foods and the competitively low price of poultry meat have acted as a Stimulus for its increased consumption (Abd El-daiem, 2004) . As Poultry

meat is fabricated into further – Processed poultry meat products , new problems are presented from the microbiological stand point . the processing operations of poultry meat increase the possibility of microbial contaminants may make apart the center of a finished product . whereas in ground , chopped or otherwise comminute meat such as Frankfurters that are very high on the list as an offender in Food- Poisoning outbreaks. There is a general agreement among the authorities that Frankfurters is important reservoir of organisms causing Food-borne disease . In addition , the growth of various spoilage bacteria , Yeast and Moulds was observed to cur in a wide range of chicken products held at (4+1^{o}C) control of micro-organisms on poultry products is the major Concern in Preparation of high-quality foods. This has initiated investigation into the combination of radiation preservation and cold storage for the purpose of extending the keeping quality of commercial ready–to–cook, chicken products. (Loahoranu, 2001) . Irradiation is a Safe technology and has been recognized as such by the .FAO/ WHO Codex Alimentarius Commission . today 40 Countries permit the irradiation of one or more food stuffs,12 countries have approved its use for pathogen control in poultry, 7 other for use in meats, chicken products , and 13 in fish and Seafood (Molins and Motarjemi, 1999 and IAEA 2002).

MATERIALS AND METHODS

Sampling :

The Samples were collected from different Local Supermarket in Sanna'a, Adin and Taiz city in Yemen . 150 Retail packages of chicken frankfurter Labeled (8 units/pag every unit 100g) . The Samples were transported to the Laboratory in an ice box and divided to small Retail vacuum-package in impermeable plastic casings (each bag contain 5 units) and divided to five groups four groups were transported to National Center for Radiation Research and Technology (N.C.R.R.T) in ice-box for irradiated at 2.5 , 4.5 , 6.5 and 8.5 K.GY doses using gamma chamber 4000A (Dose rate 9.33 KGY/hr) at Nasr city , Cairo , Egypt. The fifth groups were the control Samples. Both un-irradiated and irradiated samples were storied under Controlled Conditions at (4$\pm$1^{o}C) the Microbiological changes Carried out at zero time within 2 hr and weekly (7 days) intervals during cold storage at (4$\pm$1^{o}C) for 91 days . at each time interval 3 package were randomly sampled for analysis . until sings of spoilage appeared.

Microbiological examination :

Twenty five grams from (randomly samples) of the chicken frankfurter were blended with 225 ml of 0.1% peptone water in a sterile blender jar for 1-2 minutes and decimal dilutions prepared for testing. Numbers of viable organisms were determined by the plate count method. One ml of each

dilution was inoculated with appropriate media for the particular group of organisms to be tested as (Colony forming unit per gram (c.f.u/g) . The total Aerobic bacterial count was determined according to (APHA, 1992) using Plate count agar medium incubated at 37 ^{o}C for 3-5 days , Anaerobic bacterial count was determined by APHA, 1992 using Cooked meat agar medium with Anaerobic Jars (Gas pak system by B. BL cockysville marland 21030 USA) . Yeasts and Moulds were counted on Malt extract agar medium (Oxoid, 1985) incubated at 25-30 ^{O}C for 3-5 days as described by Pitt and Hocking, 1985 . Spore-former bacteria were determined according to method described by Chalmers, 1955 the suitable dilution were subjected to 80 ^{O}C at 20 m for 48-72 hr . proteolysis bacteria inoculation were made TGY to which 10 % (10 ml / 100 ml medium) of sterile skim med milk has been added just before pouring plates were incubated for 2-3 days at 30 ^{O}C (APHA, 1992) . Total psychrophilic bacteria count were enumerated on Plate count agar medium and incubation at 5 ^{O}C for 7 days as recommended by APHA, 1992 . Enterobacteriaceae was determined on Violet red blue dextrose agar medium after incubation at 37 ^{O}C for 20-24 hr as described by Robert *et al*., 1995 . *Bacillus* spp was counted by using Mannitol egg yolk-poly myxin (MYP) agar and incubation for 16- 24 hr at 37 ^{O}C as described by Roberts *et al* ., 1995. *Salmonella* spp was carriage out using the most probable number technique (M.P.N) according to (Iso, 1982) After enrichment at 37 ^{o}C for 24 hr in Selenite broth, the Cultures were streaked on Brilliant green agar and incubated at 37 ^{o}C for 24 hr , then colonies were biochemical examined in Triple Sugar Iron agar (TSI) and Lysine decarbonate broth . *Staphylococcus* spp was enumerated on Baird – parker medium using surface plating technique as recommended by IAEA, 1990 incubated at 37 ^{O}C for 24 hr . *Enterococci* spp was enumerated on Konamycin aesulin azide agar medium (Mossel and Tomminge, 1980) positive colonies were confirmed by Microscopic examination for the presence of short chain streptococci . Coli form group was counted used the (M.P.N) method as reported by IAEA, 1990 by inoculating Macconkey agar medium incubated at 44 ^{O}C for 24-48 hr . *Clostridum* spp used Cooked meat agar medium incubated at 37 ^{O}C for 24 hr. in anaerobic system using gas generation kit as mentioned by Craven *et al*., 1979 ; and Oxoid ,1985 .

Isolation and identification of *Bacillus* spp :

were made from total count plates (APT) agar (APHA, 1992) .colonies in opposite sectors , were picked and transferred to agar slants of the same medium. Alter purification , bacterial grouping according to morphological Characteristics and Gram stain was carried out. Gram- positive and Gram-negative groups were identified to generic and species level with the aid of (Bergey's Manual of determinative Bacteriology, 1999 ; Bergey's manual for systematic Bacteriology, 1986 and Kotzekidou, 1996) the method of identification adopted for this purpose ((Genus Bacillus)) with standard tests

and classification schemes described by Smith *et al* .,1952 in conjunction with (Holt *et al*., 1986). and examination were carried out according to (Holt *et al* ., 1986) .

RESULTS AND DISCUSSION

The total Aerobic and Anaerobic bacterial count were determined for samples of irradiated and non-irradiated Frankfurters post treatments and during storage at (4±1°C) and the results are shown in table, (1). From these results , it is clear the samples of non-irradiated Frankfurters had on initial total count 9.1 X 10^3 c.f.u/g for Aerobic and 2.2 X 10^2 c.f.u/g for Anaerobic . These results agree with (El-Shamery, 2001 ; Abd-El-latife, 2001 ; El-feky, 2002 ; and El-Eftheriadou *et al* .,2002). Subjecting samples of Frankfurters to gamma irradiation at doses of 2.5 , 4.5, 6.5 and 8.5 KGY greatly reduced the total Aerobic bacterial count by 89.01, 98.68 , 99.00 and 99.97% and their total Anaerobic bacterial count by 82.72 , 90.00 , 95.81 , and 99.45 % as the counts reached 1.0 X 10^3 , 1.2 X 10^2 , 8.3 X 10, and 2.0 c.f.u/g for Aerobic counts and reached 3.8 X 10 , 2.2 X 10 , 9.2, and 1.0 c.f.u/g for Anaerobic Count respectively . However , cold storage at (4 ± 1 °C) induced gradual increases in the total Aerobic and Anaerobic bacterial count for both irradiated and non-irradiated samples , but the rate of increase was much higher in control (non-irradiated) samples . The total Aerobic count increased to 1.0 X 10^7, 3.9 X 10^6, 2.0 X 10^6, 3.0 X 10^6 and 1.0 X 10^7 c.f.u/g and the total Anaerobic count increased to 1.4 X 10^3, 3.8 X 10^2 , 1.4 X 10^2 , 1.3 X 10 , 1.0 X 10 c.f.u/g in Samples irradiated at doses of 0.0 , 2.5, 4.5, 6.5 and 8.5 KGY after 35. 42 , 56, 70 and 91 days of cold storage . respectively. At that time samples were rejected due to increasing the total Aerobic bacterial count to more than 1 X 10^6 c.f.u/g . Indicating the importance of irradiation in extending the shelf–life of refrigerated Frankfurters Agree with (Afifi and El-Nashaby, 2001 ; AbdEl-Daiem, 2004 ; Ali, 2004 and Aycicek *et al* ., 2004) . It could be concluded , that the shelf-life of refrigerated Frankfurters be extended to more than 35 , 49 , 63 and 77 days were reached when irradiation doses of 2.5 . 4.5 , 6.5 and 8.5 KGY , respectively.

Table, (2) represents the counts of total Psychrphilic and Proteolysis bacteria in Frankfurters as affected by gamma irradiation and cold storage (4±1°C) the data in this table reveal that the initial count of Psychrophilic bacteria was 4.1 X 102 c.f.u/g and the initial count of , Proteolysis bacteria was 1.1 X 10^2 c.f.u/g in Samples of non-irradiated . Treatment by gamma irradiation at doses of 2.5 , 4.5 , 6.5 , and 8.5 KGY greatly reduced the count of total Psychrphilic bacteria by 84.39 , 89.52 , 98.00 , and 99.51 % and proteolysis bacteria by 85.45 , 92.18 , 98.63 , and 99.09% respectively . These results were agree with (El-Mongy *et al* ., 2001 ; Ibrahim *et al* ., 2004 and Kanatt *et al* ., 2004). However , storage of samples at (4 ± 1°C)

Table 1

Table 2

gradually increased the count of the total Psychrphilic and proteolysis bacteria for both irradiated and non-irradiated samples with higher rates for the Control samples . The count of psychrophilic increased to 1.8 X 10^6, 7.3 X 10^4 , 4.7 X 10^4, 8.1 X 10^3 and 2.3 X 10^3 c.f.u/g and the count of Proteolysis increased to 3.9 X 10^6, 2.1 X 10^4, 9.6 X 10^4, 1.0 X 10^4, and 6.0 X 10^3 c.f.u/g in non-irradiated samples and those received 2.5 , 4.5 , 6.5 and 8.5 KGY doses on days 35 , 42 , 56 , 70 and 84 days of storage , respectively . Similar results were observed by (Little *et al*., 2002 ; Fang *et al .,* 2003 and Lee *et al*., 2004) . Generally it could be concluded that the applied of irradiation were effective for improving the Keeping quality of irradiated Frankfurter Samples and extended their shelf-life to 42 , 56 , 70 and 84 days .

Data presented in Table, (3) Showed the average count of Spore-forming and *Bacillus* spp bacteria in chicken frankfurter samples as effected by irradiation and subsequent cold storage at (4±1^{O}C) . The results indicated that Spore-former and *Bacillus* spp were the most resistant type to irradiation , that even at dose level of 8.5 KGY considerable numbers were still recovered , due probably to their low rate content (El-Mongy *et al*., 2001 and Bennett, 2001) . During storage their total numbers increased at relatively slow rate under the unsuitable refrigerated temperature . These data were agree with (Little *et al* ., 2001; Bennett, 2001; Satin, 2002 ; Du and Ahn, 2002 and Badr , 2004).

Table, (4) indicted members of Enterobacteriaceae, *Enterococcui* spp , Coli form group, *Salmonella* spp, *Clostridum* spp, *Staphylococcus* spp Bacteria and Yeast and Moulds count were among the Micro-organism flora of frankfurter samples , recovered before irradiation , but inter latively small numbers (Table, 4) . rang from 2.0 , 10^2 to 10^3 c.f.u/g .These results were agreement with those obtained with (Roybka and Rodger, 2001 ; El-Mongy *et al* .,2001 ; Nasr, 2002 ; Fang *et al.,* 2003 ; Ali, 2004 and Aycicek *et al* ., 2004) . A During storage at (4±1^{O}C) no obvious growth was detected , due to that temperature was not suitable for their growth and proliferation , the minimal dose of radiation applied (2.5 KGY) was very effective in habiting these organism that they not recovered from the irradiated samples indicating that 2.5 KGY are quite enough to eliminate these organism . These coincides with (Barakat *et al* ., 2000 ; El-Mongy *et al*., (2001) ; El-Shamery, (2001) ; Nasr, (2002) ; Soriano *et al.,* (2002) ; Jenniber *et al* ., 2002 ; Ali 2004 and Abd El-Daiem, 2004) . reported that Gram -negative rods were the most sensitive to irradiation treatment followed by Gram- positive rods and cocci .

Isolation and Classification of Bacillus Species :

Gram positive, catalase – positive and Spore-forming rods were classified as belonging to *Bacillus* species ten group were identified . The total number of *Bacillus* spp isolated from total count plates of chicken Frankfurter belonged to the 24 species and the percent distribution of these , before and

Table 3

Table 4

after irradiation are in Tables, (5.6) *B*. Megaterium , *B*. Pumilus , *B*. Lentus, *B*. Stearothermophlis and *B*. Coagulans in that order , comprised the main flora in fresh frankfurter constited about 66.66% of the total isolates. After irradiation , there was no change in flora except that the percentage of *B*. Lichenifarm , *B*. Subtilus *B*. Macerans and *B*. Cereus decreased with the increase irradiation dose , until they were no longer encountered among the predominat organisms in samples receiving a doses 6.5 and 8.5 KGY . *Bacillus* spp were the dominating flora among the radio- resistant organisms. *B*. Pumilus and *B*. Megaterium which seemed to be the most radio resistant organism , dominated the Bacillus flora before and after irradiation , *B*. Licheniformis, and *B*. cereus on the other hang , were not recovered from samples irradiated at a dose of 6.5 and 8.5 KGY . Same results with (El-Mongy *et al* ., 2001 ; El-Shamery, 2001 and Baidr, 2004). Bacillus species present were contaminants from dust, air soil , water and animal carriers (Fang *et al* ., 2003), these species were previously isolated from different chicken parts (Smith *et al* .,1952 ; Nasr, 2002 and Ali, 2004) Since most of Sensitive species were either of public health or shelf-life Significance , the use of radiation as a preventing hazard agent , in chicken products , would be a quite satiate factory procedure from the public health and economic point of views.

Table 5

Table 6

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ازالة التلوث الميكروبيولوجي وتحسين الجودة للفرنكفورد بواسطة اشعة جا ما والتخزين بالتبريد

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المخلص العربي

يهدف هذا البحث لامكانية ازالة التلوث الميكروبيولوجي وتحسين الجودة الميكروبيولوجية للفرنكفورد المصنوع من الدجاج بواسطة استخدام اشعة جاما والتخزين بالتبريد والذي اخذت عيناته من مدينة تعز – عدن- صنعاء- (الجمهورية اليمنية) . وعوملت باشعة جاما بالجرعات (٢.٥-٤.٥ -٦.٥ -٨.٥ كيلوجراي) العينات المعاملة والغير المعاملة خزنت تحت ظروف التبريد (٤ ± ١٥° م) وخلال فترة التخزين اجريت لها الاختبارات الميكروبيولوجيه المختلفة عند نقطة الصفر وكل سابع يوم لمدة (٩١) يوماً حتى ظهور علامات الفساد عليها وقد اشارت النتائج ان الاشعاع بالجرعات المذكورة انفاً ادت الى :-

(١)- زيادة مدة الحفظ الى (٧ - ٢١ - ٣٥ - ٤٩ يوم) بدرجة تتناسب مع مقدار الجرعة الاشعاعية المستخدمة حسب الترتيب السابق للجرعات الاشعاعية مقارنة بعينات الكنترول .

(٢)- ادت الى القضاء التام على الميكروبات(انتروباكترياسيس- مجموعة الكوليفورم واجناس (السالمونيلا- الكلوستريديم- الاسيتافيلوكوكس –الانتروكوكاس) وكذلك الفطريات والخمائر.

(٣)- ادت الى التقليل من اعداد الميكروبات(الهوائية - اللاهوائية – المتجرثمة البروتوليتك - السكروفليك وجنس باسلس) وتناسب الانخفاض في الاعداد مع مقدار الجرعة الاشعاعية مقارنة بعينات الكنترول وحسب النسب الاتية :-

أ- الجرعة٢.٥ كيلو جراي:(٨٩.٠١ -٨٢.٧٢ -٨٩.٤٧ -٨٥.٤٥ – ٨٤.٣٩-٦٨.٠٠%)

ب- الجرعة٤.٥ كيلو جراي:(٩٨.٦٨ -٩٠.٠٠ - ٩٧٠١٠ - ٩٢.١٨ -٨٩.٥١-٨٨.٠٠%)

ج- الجرعة ٦.٥ كيلو جراي:(٩٩.٠٨ - ٩٥.٨١ -٩٩٠٣ - ٩٨.٦٣ -٩٨.٠٤ - ٩٥.٣٠ %)

د- الجرعة٨.٥ كيلو جراي:(٩٩.٩٧ - ٩٩.٥٤ - ٩٩٠٨٩ -٩٩.٠٩- ٩٩.٥ -٩٨.٢٠ %).

(٤)- تم عزل وتصنيف(١٠) ميكروبات تابعة لجنس باسلس من كافة العينات المختبره وهى:

***B*. subtilus, *B*. pumilus, *B*. licheniformis, *B*. megaterium, *B*. lentus, *B*. stearothermophilus, *B*. coagulans *B*. cereus, *B*. macerans, *B*. thuringiensis,**

Table (1): Effect of gamma irradiation on Total Aerobic (AE) and Anaerobic(AN)bacterial count c.f.u/g of (chicken) Frankfurters during storage at (4 ± 1 ^{o}C)

Dose (KGY)	0.0		2.5		4.5		6.5		8.5	
Microbes	AE	AN	AE	AN	AE	AN	AE	AN	AE	AN
Storage period (in days)	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g
0	$9.1x10^{3}$	$2.2x10^{2}$	$1.0x10^{3}$	$3.8x10^{1}$	$1.2x10^{2}$	$2.2x10^{1}$	$8.3x10^{1}$	9.2	2.0	1.0
7	$1.5x10^{4}$	$2.6x10^{2}$	$3.2x10^{3}$	$9.0x10^{1}$	$3.3x10^{2}$	$3.3x10^{1}$	$8.3x10^{1}$	$1.2x10^{1}$	8.0	1.0
14	$4.0x10^{4}$	$3.8x10^{2}$	$1.4x10^{4}$	$1.5x10^{2}$	$8.3x10^{2}$	$3.8x10^{1}$	$1.5x10^{2}$	$1.4x10^{1}$	$3.8x10^{1}$	2.2
21	$2.0x10^{5}$	$5.1x10^{2}$	$6.7x10^{4}$	$1.7x10^{2}$	$4.1x10^{3}$	$4.4x10^{1}$	$9.9x10^{2}$	$1.8x10^{1}$	$9.7x10^{1}$	2.9
28	$1.0x10^{6}$	$7.0x10^{2}$	$2.5x10^{5}$	$2.2x10^{2}$	$1.0x10^{4}$	$5.2x10^{1}$	$4.5x10^{3}$	$2.2x10^{1}$	$2.4x10^{2}$	3.8
35	$1.0x10^{7}$	$1.4x10^{3}$	$9.8x10^{5}$	$2.5x10^{2}$	$4.3x10^{4}$	$6.3x10^{1}$	$2.0x10^{4}$	$2.6x10^{1}$	$2.0x10^{3}$	4.4
42	R*	R*	$3.9x10^{6}$	$3.8x10^{2}$	$2.2x10^{5}$	$7.1x10^{1}$	$9.3x10^{4}$	$2.6x10^{1}$	$8.6x10^{3}$	4.4
49			R*	R*	$1.0x10^{6}$	$8.0x10^{1}$	$4.9x10^{5}$	$2.8x10^{1}$	$3.0x10^{4}$	5.1
56					$2.0x10^{6}$	$1.4x10^{2}$	$9.9x10^{5}$	$4.1x10^{1}$	$7.8x10^{4}$	5.2
63					R*	R*	$1.2x10^{6}$	$2.5x10^{1}$	$3.1x10^{5}$	6.6
70							$3.0x10^{6}$	$1.3x10^{1}$	$1.0x10^{6}$	6.6
77							R*	R*	$2.6x10^{6}$	8.7
84									$1.0 x10^{7}$	$1.0X10^{1}$
91									R*	R*

C.F.U/G =Colony forming unit per/gram R* = Unaccepted

Table (2): Effect of gamma irradiation on Psychrphilic (PS) and Proteolysis (PR) bacterial count c.f.u/g * of (chicken) Frankfurters during storage at (4 ± 1 °C)

Dose (KGY)	0.0		2.5		4.5		6.5		8.5	
Microbes	PS	PR	PS	PR	PS	PR	PS	PR	PS	PR
Storage period (in days)	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g
0	$4.1x10^2$	$1.1x10^2$	$6.4x10^1$	$1.6x10^1$	$4.3x10^1$	8.6	8.0	1.5	2.0	1.0
7	$9.3x10^2$	$8.5x10^2$	$2.7x10^2$	$6.0x10^1$	$4.4x10^1$	$2.1x10^1$	9.7	3.6	7.0	1.0
14	$2.2x10^3$	$2.8x10^3$	$7.8x10^2$	$9.8x10^2$	$4.4x10^1$	$4.0x10^1$	$1.4x10^1$	7.1	9.0	2.1
21	$4.4x10^4$	$6.8x10^4$	$1.2x10^3$	$2.4x10^3$	$5.4x10^1$	$8.6x10^1$	$1.6x10^1$	$2.2x10^1$	$1.0x10^1$	6.1
28	$8.4x10^5$	$2.9x10^5$	$2.4x10^3$	$5.4x10^3$	$9.9x10^1$	$2.7x10^2$	$3.1x10^1$	$4.6x10^1$	$1.4x10^1$	$1.2x10^1$
35	$1.8x10^6$	$3.9x10^6$	$7.2x10^3$	$9.9x10^3$	$2.0x10^2$	$7.0x10^2$	$8.4x10^1$	$8.8x10^1$	$1.8x10^1$	$1.8x10^1$
42	R*	R*	$7.3x10^4$	$2.1x10^4$	$7.1x10^3$	$1.3x10^3$	$2.2x10^2$	$3.2x10^2$	$2.2x10^1$	$3.6x10^1$
49			R*	R*	$2.8x10^3$	$4.6x10^3$	$6.2x10^2$	$7.3x10^2$	$2.7x10^1$	$5.6x10^1$
56					$4.7x10^4$	$9.6x10^4$	$9.7x10^2$	$3.5x10^3$	$5.2x10^4$	$9.5x10^1$
63					R*	R*	$5.2x10^3$	$7.1x10^3$	$8.1x10^5$	$2.8x10^2$
70							$8.1x10^3$	$1.0x10^4$	$3.3x10^2$	$7.6x10^2$
77							R*	R*	$9.8x10^2$	$2.9x10^3$
84									$2.3x10^3$	$6.0x10^3$
91									R*	R*

C.F.U/G =Colony forming unit per/gram R* = Unaccepted

Table (3): Effect of gamma irradiation on Spore-form (SP) and *Bacillus* spp (Ba) bacterial count C.F.U/G * of (chicken) Frankfurters during storage at (4 ± 1 °C)

Dose (KGY)	0.0		2.5		4.5		6.5		8.5	
Microbes	SP	Ba	SP	Ba	SP	Ba	SP	Ba	SP	Ba
Storage period (in days)	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g
0	$9.5x10^2$	$1.0x10^2$	$1.0x10^2$	$3.2x10^1$	$2.8x10^1$	$1.2x10^1$	5.9	4.7	1.0	1.8
7	$1.7x10^3$	$1.4x10^2$	$1.5x10^2$	$4.6x10^1$	$2.8x10^1$	$1.4x10^1$	6.6	6.0	2.0	2.0
14	$3.7x10^3$	$1.9x10^2$	$2.9x10^2$	$6.0x10^1$	$5.9x10^1$	$1.8x10^1$	$1.6x10^1$	5.0	4.4	2.4
21	$8.4x10^3$	$2.2x10^2$	$6.9x10^2$	$7.4x10^1$	$9.8x10^1$	$1.9x10^1$	$5.0x10^1$	6.8	6.5	3.1
28	$2.8x10^4$	$3.6x10^2$	$2.5x10^3$	$9.0x10^1$	$2.8x10^2$	$2.0x10^1$	$1.2x10^2$	8.1	$1.3x10^1$	3.5
35	$2.8x10^5$	$5.8x10^2$	$5.2x10^3$	$1.1x10^2$	$6.5x10^2$	$2.6x10^1$	$2.9x10^2$	9.5	$2.1x10^1$	4.0
42	R*	R*	$9.1x10^3$	$1.2x10^2$	$1.3x10^3$	$3.2x10^1$	$5.9x10^2$	$1.2x10^1$	$3.8x10^1$	4.4
49			R*	R*	$2.3x10^3$	$4.1x10^1$	$1.4x10^3$	$1.2x10^1$	$6.5x10^1$	5.2
56					$5.9x10^3$	$5.1x10^1$	$4.6x10^3$	$1.2x10^1$	$9.9x10^1$	5.9
63					R*	R*	$1.4x10^4$	$1.5x10^1$	$1.9x10^2$	6.5
70							$2.2x10^4$	$1.7x10^1$	$4.8x10^2$	7.0
77							R*	R*	$8.8x10^2$	8.1
84									$9.6x10^3$	$1.4x10^1$
91									R*	R*

R* = Unaccepted C.F.U/G = Colony forming unit per/gram

Table (4): Effect of gamma irradiation on Yeast and Moulds (Y.M), Enterobacteriaceae (EN), Coli form group (Coli), *Salmonella* spp {Sal}, *Staphylococcus* spp {Stph}, *Enterococcus* spp {ENT}, and *Clostridium* spp{Clos} Bacterial count C.F.U/G of (chicKen) Frankfurters during storage at (4±1°C)

Mic	Y.M		EN		Coli		Sal		Stph		ENT		Clos	
Dos	0.0	2.5	0.0	2.5	0.0	2.5	0.0	2.5	0.0	2.5	0.0	2.5	0.0	2.5
Storage period in days	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g	Count/g
0	$8.9x10^1$	—	$2.4x10^2$	—	1.1	—	2.0	—	$7.0x10^1$	—	$1.1x10^1$	—	$1.0x10^1$	—
7	$2.8x10^2$	—	$5.1x10^2$	—	1.1	—	2.0	—	$8.7x10^2$	—	$2.1x10^2$	—	$1.8x10^1$	—
14	$6.2x10^2$	—	$9.8x10^2$	—	2.2	—	2.0	—	$1.5x10^3$	—	$2.1x10^2$	—	$2.0x10^1$	—
21	$9.8x10^2$	—	$1.6x10^3$	—	2.2	—	3.0	—	$3.0x10^3$	—	$4.1x10^2$	—	$2.4x10^1$	—
28	$3.0x10^3$	—	$3.6x10^3$	—	4.4	—	3.0	—	$9.8x10^3$	—	$6.6x10^2$	—	$2.7x10^1$	—
35	$4.7x10^3$	—	$6.6x10^3$	—	5.9	—	4.0	—	$1.0x10^4$	—	$1.5x10^3$	—	$3.0x10^1$	—
42	R*	R*	R*	R*	R*	R*	R*	R*	R*	R*	R*	R*	R*	R*
49														
56														

R* = Unaccepted — = No vailable count * C.F.U/G = Colony forming unit per gram

Table (5) : Numbers and percent distribution of *Bacillus* species isolated from Chicken Frankfurters .

% Distribution											
Doses (KGY)		**0.0**		**2.5**		**4.5**		**6.5**		**8.5**	
Numbers Group	**Organism**	**No.of Isolates**	**Percent of total Isolates**	**No.of Isolates**	**Percent of total Isolates**	**No.of Isolates**	**Percent of total Isolates**	**No.of Isolates**	**Percent of total Isolates**	**No.of Isolates**	**Percent of total Isolates**
1	B. subtilus	2.0	8.330	2.0	10.00	1.0	5.550	0 .0	00 .00	0.0	00 .00
2	B. pumilus	3.0	12.50	3.0	15.00	3.0	16.66	3.0	23.07	3.0	30.00
3	B. licheniformis	1.0	4.160	1.0	05.00	1.0	5.550	0 .0	00 .00	0.0	00 .00
4	B. megaterium	4.0	16.66	3.0	15.00	3.0	16.66	3.0	23.07	3.0	30.00
5	B. lentus	3.0	12.50	2.0	10.00	2.0	11.11	2.0	15.38	1.0	10.00
6	B. macerans	2.0	8.330	1.0	05.00	1.0	5.550	0 .0	0 0.00	0 .0	00 .00
7	B. thuringiensis	2.0	8.330	2.0	10.00	2.0	11.11	1.0	07.69	1.0	10.00
8	B. cereus	1.0	4.160	1.0	05.00	1.0	5.550	0 .0	00 .00	0 .0	00 .00
9	B. stearothermophilus	3.0	12.50	2.0	10.00	2.0	11.11	2.0	15.38	1.0	10.00
10	B. coagulans	3.0	12.50	3.0	15.00	2.0	11.11	2.0	15.38	1.0	10.00
Total No. of *Bacillus* spp		**24**	**100%**	**20**	**100%**	**18**	**100%**	**13**	**100%**	**10**	**100%**

Table (6): Physiological and biochemical characteristics of the bacterial isolates (*Bacillus* spp) obtained from samples under studies .

Characters / Group of *Bacillus* spp		Roade-shape	Gram stain	Endospore formation	Anaerobic growth	V.P. test	Acid from D-glucose	Acid from L-arabinose	Acid from D-xylose	Acid from D-mannitol	Hydrolysis of Casein	Hydrolysis of Gelatin	Hydrolysis of Starch	Utilization of citrate	Reduction of nitrate to nitrite	Formation of indole	Reduction of lecithinase	Growth at pH 6.8 (NB)	Growth at pH 5.7 (NB)
G1.	B. subtilus	+	+	+	---	+	+	+	+	+	+	+	+	+	+	---	---	+	+
G2.	B. pumilus	+	+	+	---	+	+	+	+	+	+	+	---	+	---	---	---	+	+
G3 .	B. licheniformis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	---	+	+
G4 .	B. megaterium	+	+	+	---	---	+	---	---	---	+	+	+	+	---	---	---	+	---
G5.	B. lentus	+	+	+	---	---	+	+	+	+	---	+	+	---	+	---	---	+	---
G6.	B. macerans	+	+	+	+	---	+	+	+	+	---	+	+	---	+	---	---	+	+
G7.	B. thuringiensis	+	+	+	+	---	+	---	---	---	+	+	+	+	+	---	+	+	+
G8.	B. cereus	+	+	+	+	+	+	---	---	---	+	+	+	+	+	---	---	+	+
G9.	B. stearothermophilus	+	+	+	---	---	+	---	+	---	+	+	+	---	---	---	---	+	--
G10	B. coagulans	+	+	+	+	+	+	---	---	+	---	---	+	---	+	---	---	+	+

(+) = Presence **(-) = Absent**