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Incidence of Aflatoxin M1 in milk powder with special reference to the effect of Gamma irradiation and Ultra violet rays on its level and their further effects on the examined samples

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ABSTRACT

Milk is a highly nutritious food, and it is a source of macro and micronutrients which necessary for growth, development and maintenance of human health. On the other hand, it also considered to be a good environment for a lot of food contaminants that may cause many diseases. In this study, random samples from milk powder were collected from 5 hyper markets in Alexandria governorates, Egypt (15 samples from closed containers and 15 samples from opened containers). All samples were examined for the incidence and enumeration of both aerobic bacteria and mould to judge the microbiological condition. Also, aflatoxin M1 determination. The result revealed that the clearance of all samples which collected from closed containers examined for aerobic bacteria, mould and aflatoxin M1. Results of samples which collected from opened containers revealed that 60% of samples were positive for aerobic bacteria with mean ± SEM ($8.1x10^3 \pm 1.7x10^4$), 33.3% were positive for mould with mean ± SEM ($6 x10^2 \pm 1x10^3$) and 13.3 % were positive for aflatoxin M1 with mean ± SEM (1.53 ± 0.28). Results of aerobic bacteria enumeration in all contaminated samples from opened containers were within the accepted limits according to Egyptian standard no.8073\2017. On the other hand, results of aflatoxin M1 exceeded the limits based by Egyptian, European and U.S standards. Two contaminated samples with aflatoxin M1 were subjected to gamma irradiation and ultraviolet treatment, the results showed that the bacterial growth was eliminated in the first sample after 45 minutes UV treatment while reduced by 99.8% in the second sample. Fungal growth was eliminated in the first sample and reduced by 98.75 %in the second sample after 60 minutes of UV radiation treatment. Moreover, aflatoxin M1 reduced by 67.2 % and 72.9 % for the first and second samples respectively after 60 minutes of UV radiation treatment. By using gamma irradiation, bacterial and fungal growth

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in the two treated samples were significantly reduced by the 1st treatment dose (5 kGy), while aflatoxin M1 were gradually reduced in both samples by increasing treatment dose without reaching to regulations acceptable limits. It was noticed that some changes on treated samples colour after 60 minutes of UV treatment and 40 kGy gamma irradiation as a side effect from irradiation treatment. Treatment with elevated doses of gamma irradiation had more effect on reduction of aflatoxin M1 than UV irradiation in spite of the both treatment failed to reduce aflatoxin M1 of contaminated samples to the regulations acceptable limit. In addition, irradiation treatment showed side effect on the nutritional values of the two treated milk powder samples including protein, fat, vitamin D, Calcium and total sugar amount. The results showed that most of nutritional values were not affected by UV treatment up to 45 minutes of exposure, while showed slight effect after 60 minutes of exposure. On the other hand, gamma irradiation showed noticeable changes on most of nutritional values from the first dose of exposure.

This study emphasized the importance of good hygienic packaging of milk powder in closed packed containers to keep it safe. Also, the study revealed that treatment either by UV (up to 60 minutes) or elevated doses of Gamma irradiation (up to 100 kGy) had a potential effect on reduction of microbial growth in milk powder samples with insignificant effect on reduction of aflatoxin M1 level to the acceptable limit based by standards, with some changes in samples physical properties and nutritional values. As this study proved that even high doses of irradiation treatment could not eradicate the present toxin. So that, the application of hygienic measures in packaging of milk powder is considered the gold standard for safe products.

INTRODUCTION

The concept of safe and wholesome food from a food safety aspect, is food that is free not only from toxins, pesticides, chemicals, and physical contaminants but also from microbiological pathogens or their toxins that can cause food born disease **(Roberts, 2001).**

Milk contains all the essential nutrients for all physiological functions of the body systems. Milk is a good source of calcium, phosphorus and fat-soluble vitamins (A, D, E and K). For this reason, it is the nature's most nearly perfect food. Milk is highly perishable agricultural product because its support to luxuriant growth of almost all kinds of microbes. **(Kajal et al. 2012).**

Packaging of milk and milk products is very important for protecting milk against external microbes and bacteria. It can also help to preserve milk and ensure it doesn't spoil. After packaging is removed, the shelf life of milk product is greatly reduced.

Sterilization is defined as any process that effectively kills or eliminates almost all microorganisms like fungi, bacteria, viruses, spore forms. There are many different sterilization methods depending on the purpose of the sterilization and the material that will be sterilized. The choice of the sterilization method alters depending on materials and devices for giving no harm. These sterilization methods are mainly: dry heat sterilization, pressured vapour sterilization, ethylene oxide (EtO) sterilization, formaldehyde sterilization, gas plasma (H2O2) sterilization, per acetic acid sterilization, e-beam sterilization and gamma sterilization. **(Kátia Aparecida da Silva Aquino Federal, 2020**).

It is hard to assess a perfect sterilization method because every method has some advantages and disadvantages. For this reason, sterilization process should be selected according to the chemical and physical properties of the product.

The recent years have seen a great number of instances when ultraviolet (UV) radiation was used in the preservation process of all sorts of foods. UV radiation is a non-ionizing radiation from whose spectrum (140–400 nm). The wave lengths between 250 and 280 nm can be utilized as a germicide since the light of this wavelength can be absorbed by both nucleic acids and most proteins containing aromatic amino acids as well, the subsequent transformation having the potential to destroy microorganisms **(Koutchma et al. 2009).**

UV treatment is a competitive method for decreasing the number of harmful microbes in foods. At the same time, UV treatment may adversely affected food composition since a number of harmful photochemical reactions may be activated following the formation of free radicals, which may reduce the number of valuable food components (**Lu et al. 2011).**
Gamma radiation sterilization is mainly used for the sterilization of pharmaceuticals. Gamma radiation delivers a certain dose that can take a period of time from minutes to hours depending on the thickness and the volume of the product. Thus, the sterilization method chosen must be compatible with the item to be sterilized to avoid damage **(Shintani H., 1992).**
Gamma radiation can be used to inactivate aerobic bacteria, yeasts, and moulds. Application of gamma radiation altered the physicochemical and structural properties of whey proteins. Protein-protein interactions occurred after the gamma radiation treatment, which was seen in the increased viscosity and turbidity of the solutions and decreased nitrogen solubility.
So, this study aimed to illustrate the role of food packaging in reduction of contamination risk, effect of irradiation treatment on microbial and aflatoxin M1 contamination and further effect of irradiation treatment on the nutritional value of examined samples.

2. MATERIAL AND METHODS

2.1 Samples collection: a total 30 random samples of milk powder were collected from 5 hyper markets located in Alexandria governorates, Egypt (15 milk powder samples from closed containers and 15 milk powder samples from opened containers) from January 2023 till April 2023. Each sample was collected under a complete aseptic technique, kept in an ice box and transferred rapidly to the lab, and processed as quickly as possible.

2.2 Determination of microbial contamination:

2.2.1 Sample preparation (ISO 6887-5: 2020)

Twenty-five grams of milk powder sample were added to 225 ml of sterile buffered peptone then mixed thoroughly by sterile blender for 1-1.5 min. The prepared samples were subjected to further examination technique.

2.2.2 Enumeration aerobic microorganisms:

Enumeration of microorganisms by counting the colonies growing on plate count agar after 3 days aerobic incubation at 30^{0}c by pour plate technique according to (ISO 4833-1:2013 Amd 1:2022).

2.2.3. Enumeration of mould:

Enumeration of viable moulds by means of the colony count technique after 5 days aerobic incubation at 25 °C ± 1 °C using DG18 agar medium according to (ISO 21527-2, 2008).

2.3 Determination of aflatoxin M1

Aflatoxin M1 was determined by using LC -MSMS according to Food control 50 (2015), in the central lab of residue analysis (QCAP) Dokki, Giza.

2.4 Treatment of contaminated samples by two types of radiation

2.4.1 Ultraviolet irradiation treatment

254 nm ultraviolet light used for treat contaminated milk powder samples at distance of 10 cm between the sample and UV lamb with 3 mm sample depth. Irradiation was carried out at the National Center for Radiation Research and Technology (NCRRT) according to **Thu Nguyen et al. (2022).**

2.4.2 Gamma irradiation treatment

Milk powder samples were exposed to gamma radiation at different doses 5, 10, 20, 40, 70 and 100 kGy (kilo Gray). Irradiation was carried out at the National Center for Radiation Research and Technology (NCRRT), using ^{60}Co gamma irradiation source of gamma chamber (4000A) with a dose rate 0.925 kGy / h. at the time of the experiment according to **Oueke and Oluwakemi B., 2019**.

2.5 Determination of the nutritional values of treated samples

2.5.1 Quantitative determination of calcium

Milk powder samples were analyzed for the presence and quantity of Calcium at the central laboratory of residue analysis of pesticides and heavy metals in food (QCAP) in Egypt according to Food additives and contaminants, 2003. Using inductively coupled plasma optical emission spectrometry (ICP-OES), specifically the Optima 8300 ICP-OES from Perkin Elmer with limit of quantification (LOQ) 0.5 mg/kg.

2.5.2 Determination of Protein %

Milk powder samples were analyzed for the percentage of Protein using Kjeldahl technique according to ISO 1871:2009.

2.5.3 Determination of fat %

Fat is extracted with mixture of ethers from known weight of milk. Ether extract is decanted into pre-weighed dry weighing dish, and ether is evaporated. Extracted fat is dried to constant weight. Result is expressed as % fat by weight according to AOAC 989.05:2019.

2.5.4 Determination of Total sugar % according to (ISO /DIS 22184, 2021)

Milk powder samples were analyzed for the determination of total sugar department of at milk science, faculty of agriculture, Alexandria University by High performance anion exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) used to determine total sugar amount through most important sugars in milk.

2.5.5 Determination of Vitamin D

Milk powder samples were analyzed for the presence and quantity of Vitamin D3 at the central laboratory of residue analysis of pesticides and heavy metals in food (QCAP) in Egypt according to J.AOAC vol. 90, 2007. Using high pressure liquid chromatography with limit of quantification (LOQ) 5 mg/kg.

2.6. Statistical analysis

The data collected were subjected to analysis by t-independent test and Fischer Exact Probability test using SAS, 2004) software, the data was considered significant at $P < 0.05$.

RESULTS

Table 1. Incidence and Enumeration of aerobic bacteria in examined milk powder samples (CFU/g).

Type of samples	No. of examined samples	Positive samples No.	Positive samples %	Minimum	Maximum	Mean ± SEM	Accepted range acc. To ES 8073/2017
Milk powder (From closed containers)	15	zero	zero	zero	zero	zero	$< 10^5$
Milk powder (From opened containers)	15	9	60	2.1 x10	5.5 x10^4	$8.1x10^3 ± 1.7x10^4$	

CFU/g = colony forming unit \ gram

Table 2. Incidence and Enumeration of mould in examined milk powder samples (CFU/g).

Type of samples	No. of examined samples	Positive samples No.	Positive samples %	Minimum	Maximum	Mean ± SEM
Milk powder (From closed containers)	15	zero	zero	zero	zero	zero
Milk powder (From opened containers)	15	5	33.3	5.5 x10	2.4 $x10^3$	6 $x10^2$±$1x10^3$

CFU/g = colony forming unit \ gram

Table 3. Incidence and Determination of aflatoxin M1 level in examined milk powder samples (µg/kg)

Type of samples	No. of examined samples	Positive samples No.	Positive samples %	Minimum	Maximum	Mean ± SEM	Accepted range acc. To Egyptian Regulation (7136.2010)
Milk powder (From closed containers)	15	zero	zero	Not detected	Not detected	-	0.05 µg/kg
Milk powder (From opened containers)	15	2	13.3	1.72	1.33	1.53±0.28	

µg/kg =ppb = microgram\ kilo gram

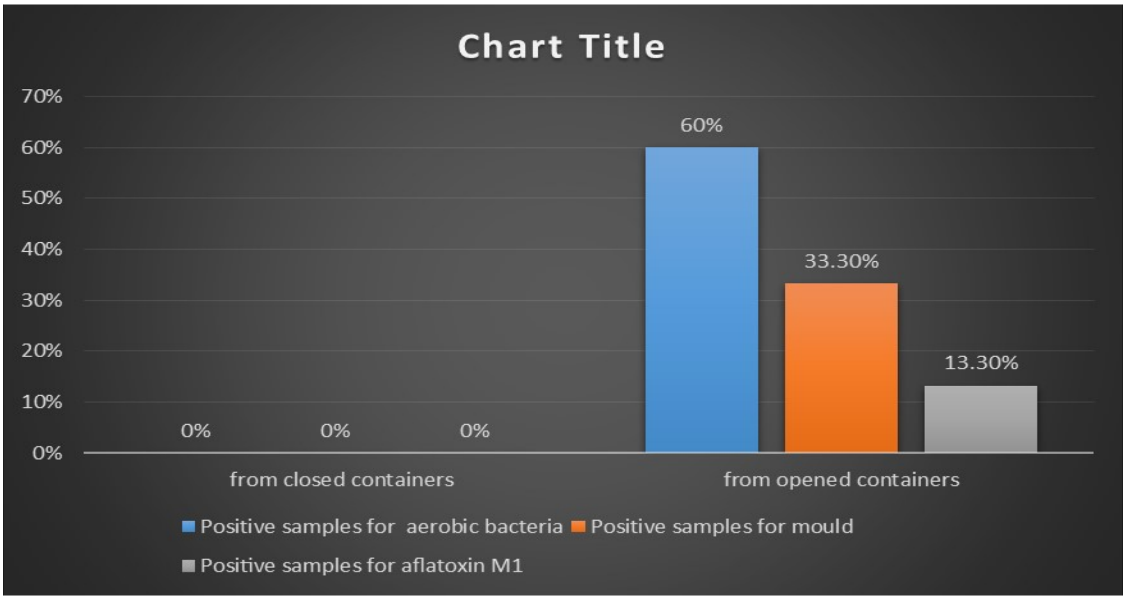

Figure 1: I incidence of aerobic bacteria, mold and aflatoxin M1 in milk powder samples

Table 4.Aflatoxin M1 level (µg/kg) in examined milk powder samples and existing regulations limits.

Type of samples	Positive samples	Exceeding Egyptian regulations(7136.2010) (0.05 µg/kg)		Exceeding EU regulations (0.05 µg/kg)		Exceeding US regulations (0.50 µg/kg)	
		No. of samples	Range	No. of samples	Range	No. of samples	Range
Milk powder (From closed containers)	zero	zero	No range	zero	No range	zero	No range
Milk powder (From opened containers)	2	2/15 (13.3%)	1.33-1.72	2/15 (13.3%)	1.33-1.72	2/15 (13.3%)	1.33-1.72

EU = European standards US = United state standards

Table 5 \ Figure 2: Effect of Ultraviolet and Gamma irradiation treatment on enumeration of aerobic bacteria and mould in the 1st contaminated milk powder sample by aflatoxin m1.

Criteria	Before treatment	After treatment by UV 15 min	UV 20 min	UV 30 min	UV 45 min	UV 60 min	Gamma 5 KGy	Gamma 10 KGy	Gamma 20 KGy	Gamma 30 KGy	Gamma 40 KGy	Gamma 70 KGy	Gamma 100 KGy
Aerobic bacterial count (CFU/g)	1100	910	820	660	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Mould count (CFU/g)	350	320	310	120	70	< 10	30	< 10	< 10	< 10	< 10	< 10	< 10

UV = Ultraviolet KGy= Kilo gray min= minutes 1st contaminated milk powder sample= sample contaminated with 1.72 ppb aflatoxin M1

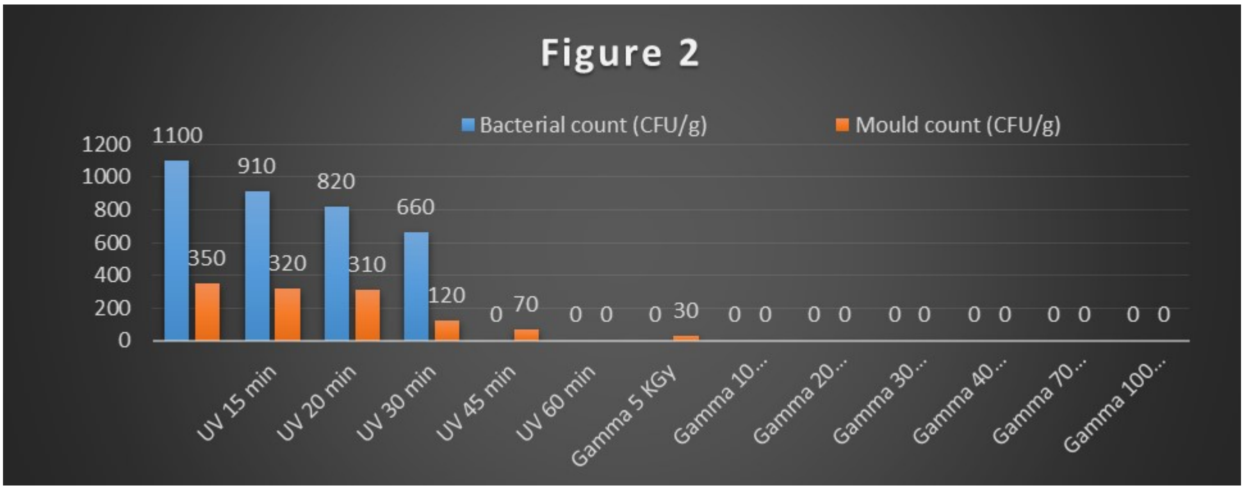

Table 6 \ Figure 3 Effect of Ultraviolet and Gamma irradiation treatment on aflatoxin M1 level in the 1st contaminated milk powder sample by aflatoxin m1

Criteria	Before treatment	After treatment by											
		UV 15 min	UV 20 min	UV 30 min	UV 45 min	UV 60 min	Gamma 5 KGy	Gamma 10 KGy	Gamma 20 KGy	Gamma 30 KGy	Gamma 40 KGy	Gamma 70 KGy	Gamma 100 KGy
Aflatoxin M1 level (µg/kg)	1.72	0.63	0.6	0.59	0.576	0.563	0.64	0.62	0.605	0.59	0.531	0.5	0.32

1st contaminated milk powder sample= sample contaminated with 1.72 ppb aflatoxin M1

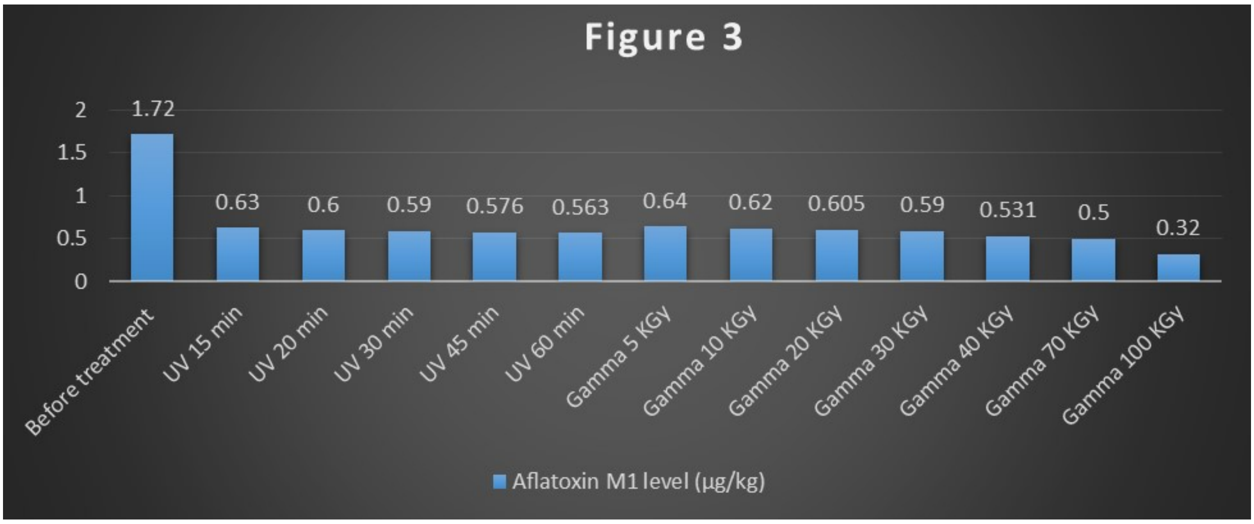

Table 7 \ Figure 4: Effect of Ultraviolet and Gamma irradiation treatment on enumeration of aerobic bacteria and mould in the 2nd contaminated milk powder sample by aflatoxin m1.

Criteria	Before treatment	After treatment by											
		UV 15 min	UV 20 min	UV 30 min	UV 45 min	UV 60 min	Gamma 5 KGy	Gamma 10 KGy	Gamma 20 KGy	Gamma 30 KGy	Gamma 40 KGy	Gamma 70 KGy	Gamma 100 KGy
Aerobic bacterial count (CFU/g)	55000	8100	4300	730	240	80	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Mould count (CFU/g)	2400	1000	670	210	90	30	90	< 10	< 10	< 10	< 10	< 10	< 10

2ndt contaminated milk powder sample= sample contaminated with 1.33 ppb aflatoxin M1

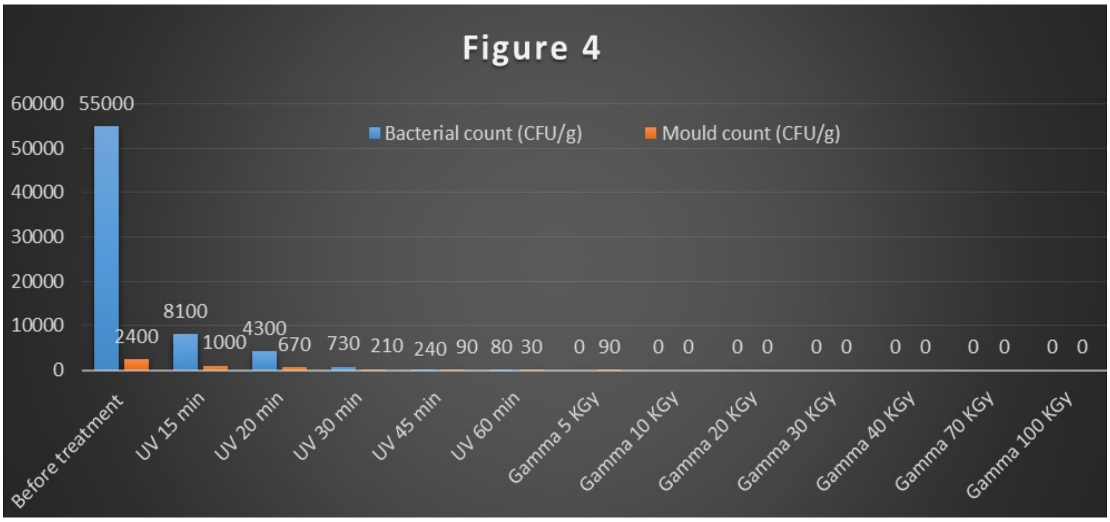

Table 8 \ Figure 5: Effect of Ultraviolet and Gamma irradiation treatment on aflatoxin M1 level in the 2nd contaminated milk powder sample by aflatoxin m1.

Criteria	Before treat-ment	After treatment by											
		UV 15 min	UV 20 min	UV 30 min	UV 45 min	UV 60 min	Gamma 5 KGy	Gamma 10 KGy	Gamma 20 KGy	Gamma 30 KGy	Gamma 40 KGy	Gam-ma 70 KGy	Gam-ma 100 KGy
Afla-toxin M1 level (µg/kg)	1.33	0.47	0.44	0.41	0.38	0.36	0.38	0.36	0.31	0.3	0.27	0.22	0.19

2nd contaminated milk powder sample= sample contaminated with 1.33 ppb aflatoxin M1

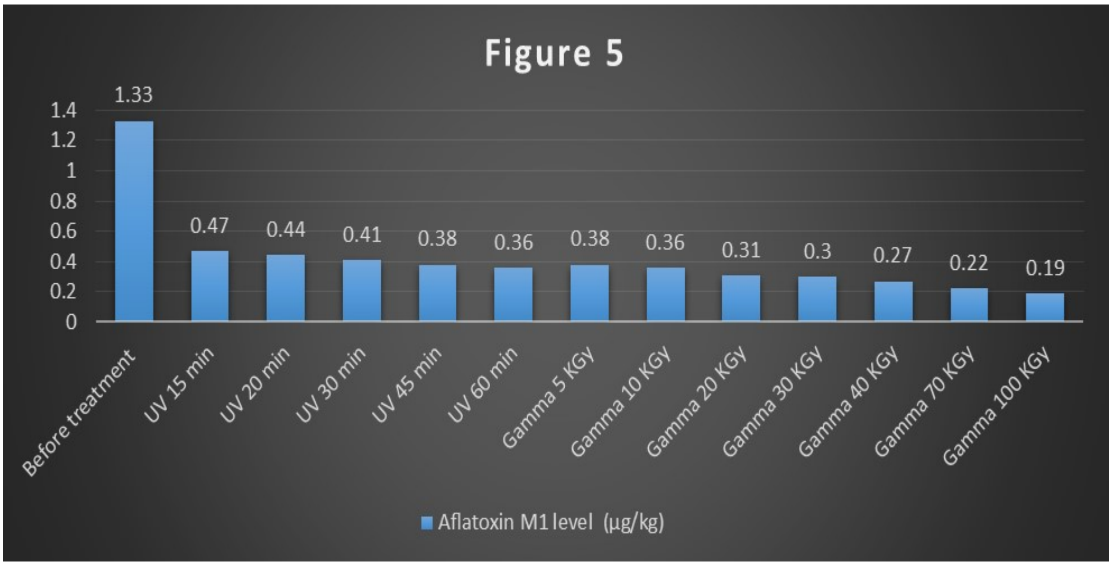

Table 9 \ Figure 6: Effect of Gamma and ultraviolet irradiation treatment on calcium level of the two contaminated milk powder samples

15 min		20 min		30 min		45 min		60 min		5 KGy		10 KGy		20 KGy		30 KGy		40 KGy		70 KGy	
1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1200	970	1200	970	1200	970	1197	970	1189	964.08	1200	970	1190	970	1154	949.63	1057	890.46	1050	878.82	982	841.96

Ppm = mg\1000 g 1 = 1st contamined milk powder sample with 1.72 ppb aflatoxin M1 2= 2nd t contaminated milk powder sample with 1.33 ppb aflatoxin M1

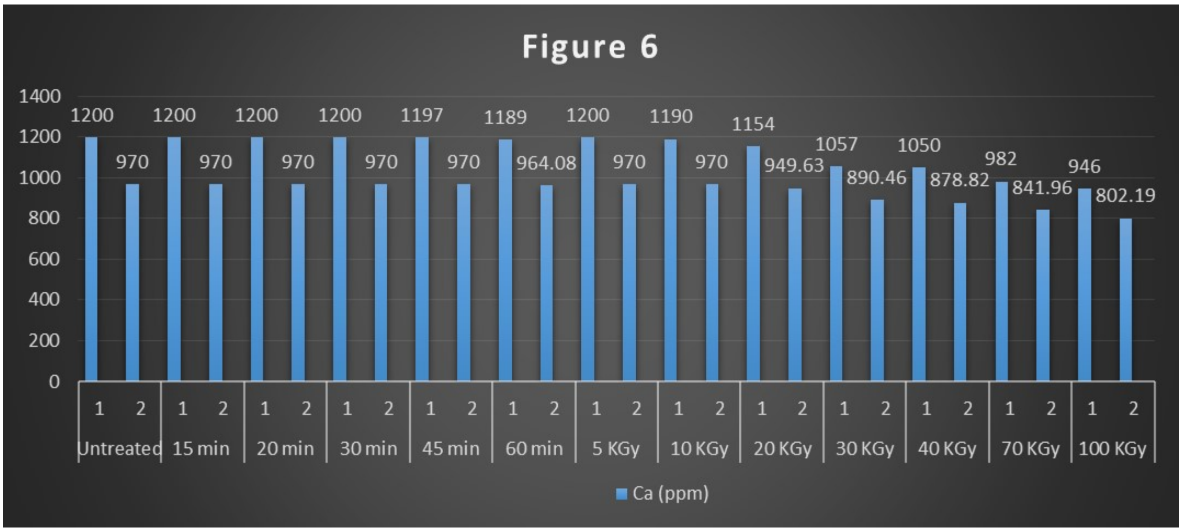

Table 10 \ Figure 7: Effect of Gamma and ultraviolet irradiation treatment on protein level of the two contaminated milk powder samples

15 min		20 min		30 min		45 min		60 min		5 KGy		10 KGy		20 KGy		30 KGy		40 KGy		70 KGy	
1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
23.9	26	23.9	26	23.9	26	23.80	26	23.71	25.67	23.71	25.95	23.70	25.91	22.74	25.40	22.10	24.64	21.22	23.81	18.56	21.02

1 = 1st contamined milk powder sample with 1.72 ppb aflatoxin M1 2= 2nd t contaminated milk powder sample with 1.33 ppb aflatoxin M1

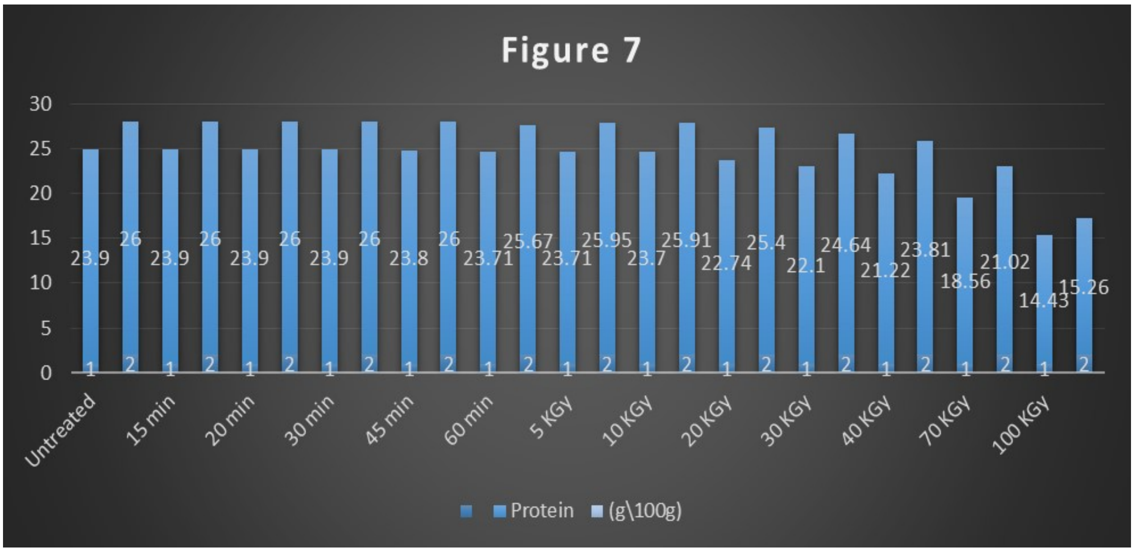

Table 11\ Figure 8: Effect of Gamma and ultraviolet irradiation treatment on fat level of the two contaminated milk powder samples

15 min		20 min		30 min		45 min		60 min		5 KGy		10 KGy		20 KGy		30 KGy		40 KGy		70 KGy	
1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
25.4	26.8	25.4	26.8	25.4	26.8	25.4	26.8	25.29	26.66	25.4	26.8	25.4	26.8	25.3	26.58	25.22	26.45	25.12	26.23	25.04	26.04

1 = 1st contamined milk powder sample with 1.72 ppb aflatoxin M1 2= 2nd t contaminated milk powder sample with 1.33 ppb aflatoxin M1

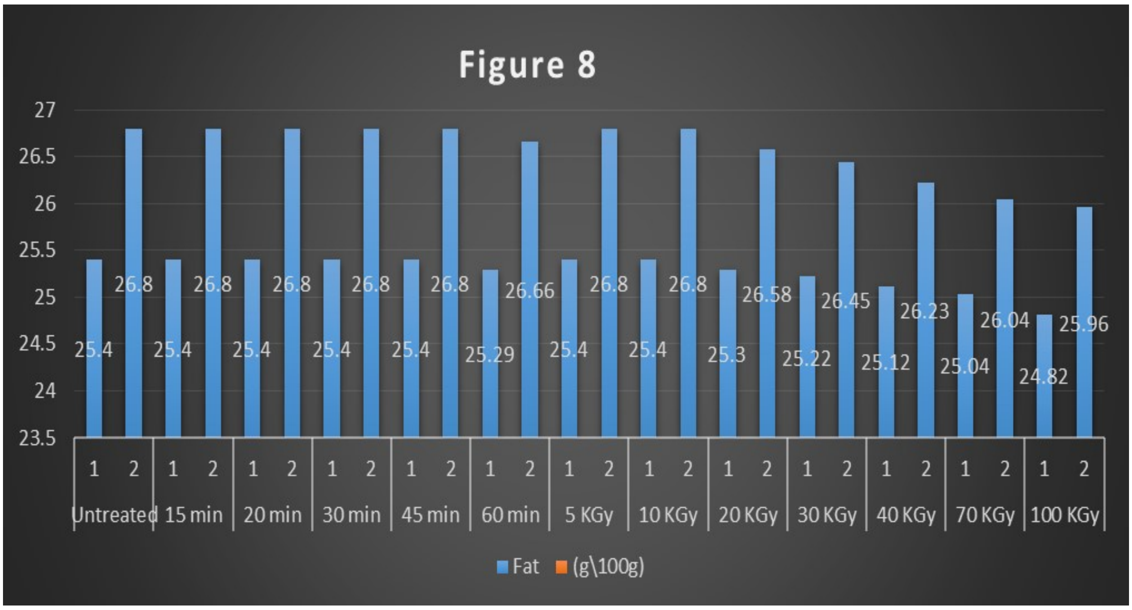

Table 12 \ Figure 9: Effect of Gamma and ultraviolet irradiation treatment on sugar level of the two contaminated milk powder samples

15 min		20 min		30 min		45 min		60 min		5 KGy		10 KGy		20 KGy		30 KGy		40 KGy		70 KGy
1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
38.8	39.3	38.8	39.3	38.8	39.3	38.8	39.3	39.03	39.63	38.45	38.86	39.38	40.63	40.42	41.46	41.16	42.36	41.90	42.95	43.06

1 = 1st contamined milk powder sample with 1.72 ppb aflatoxin M1 2= 2nd t contaminated milk powder sample with 1.33 ppb aflatoxin M1

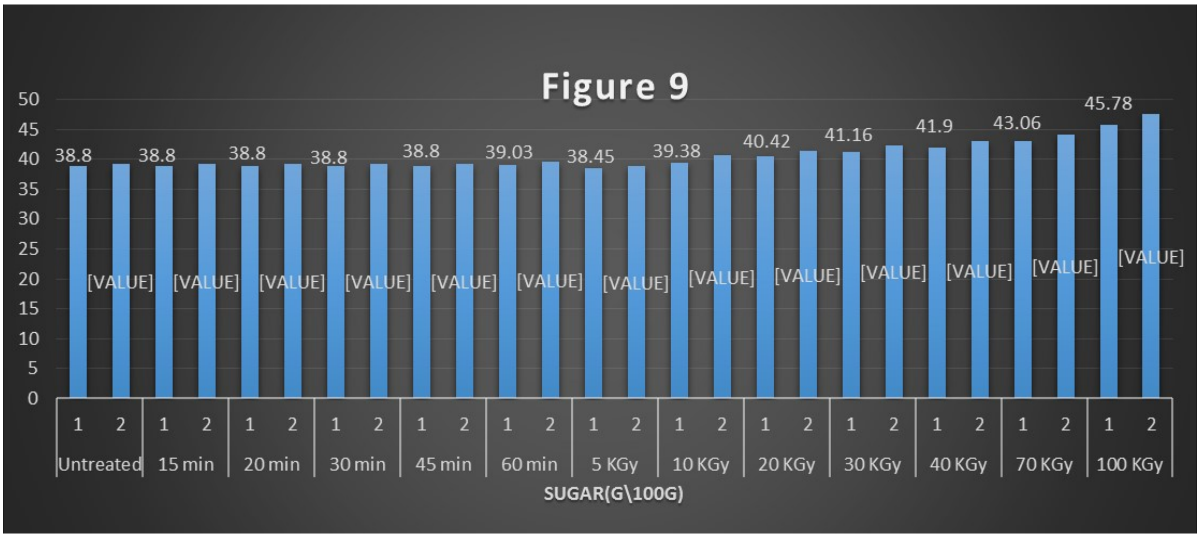

Table 13\ Figure 10: Effect of Gamma and ultraviolet irradiation treatment on vitamin D level of the two contaminated milk powder samples

	Untreated		15 min		20 min		30 min		45 min		60 min		5 KGy		10 KGy		20 KGy		30 KGy		40 KGy		70 KGy		100 KGy	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Vit D (Ug\100g)	4.9	8	4.9	8	4.9	8	4.9	8	4.9	8	4.9	8	4.9	8	4.9	8	4.9	8	4.9	7.94	4.89	7.92	4.86	7.87	4.86	7.76

1 = 1st contamined milk powder sample with 1.72 ppb aflatoxin M1 2= 2nd t contaminated milk powder sample with 1.33 ppb aflatoxin M1

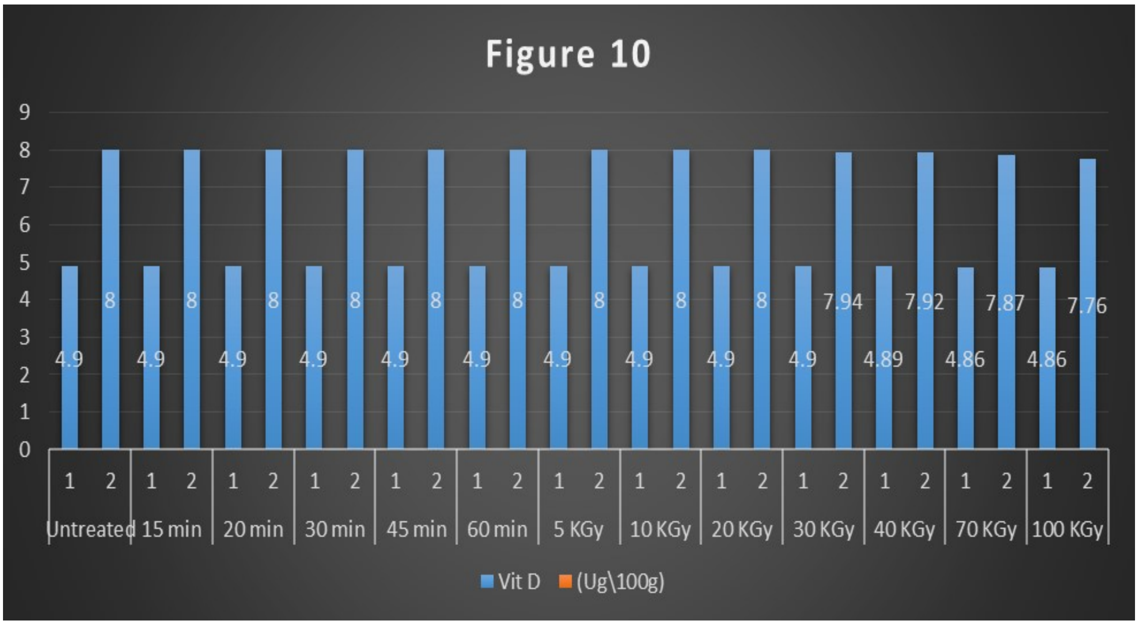

Table 14. reduction \ increasing % on the nutritional value of the 1st contaminated milk powder sample as a result of Ultraviolet and Gamma irradiation treatment

Nutritional factor	Ultraviolet treatment effect					Gamma treatment effect						
	15 min	20 min	30 min	45 min	60 min	5 KGy	10 KGy	20 KGy	30 KGy	40 KGy	70 KGy	100 KGy
Calcium	zero%	zero%	zero%	↓ (0.21%)	↓ (0.9 %)	zero%	↓(0.8%)	↓(3.8%)	↓ (11.89%)	↓(12.5%)	↓(18.1%)	↓(21.1%)
Protein	zero%	zero%	zero%	↓ (0.41%)	↓ (0.82%)	↓ (0.23%)	↓ (0.81%)	↓ (4.82%)	↓ (7.51%)	↓ (11.20%)	↓ (22.32%)	↓(39.61%)
Fat	zero%	zero%	zero%	zero%	zero%	zero%	zero%	↓ (0.4%)	↓ (0.7%)	↓ (1.1%)	↓ (1.4%)	↓ (2.3%)
Sugar	zero%	zero%	zero%	zero%	↑(0.6%)	↓ (0.9%)	↑ (1.5%)	↑ (4.2%)	↑ (6.1%)	↑ (8%)	↑ (11%)	↑ (18%)
Vit. D	zero%	zero%	zero%	zero%	zero%	zero%	zero%	zero%	zero%	↓ (0.2%)	↓ (0.8%)	↓ (0.8%)

↓ means reduction rate ↑ means increasing rate

Table 15. reduction \ increasing % on the nutritional value of the 2nd contaminated milk powder sample as a result of Ultraviolet and Gamma irradiation treatment

Nutritional factor	Ultraviolet treatment effect 15 min	20 min	30 min	45 min	60 min	Gamma treatment effect 5 KGy	10 KGy	20 KGy	30 KGy	40 KGy	70 KGy	100 KGy
Calcium	zero%	zero%	zero%	zero%	↓(0.61%)	zero%	zero%	↓(2.1%)	↓ (8.2%)	↓ (9.4%)	↓ (13.2%)	↓ (17.3%)
Protein	zero%	zero%	zero%	zero%	↓ (0.9%)	↓(0.20%)	↓(0.34%)	↓(2.3%)	↓ (5.2%)	↓(8.41%)	↓(19.12%)	↓ (41.32%)
Fat	zero%	zero%	zero%	zero%	↓ (0.5%)	zero%	zero%	↓(0.8%)	↓ (1.3%)	↓ (2.1%)	↓ (2.8%)	↓ (3.1%)
Sugar	zero%	zero%	zero%	zero%	↑(0.83%)	↓(1.1%)	↑(3.4%)	↑(5.5%)	↑ (7.8%)	↑ (9.3%)	↑ (12.1%)	↑ (20.8%)
Vit. D	zero%	zero%	zero%	zero%	zero%	zero%	zero%	zero%	↓(0.7%)	↓ (0.9%)	↓ (1.6%)	↓ (3%)

↓ means reduction rate ↑ means increasing rate

DISCUSSION

Powder milk has a much longer keeping quality and can be held in un-refrigerated storage condition. Much less storage space is required per unit of solids. Distribution is possible to the countries particularly those with unfavourable conditions of the perishable dairy products to be impractical. Consequently, dry milk has superiority both in economy and convenience. Powder milk is advantages for its concentrated source of many essential nutrients. Powder milk (whole and non-fat) are used in manufacturing ice cream, infant foods, bakery goods, confections and sausages and they are utilized by flour millers, and cheese processors. These are also used for convalescents and in the preparation of many other sweetmeats **(MFI. Kajal, et al. 2012**).

There is broad consensus that both traditional and novel pasteurization procedures need to be validated, and it must be made certain that these methods will indeed lead to the destruction of the pathogenic microorganisms most relevant in terms of human health, which are followed by the authorities' (in the USA: NACMCF – National Advisory Committee on Microbiological Criteria for Foods, 2005) licensing procedures for the different foodstuffs **(Csap´ et al. 2019).**

In this study, 15 samples of milk powder sold as closed containers and other 15 samples from opened containers were examined for the incidence and enumeration of both aerobic bacteria and mould to judge the microbiological condition of both samples of milk powder. The result revealed the clearance of the samples from closed containers from both aerobic bacteria and moulds while 60% of the samples from opened containers were positive to aerobic bacteria enumeration with mean ± SEM ($8.1x10^3 \pm 1.7x10^4$) as showed in table (1) , also 33.3% of the same samples were positive for mould enumeration with mean ± SEM ($6 x10^2 \pm 1x10^3$) as showed in Table (2) .

Also, the same samples were examined for the presence of Aflatoxin M1 which is considered as one of the dangerous fungal toxins that is produced in the presence of humidity and heat and lack of proper storage conditions.

The results in table (3) showed that 13.3% of the samples from opened containers were positive for the presence of aflatoxin M1 with Mean ± SEM (1.53±0.28) and their level Exceeding Egyptian regulations no.7136.2010 (0.05 µg/kg), EU regulations (0.05 µg/kg) and US regulations (0.50 µg/kg) ranging from1.33 to 1.72 (table no. 4). While samples from closed containers were negative for the presence of aflatoxin M1.

After evaluation of milk powder samples from opened containers compared with the closed clean and safe milk powder containers, there was a need for a preservation method which shall to be safe and would not affect the physical properties and nutritive values of milk.

The risk of contamination expected to be high in the milk powder samples from opened containers. As, it considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms **(Yousef, 2004 and Dawood, et al. 2006).**

It's necessary to apply of new food preservation techniques that provide microbiological safety, increasing the commercial validity, and still provide minimal biochemical alterations, promoting the maintenance of the nutritional and sensorial quality of the products **(Leinstner and Gorris, 1995)**.Gamma rays are generally used for the sterilization of gaseous, liquid, solid materials, homogeneous and heterogeneous systems and medical devices, such as syringes, needles, cannulas, etc. Gamma irradiation is a physical means of decontamination, because it kills bacteria by breaking down bacterial DNA, inhibiting bacterial division **(Farkas and Farkas, 2011).**

To be effective, gamma sterilization requires time, contact and temperature. The effectiveness of any method of sterilization is also dependent upon other factors like the type of microorganism present or their toxins. Some microorganisms are difficult to be eradicated while others might be more affected **(Kátia Aparecida, 2012).**

Ultraviolet (UV) radiation was discovered in 1801 by the German scientist Johan Ritter, the use of UV-light as a non- chemical disinfection method is increasingly gaining acceptance by its germicidal power. The UV-light can be applied as a continuous or pulsed mode. The continuous mode is the conventional method, the light being emitted continuously without interruption. In pulsed UV-light mode, the UV-light is released as intermittent pulses using a capacitor, which allow to increase the energy intensity per pulse. Therefore, the pulsed mode is more effective for microbiological inactivation and the most used method **(Krishnamurthy et al. 2005).**

Irradiation techniques have been widely studied, and like most food processing techniques it can induce some changes that are able to modify the chemical and nutritional characteristics on foods. These changes are dependent on some factors such as the radiation dose, the constitution of the irradiated food, the type of packaging, and how it was processed, besides the variables of the process as temperature and oxygen saturation on the atmospheric **(Dionísio, et al. 2009).**

So, in this study we focused on the effect of both ultraviolet and gamma treatment on the incidence and level of aerobic bacteria, mould and aflatoxin M1 in the contaminated milk powder samples by aflatoxin M1 from opened containers. Firstly, we studied the effect of these radiation on the incidence and level of aerobic bacteria and mould in first contaminated milk powder sample (table no.5, figure no. 2). The result revealed that the UV treatment for 45 min. give the best result in eliminating the aerobic bacteria while for mould growth eradication we needed to UV treatment for 60 minutes. On the other hand, 5 and 10 kGy of gamma irradiation was enough to get rid of all aerobic bacteria and mould, respectively.

Salwa, et al. (2012) evaluated the effect of gamma irradiation on the quality of Egyptian karish cheese and their study revealed that it could be concluded that increasing the dose of irradiation up to 5 kGy had high reduction percentages for bacterial counts with no effects on either sensory or chemical characteristics and their results suggested that karish cheese manufacturers could use gamma irradiation to improve the safety and quality of this product.

The effect of UV and gamma irradiation on aflatoxin M1 in the 1st contaminated milk powder sample showed in Table (6) and Figure (3). The results showed the maximum reduction of aflatoxin M1 level from 1.72 µg/kg to 0.563 µg/kg under the effect of UV rays for 60 min, while to 0.32 µg/kg by using 100 kGy gamma irradiation. This effect considered to be limited and not effective.

The results obtained in table (7), figure (4) showed that 60 minutes exposure to UV treatment cannot eliminate all bacterial and mould in 2nd contaminated milk powder sample. While, 5 kGy and 10 kGy of gamma irradiation was enough to eliminate all bacterial and mould in the sample, respectively.

The effect of UV and gamma irradiation on aflatoxin M1 in the 2nd contaminated milk powder sample showed in Table (8) and Figure (5). The results showed the maximum reduction of aflatoxin M1 level from 1.72 µg/kg to 0.36 µg/kg under the effect of UV rays for 60 min, while to 0.19 µg/kg by using 100 kGy gamma irradiation. This effect considered to be limited with noticeable changing in the treated milk powder colour by increasing the treatment doses.

Using UV and gamma irradiation treatment to eliminate aflatoxin M1 from contaminated milk powder samples was not effective, as aflatoxin M1 level was slightly decreased but not to the safe level which reported by regulations standard.

The typical doses for insects' disinfestation or parasite inactivation are up to 1 kGy and to reduce or eliminate spoilage or disease-causing pathogenic microorganisms the common doses used are up to 10 kGy (**Molins, 2001**).

Milk is a combination of different nutritive factors which formulate its structure. Calcium is an essential element that is very important for the milk consumers so in this study we studied the effect of UV and gamma radiation treatment on the calcium level (table 9, figure 6). The results showed that calcium level decreased after 45, 60 minutes of UV radiation treatment in the first and second treated samples, respectively. While, by using gamma irradiation, calcium level was nearly constant up to 10 kGy treatment dose.

Milk contains various types of proteins that play a major role in milk structure **(Van Lieshout GA, et al؛ 2020).**

Results obtained from table (10), figure (7) showed the effect of UV and gamma radiation treatment on protein level of the two treated samples. Protein was decreased after 45 and 60 minutes of UV treatment of the first and second sample, respectively. While decreasing effect of gamma radiation began from treatment dose of 5 kGy on both treated samples.

Fat level of two treated samples was decreased after 60 minutes of UV treatment and 20 kGy of gamma irradiation as showed in table (11), figure (8).

Total sugar of two treated samples was decreased after 5 kGy of gamma irradiation, while increased after 60 minutes of UV treatment and 10 kGy of gamma irradiation (table 12, figure 9). That is agreed with which reported by Carla Pereiraa, et al., 2000 who emphasized that the sample which is irradiated at 10 kGy showed the highest amount of sugars that also contributed to the highest total sugars content (8.63 g/100 g). This also is compatible with **Byun, Kang, et al., 1996** previous study which explained this increasing through the depolymerization or degradation of polysaccharide molecules and glucoside group due to gamma irradiation in soybeans. **Tissot, et al. 2013** reported the potential of ionizing radiation on facilitating the breakdown of cellulose into simple sugars.

Table (13) showed that there is no significant effect of both UV and gamma irradiation on the level of Vitamin D in two treated samples.

Tables (14 & 15) summarized the effect of both UV and gamma irradiation on nutritive values of the two treated milk powder samples.

Food irradiation arises as one of the most promising decontamination methods for many foodstuffs, reducing the reliance on chemical fumigants used by the food and pharmaceutical industries and other chemical fumigants like gaseous ethylene oxide or methyl bromide for decontamination or sterilization, that are carcinogenic and increasingly restricted in several countries, due to health, environmental or occupational safety reasons. Among the different types of radiation sources allowed for food processing (gamma, UV,), gamma irradiation represents an effective and environment friendly technology to avoid the re-contamination and reinfestation of the product, since it can be done after packaging **(Aouidi, Samia, et al. 2011).**

CONCLUSION

This study emphasized the importance of good hygienic packaging of milk powder in closed packed containers to keep it safe. Also, the study revealed that treatment either by UV (up to 60 minutes) or elevated doses of Gamma irradiation (up to 100 kGy) had a potential effect on reduction of microbial growth in milk powder samples with insignificant effect on reduction of aflatoxin M1 level to the acceptable limit based by standards, with some changes in samples physical properties and nutritional values. As this study proved that even high doses of irradiation treatment could not eradicate the present toxin. So that, the application of hygienic measures in packaging of milk powder is considered the gold standard for safe products.

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