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EFFECT OF IRRADIATION AND NATAMYCIN ON DECONTAMINATION OF FUNGI FROM LABORATORY INOCULATED MINCED MEAT

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ABSTRACT

This study was carried out on 60 samples of fresh minced meat (200 g) for each to evaluate the effect of both gamma irradiation and 0.1% natamycin on elimination of total mould and yeast count. Samples were divided into 5 groups (12 for each), all samples were sterilized by using gamma irradiation at dose 10 kGy then artificially inoculated with *Candida albicans and Aspergillus niger* at infected dose 10^5 cfu/g,1st group not treated (control), 2nd, 3rd, and 4th groups were exposed to gamma irradiation at doses of (2.5, 4.0, 6.0 kGy) respectively. While 5th group treated with 0.1% natamycin by spraying. All samples chilled at (4°C) for 15 days. Total mould and yeast count were detected at zero, 5, 10, 15 days of storage. The results showed that there was a significant difference between non-irradiated (control) samples and irradiated samples at (2.5, 0.4, 0.6 kGy) at zero, 5 and 10 days of storage and there was a significant difference between doses of 4.0 and 6.0 kGy at15 days of storage as the control samples were spoiled, the samples exposed to 6.0 kGy were contained less mould and yeast growth than that exposed to 4.0 kGy irradiated dose. Samples treated with natamycin 0.1% showed a significant decrease in total mould and yeast count in comparison with untreated (control) group at 5, 10 days of storage day. As well as there was significant differences between treated samples at 15 days of storage and each treated samples at 5 and 10 days of storage.

Key Words: Irradiation, Natamycin, Decontamination, Fungi, Minced Meat.

INTRODUCTION

Meat is a source of animal protein which contains all essential amino acids required for human nutrition. Contamination of meat and meat products by fungi originated from different sources as additives spices (Farkasm, 1989), contamination by food handlers (Soriano *et al.*, 2000), from water, processing environment and microbial fecal contamination (Seri and Mohd, 2006 and Thalita *et al.*, 2014). A wide spectrum of filamentous fungi and yeasts is often found in various food commodities, where they can cause extensive damage and lead to sizable economic loss. Fungal infection leads to meat spoilage such as off-flavors, discoloration, rotting and disintegration of the food structure. The very important aspect involved in spoilage of food by fungi is also the formation of toxic secondary metabolites – mycotoxins which produced by some filamentous fungi. Concerning the importance and diversity of their toxic effects carcinogenic, teratogenic, mutagenic, immunotoxic, neurotoxic, nephrotoxic and hepatotoxic, the occurrence of mycotoxinogenic moulds in foods constitutes a high risk for human and animal health (Sirion, *et al.*, 2005, Anandarja *et al.*, 2006 and Ma *et al.*, 2011, Singh *et al.*, 2012, Dahama *et al.*, 2013 and Nurzhan, 2015). Although prevention of fungal growth and mycotoxins production in feedstuffs is usually considered as the best approach to impede the harmful effects on animal and human health, decontamination and detoxification of contaminated products is also of prime importance (Varga *et al.*, 2005). Several physical and chemical techniques are used for the preservation of meat and meat products. Drying, freeze, cold storage, modified atmosphere storage and heat treatments are all means of physical methods of food preservation. Several chemical additives also are used as preservatives, and those are organic acids - acetic, lactic, sorbic, benzoic and propionic, as well as some antibiotic, such as natamycin. Consumers required high quality meat that has fresh appearance with natural flavor and taste. Therefore there is an interest for new non thermal technologies.

Food irradiation is a physical method of food processing that involves exposing prepackage food to ionizing energy, this process is sometime called cold pasteurization because the inactivation of

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microorganism is achieved at low temperatures unlike the traditional heat pasteurization (Thalita, *et al.,* 2014) Gamma rays are a short length waves with extensive penetration and lethality and are produced by radioactive, such as cobalt 60(60co) and cesium 137(137cs). Using a low dose of ionizing radiation eliminate or reduce the presences of bacteria , mould and mould spores in meat and meat products prolonged its shelf life (Petwal *et al.,* 2004, Vural *et al.,* 2006, Seri *et al.,* 2012 and Balakrishnan, 2015). The high penetrative ionizing energy have the ability to inactive spoilage and disease causing microorganisms without causing harmful changes to the products, the ionizing energy passes completely through products and their packaging Other food preservation methods such as chemicals and heat treatment kill microorganisms including pathogens. However chemicals leaves residues and heating can change the texture, colour and flavor of the products (Kyzlink, 1990), as well as some enzymes and bacterial spores are thermo-tolerant which require the application of extreme heat treatment that affect nutrition and organoleptic properties of meat (Raso and Barbosa, 2003). Additional advantages of irradiation technology include the possibility of irradiating packed food at its fresh and frozen state, the ionizing energy passes completely through products and their packaging (Sridhar, and Bhat, 2008, Stewart, and Padalia, 2015). Irradiation elemis pathogenic and non pathogenic microorganisms, insects and parasites (Stefanova *et al.,* 2010). Furthermore, irradiation may be considered environment friendly because this method does not consume water and has lower electrical energy demands than other food preservative methods. Irradiation does not increase human exposure to radiation since energy used is not strong enough to cause food to become radioactive (Abid Sarwar *et al.,* 2014). The first international safety recommendation was presented in 1981 whsen a committee of experts considered that the irradiation of food up to an overall average dose of 10 kgy introduces no special nutritional or microbiological problems (FAO/IAEA/ WHO, 1981). The energy from electrons has to target and inactive fungal nucleic acid, this damage occure directly as a results of electron and pheton interaction with DNA and RNA and indirectly through the radio lytic products of water as H, OH these free radicals split carbon bonds of macromolecules in the living microorganisms, thereby killing them (Ingram and Farkas 2008, Stewat and Padalia 2015). Energy required to control microorganisms in food varies according to the type of species to be eliminated, according to their population numbers and according to their development state. Other factors such as the composition and moisture content of food, the frozen or fresh state of food, the temperature and level of oxygen present during irradiation, may also influence the resistance of microorganisms to radiation (Farkas, 2006).

Natamycine is a nature antifungal substances produced during the fermentation of the bacterium Streptomyces natalensis (Zeuthen and Bogh-Sorensen, 2003). Natamycin is one of the globally permitted food preservatives to protect a wide variety of food products, its application has a huge potential to extend shelf life and prevent the growth of mould and yeast till the end of storage (El-Diasty *et al.,* 2009). It has been approved as a food additive in over 40 countries and has been considered as a GRAS (generally recognized as safe) product by the Food and Drug Administration (FDA) (El-Daly, 2000and Koontz *et al.,* 2003). Natamycin is a widely used in the food industry for the prevention of mould contamination in meat (Var *et al.,* 2004 Jay *et al.,* 2005 and Welscher *et al.,* 2008), such as *Aspergillus carbonarius* and ochratoxin production (Medina *et al.,* 2007), and *Aspergillus niger, Aspergillus versicolor Penicillium chrysogenum, Penicillium glabrum, Penicillium commune, Penicillium verrucosum* (Stark, 2003). Recently, the European Food Safety Authority (EFSA) has published a favorable scientific opinion on the use of natamycin as a food additive (EFSA, 2009). its superiority over other natural antifungal has been attributed to its wide spectrum of antifungal activity at low concentration and it is white tastless and odorless powder so it induce its effect without changing organoleptic characteristics of food products such as cheese, meat and juices (Dzigbordi *et al.,* 2013). Natamycin act by interacts with sterols which present in cellular membrane of fungi and destruct the selective permeable memberane causing leakage and even lysis of cells (Jay, 2000), it slow down the fungal growth for up to six months but it can not completely inhibit it. On the other hand natamycin has no antibacterial activity due to lack of ergosterol compound in the bacterial cell memberane (Delves *et al.,* 2006), this makes it useful for the application on such products which require bacterial processes such as dry sausages (Stark, 2004). It is active at a low concentration (0.1%-0.2%) and could be applied either by dipping or spraying (Stibing and Oberhaus, 2001, Delves *et al.,* 2006, El-Abbasy, 2007 and Petr, *et al.,* 2010). It is crystalline in structure and melts at temperature 180°C and effectively acts at a wide range of pH from 3 - 9 (Beuchat, 1998). Natamycin concentration of 10 ppm completely inhibited 16 common food spoilage yeasts and moulds by an agar plate method (Klis *et al.,* 1959). Bullerman, (1977) demonstrated that 5 ppm of natamycin prevented growth of all the mould isolates from natural cheese, the majority of which were *Penicillium spp,* growth of 96% of the mold isolates was prevented for at least 7 days. Yeasts possess an even greater sensitivity to natamycin than mould. Natamycin appeared to be more effective at inhibiting growth of *Aspergillus ochraceus* in liquid media than in olive paste (Gourama and Bullerman, 1988). Natamycin has been observed to have an inhibitory effect that is greater on toxin production than on growth for all of the

toxigenic molds. In studies using laboratory media, a 1 ppm concentration of natamycin inhibited toxin production of aflatoxin B_1 by 25%. Natamycin inhibited mycelial growth of *Aspergillus. ochraceus* by 16 to 52% at concentrations of 1 to 50 ppm, but at 10 ppm ochratoxin production was completely inhibited. The use of natamycin is advantageous for such minced meat as it cannot interfere with the starter bacteria as it has no antibacterial activity (Stark, 2004 and Petr *et al.*, 2010). Natamycin cause defects in the permeability of memberane of the fungi as it interact with sterols, which are present in the cell membrane of the fungal cells and thus it destructs the selective permeable membrane (Jay, 2000 and Adams, and Moss, 2008).

So, the ojective of this review is to highlight the importance of irradiation technology and natamycin to prevent fungal contamination in minced meat and thus enhance the meat quality and increase its shelf life.

MATERIALS AND METHODS

A total of 60 random samples of fresh minced meat (200gm for each) were purchased from different supermarkets with different sanitation levels in Dakahlia governorates. Samples were divided into 5 equal groups (12 for each). All samples were placed in sealed polyethylene bags and exposed to 10 kGy of gamma radiation for sterilization at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt.

Preparation of inoculums: Strains of *Candida albicans and Aspergillus niger* were obtained from Department of Microbiology, Faculty of Veterinary Medicine, Mansoura University, then prepared to achieved stock culture that was aproximately 10^9according to Cruickshank *et al.* (1975) and artificially inoculated into all examined meat samples to give a final concentration 10^5 according to APHA, (1985).

After inoculation, the first group (control group) was not treated, the second, third and fourth groups were irradiated at doses of (2.5, 4.0, 6.0 kGy) respectively according to Baher, (2010), while the 5^{th} group were treated with 0.1% natamycin which was applied by spraying according to Petr *et al.* (2010).

Preparation of samples: All samples were chilled at 4°C for 15 days. Total yeast and mould count, were applied at 0, 5, 10, 15 days of storage as following: twenty five grams of each samples were homogenized with 225 ml of 1% peptone water in stomacher for 1 minute and serial dilutions were prepared. One ml of selected dilutions was transferred (in replicates) onto petri dishes and Sabauroud´s dextrose agar was poured, the plates were incubated at 30°C for 3-5 days then the count of total mould and yeast was recorded (cfu/gm) according to Cruickshank *et al.* (1975).

Statistical analysis: The obtained data were analysis using analysis of variance (one way a Anova) SPSS according to Sabine and Brian, (2014).

RESULTS

The effect of gamma irradiation on total mould and yeast counts in minced meat are presented in table (1) and Fig (1-4) which revealed that non-irradiated samples (control group) had ($2.9\times10^5\pm8.4\times10^4$) cfu/g of microbial load which increased to ($1.9\times10^6\pm6.3\times10^5$), ($2.9x10^5\pm8.4x10^6$) cfu/g on 5 and 10 days of storage, respectively and became spoiled on 15 day of storage. The total yeast and mould counts were reduced by 2.5 kGy irradiation dose to ($1.6\times10^4\pm7.8\times10^3$), ($9.9\times10^4\pm3.4\times10^4$), ($6.2\times10^5\pm2.5\times10^5$) on zero, 5 and 10 days of storage, respectively while samples became reject at 15 day of storage.

Our results revealed that the irradiated samples at 4.0 kGy showed decrease in total mould and yeast count to ($4.3\times10\pm2.3\times10$), ($2.7\times10\pm1.8\times10$), ($4.4\times10\pm2.8\times10$), ($8.6\times10^2\pm3.2\times10^2$) cfu/gon 0, 5, 10 and 15 days of storage, respectively. Whereas irradiated samples at 0.6 kGy, the mould and yeast count were recorded to non detectable level during 0, 5, 10 days of storage while at 15 day there was a very low mould and yeast count (9.2 ± 8.3). There was a significant difference between control samples and each radiated samples at 4.0 and 6.0 kGy on 0, 5 and 10 days of storage respectively. Also there was a significant difference between doses of 4.0 and 6.0 kGy at 15 days of storage as the control samples were already spoiled before this period and the samples exposed to 6.0 kGy were contained less mould and yeast growth than that exposed to 4.0 kGy radiation dose.

The effect of 0.1% natamycin on total mould and yeast count was illustrated in table (2) and Fig (5) which showed that, the initial total mould and yeast count in untreated (control) samples were ($2.9x10^5\pm8.4x10^6$ cfu/g) increased to ($1.9x10^6\pm6.3x10^5$, $3.0X10^6\pm5.7X10^5$cfu/g) on 5 and 10 days of storage respectively while became spoiled (rejected) on 15 day of storage. Samples treated with 0.1% natamycin showed decrease in total mould and yeast count to ($8.1X10^3\pm1.4X10^3$, $3.1X10^4\pm6.8x10^3$, $7.9X10^4\pm2.2X10^4$, $5.0X10^5\pm1.0X10^5$ cfu /g) on zero, 5, 10 and 15 days of storage respectively. The Statistical analytical results showed that, there was a significant differences between treated samples and untreated (control) samples at 5 and 10 days of storage as well as there was a significant differences between treated samples at 15 day of storage and each treated samples at zero, 5 and 10 days.

Table 1: Statistical analytical results of total mould and yeast count in non-irradiated (control) and irradiated minced meat (N=12 for each).

	0 kGy Control samples			2.5 kGy			4 kGy			6 kGy		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
O day	$2.9x10^5 \pm 8.4x10^{4\ A}$	$1.4x10^4$	$9.0x10^5$	$1.6x10^4 \pm 7.8x10^{3\ a}$	$1.0x10^3$	$9.0x10^4$	$4.3x10^1 \pm 2.3x10^{1\ a}$	0	$2.0x10^2$	0	0	0
5 days	$1.9x10^6 \pm 6.3x10^{5\ B}$	$1.1x10^5$	$6.0x10^6$	$9.9x10^4 \pm 3.4x10^{4\ b}$	$4.5x10^3$	$3.5x10^5$	$2.7x10 \pm 1.8x10^{\ b}$	0	$2.0x10^2$	0	0	0
10 days	$3.0x10^6 \pm 5.7x10^{5\ C}$	$5.2x10^5$	$7.5x10^6$	$6.2x10^5 \pm 2.5x10^{5\ c}$	$1.5x10^4$	$3.1x10^6$	$4.4x10^1 \pm 2.8x10^{1\ c}$	0	$3.2x10^2$	0	0	0
15 days	R	R	R	R	R	R	$8.6x10^2 \pm 3.2x10^{2\ D}$	$1.1x10^2$	$4.0x10^3$	9.2 ± 8.3^d	0	$1.0x10^2$

R=rejected. (A&a/B&b/C&c) = There were significant differences between the small and capital litters

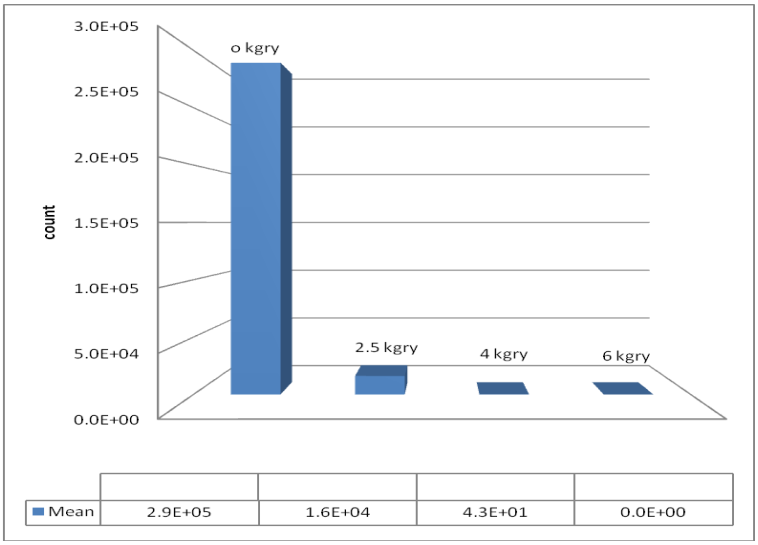

Fig. 1: Statistical analytical results of total mould and yeast count in irradiated and non-irradiated samples on 0 day of storage

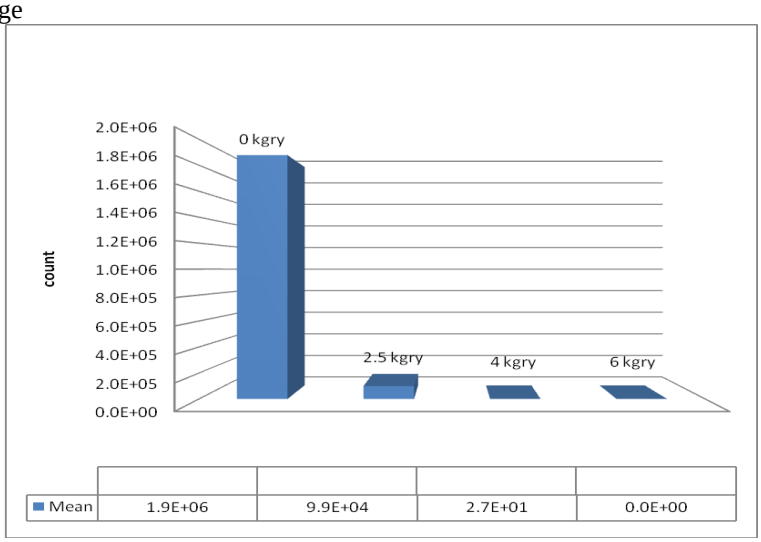

Fig. 2: Statistical analytical results of total mould and yeast count in irradiated and non-irradiated samples on 5 day of storage

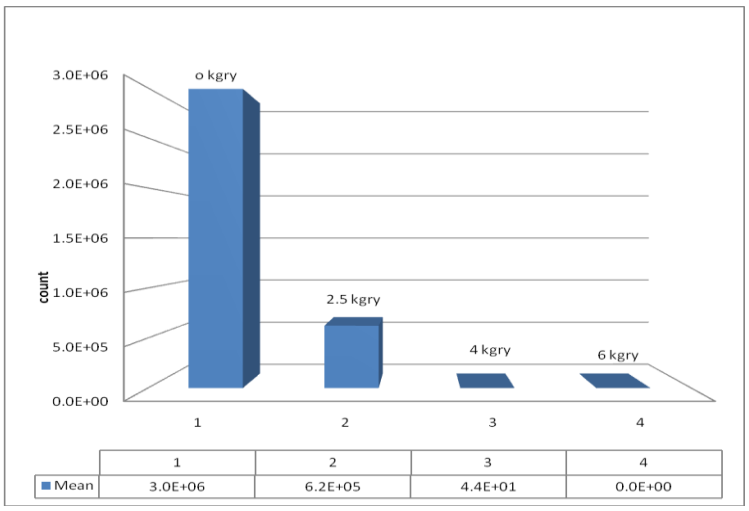

Fig. (3): Statistical analytical results of total mould and yeast count in irradiated and unirradiated samples on 10 day of storage

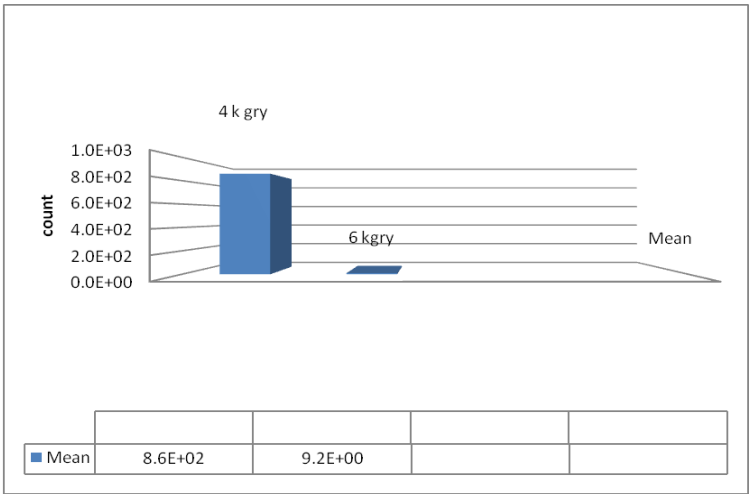

Fig. (4): Statistical analytical results of total mould and yeast count in irradiated and non-irradiated samples on 15 day of storage

Table 2: Statistical analytical results of total mould and yeast count in non treated (control) and treated samples with natamycin (12= of each).

	0 day			5days			10 days			15 days		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Before treatment	$2.9x10^5$ $\pm 8.4xI0^4$ b	1.4 $x10^4$	9.0 $X10^5$	$1.9x10^6$ $\pm$ $6.3x10^{5\,a}$	1.1 $x10^5$	6.0 $x10^6$	$3.0x10^6$ $\pm$ $5.7x10^{5\,a}$	5.2 $x10^5$	7.5 $x10^6$	R	R	R
After treatment	$8.1x10^3$ $\pm$ $1.4x10^{3b}$	5.4 $x10^3$	9.0 $x10^4$	$3.1x10^4$ $\pm$ $8.6x10^{3b}$	5.4 $x10^3$	9.0 $x10^4$	$7.9x10^4$ $\pm$ $2.2x10^{4\,b}$	1.0 $x10^4$	3.0 $x10^5$	$5.0x10^5$ $\pm$ $1.0x10^{5a}$	1.0 $x10^5$	9.9 $x10^5$

R=rejected each value represented mean ± S.E. Different letters mean significant difference at ($p \leq 0.05$) while the same letters mean non significant difference

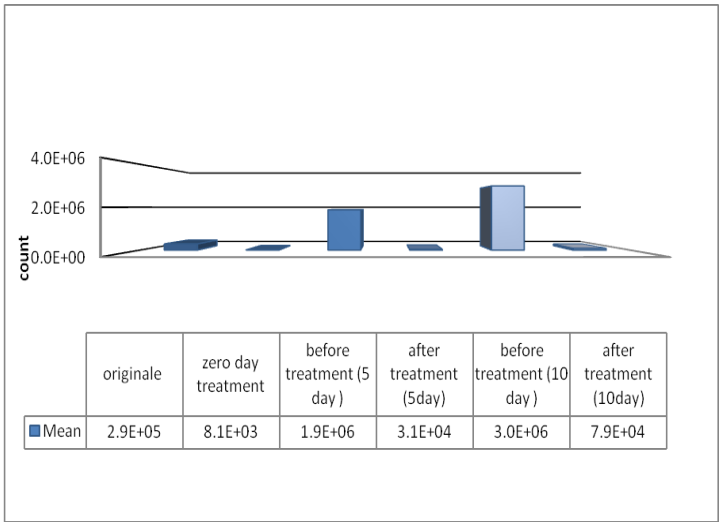

Fig. 5: Statistical analytical results of total mould and yeast count in non treated (control) and treated samples with natamycin

DISCUSSION

The total yeast and mould counts were reduced by 2.5 kGy irradiation dose on zero, 5 and 10 day of storage, respectively while samples became spoiled at 15 day of storage. The statistical analytical results showed that there was a significant difference ($p < 0.01$) between non-irradiated (control) samples and each of irradiated samples at 2.5, 4.0,6.0 kGy on zero and 5 day of storage, respectively**.**

These findings are in agreement with Abid Sarwar *et al.* (2014) who found that the 2.5 kGy treated poultry and beef samples showed decreasing trend in the development of fungi. Also these findings are agree with Jay, *et al.*, (2005) who reported that low doses of irradiation (less than 3kGy) could eliminate pathogens inoculated into ready to eat meat. Also our findings are nearly similar to those obtained by Vural, *et al.* (2006) who worked on low irradiation doses 1, 2, 3 kGy in elimination the microorganisms count in raw Turkish meat and found that, the microorganisms count were decreased parallel with the increased doses of radiation. Mould and yeast count were reduced under detectable value by application of 3 kGy. Our results revealed that the samples irradiated at 4.0 kGy showed decrease in total mould and yeast count on 0, 5,10 and 15 days of storage .Whereas samples irradiated at 0.6 kGy, the mould and yeast were recorded to non detectable level during 0,5,10 days of storage while at 15 day there was a very low mould and yeasts count. These findings are disagree with Seri *et al.* (2012) who mentioned that there was no microbial load in backed ccked chicken sausage irradiated at 3.5 kGy and chilled for 3 month. Chicken burger samples irradiated at 3.5 kGy and later put to chilled storage at 0-3°C had initial microbial count $2.1x1o^3$cfu/g, which increases to $1.8x10^5$ cfu/g after 2 weeks and became spoiled after 1 months. He added the irradiated products with doses of 5.5 and 10.0 kGy were free from microbial load including from mould and yeast at chilled storage 3 months. Also our results are nearly agree with Abid Sarwar *et al.* (2014) who uses of gamma irradiation to eliminate mould and yeast count from poultry and beef samples and found that total mould and yeast count were reduced under detectable value after application of 5 kGy irradiated dose. From our results it was clear that treatment of minced meat with low doses of ionizing radiation (2.5, 4.0, 6.0) kGy are considered as an effective procedure for meat decontamination and shelf life extension as the total mould and yeast counts were slowly and gradually increased in all irradiated samples during storage period in comparison with the highly increased in unirradiated (control) samples. Irradiation extend the shelf life of food without causing harmful changes in the texture, colour, and flavor of the products (Stefanova *et al.*, 2010, Petwal *et al.*, 2004, and Sofos, 2009). The killing effect of irradiation is attributed to breaking of chemical bond of DNA orto formation of highly reactive redical which split carbon bond of macromolecules in living microorganism so, killing them (Ingram and Farkas 2008).

Samples treated with 0.1% natamycin showed decrease in total mould and yeast count at zero, 5, 10and 15 days of storage. These results are in agreement with El-Tawab, (2014) who reported that natamycin (0.1%) had a higher inhibitory effect than potassium sorbate (2%) as total mould count (log cfu/g) was reduced from 4.69 in control group to 2.96 and 3.39 in natamycin and potassium sorbate treated groups respectively. In addition our results agreed with Petr *et al.* (2010) who reported that natamycin (0.1 and 0.2%) proved to be the most efficient mould suppression on the surface of dry sausage (both heated and fermented). Also our results in accordance with El-Abbasy, (2007) who stated that the

application of 0.1natamycin on quail carcass reduced the total yeast and mould count. (Hassan and El Lawandy, 2006) found that natamycin (50ppm) reduced the total mould count (log cfu/g) from 3.44 in ordinary formulated sausage to 2.28 in natamycin treated sausage and from 3.10 in ordinary formulated Frankfurt to 1.96 in natamycin treated frankfurters. Salem *et al.* (2016) reported that natamycin 3oo ppm proved to be efficient in suppression of *A. niger* growth in minced meat so, the use of natamycin (300ppm) is recommrnded to improve safety of meat products.

CONCLUSION

We can concluded that gamma irradiation at low doses (2.5, 4.0, 6.0) kGy are sufficient in reduction of total mould and yeast count in fresh minced meat chilled at 4C° This reduction was proportional with the irradiation doses. The irradiated samples at 2.5 kGy spoiled at 10 day of storage while samples irradiated at 6.0 kGy contain less number of microorganisms than that exposed to 4.0 kGy on 15 day of storage, as during storage, the count of examined microorganisms increased but the rate of increasing was slower as the irradiation dose increased. As well as natamycin 0.1% are efficient in reduction of total mould and yeast count at 0, 5, 10 and 15 day of storage. So the use of irradiation and natamycin, are recommended to improve safety of meat and meat products.

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تأثير التشعيع والنتاميسين على ازالة التلوث بالفطريات من اللحوم المفرومة المحقونة معمليا

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أجريت هذه الدراسة على عدد ٦٠ عينة من اللحوم المفرومة الطازجة (٢٠٠جرام لكل عينة) والتي استخدمت في تقييم تأثير كل من التشعيع والنتاميسين فى تقليل الفطريات و الخمائر و تم تقسيم كل العينات إلى ٥ أقسام (١٢ عينة لكل مجموعة) تم تعقيم كل العينات باستخدام التشعيع عند جرعة ١٠ كيلو جراى ثم تم حقنها صناعيا بجرعة معدية (١٠)° من الكانديدا البيكانز والاسبيرجلس نيجر المجموعة الأولى كانت (ضابطة) والمجموعة الثانية والثالثة والرابعة تم تعرضها لأشعة جاما بجرعات (٢.٥، ٤.٠، ٦.٠) كيلو جري على التوالي بينما المجموعة الخامسة تم علاجها بالنتاميسين ٠.١% عن طريق الرش تم تخزين العينات بالتبريد عند ٤ درجة مئوية ثم تم عمل العد الكلى للفطريات والخمائر عند (٠، ٥، ١٠، ١٥) يوم من التخزين وأسفرت النتائج عن وجود أختلاف معنوى بين العينة الضابطة والعينة التي تم تعرضها للتشعيع عند الجرعات (٢.٥، ٤.٠، ٦.٠) كيلو جرى في اليوم (0,٥, 10) من التخزين وكان هناك فرق معنوى بين العينة الضابطة والعينات التي تم تشعيعها عند (٤.٠، ٦.٠) كيلو جراى في اليوم الخامس عشر من التخزين حيث ان العينة الضابطة اصبحت ملوثة وقد احتوت العينات التي تعرضت للتشعيع بجرعة ٦.٠ كيلو جرyي علي نمو للفطريات والخمائر أقل من التي احتوتة العينات التي تعرضت للتشعيع بجرعة ٤.٠ كيلو جري وقد أظهرت النتائج التي تم علاجها بالنتاميسين وجود أختلاف معنوي في عدد الفطريات والخمائر عن الموجودة في المجموعة الضابطة وهذا عند اليوم (٥، ١٠) من التخزين وأصبحت العينات فاسدة عند اليوم ال١٥ من التخزين. وكان هناك فرق معنوي بين العينة التي تم علاجها بالبتاميسين عند اليوم ال١٥ من التخزين وكل من العينات التي تم علاجها عند اليوم (٥,١٠) من التخزين