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Influence of Gamma Irradiation on Microbial Load of Peanut (*Arachis hypogaea* L) Kernels

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A B S T R A C T

The influence of gamma irradiation on microbial load of different peanut varieties has been assessed. The healthy and mature kernels of peanut variety popular in saurastra region of India *viz.* GG-20 and TG-37A were naturally contaminated and conditioned with three initial moisture contents (6.0%, 8.5% and 11.0%), w.b. and packed in Poly Propylene (PP) bags of 50µ thickness. These samples were irradiated with different gamma irradiation dose (0 kGy, 2.5 kGy, 5.0 kGy, 7.5 kGy and 10.0 kGy) and stored at ambient temperature for three months. Microbial load including total plate count, yeast and mold count, *E.coli*and *salmonella* were determined for irradiated and non-irradiated peanut kernels. Microbial load of both peanut varieties for tested microorganism significantly increase in non-irradiated samples whereas irradiated samples showed decrease in population for all the conditioned moisture content as storage time progressed. The research data discovered that gamma irradiation dose of 7.5 kGy is the recommended dose for complete elimination of total plate count as well as yeast and mold; whereas the 5.0 kGy is sufficient for complete killing of *E. Coli.*in kernels of both peanut varieties. Salmonella was not noticed in any of the samples before irradiation and during whole storage period. There was found no steady pattern in variation in D_{10} value, for all the tested microorganisms with respect to initial moisture in kernels of both the peanut varieties.

Introduction

Groundnut or peanut (*Arachis hypogaea L.)* commonly known as poor people's nut. In most of the emerging countries, peanut kernels are stored as dry seeds and form a massive serve of food; however huge quantities of seeds are lost annually as a result of microbial load as well as insects' attacks. Presently, the inhibition of microbial infestation in food materials is the most persistent food safety problems facing by the food industry. People, all over the globe are demanding for nontoxic food as we all become more concerned about the quality of the food.

Therefore, a range of preservative strategies are used to prevent and reduce infestation of molds and insects. Chemical fumigation with pesticides, such as methyl bromide and phosphine, is practiced for protection of stored seeds from pest infestation. However, as necessary as we believe, the excessive use of these chemicals leads to have hostile effects on both food and environment, related with residues and ozone depletion. In addition, the high cost of insecticides, non-availability of suitable formulation of chemical and packaging, along with the increasing incidence of insecticides necessitate an alternative approach to post harvest pest control.

Fortunately, radiation processing, which is basically a cold process, can kill microorganisms and insects, reduce post-harvest losses, and enhance food safety and quality (Loaharanu and Thomas, 2001). Consequently, irradiation is increasingly being applied to disinfest and decontaminate foods and other products, while gaining recognition as an effective quarantine treatment for agricultural produce (IAEA, 2002). Besides drying, radiation is the only alternative to cold processing for food preservation that has a lethal effect on micro-organisms (Gojiya *et al.,* 2019). Further, it is the novel method of food preservation suggested for many countries.

Purpose of study

India's key export markets include the US, the EU, Asian, SAARC countries and West Asia. Although there is a solid assurance from the Government to promote exports of fresh and processed food products, and a strong preparedness on part of the exporters and farmers to export; Indian exports of food products are getting rejections and bans in key markets on surroundings of lack of obedience with food safety and health standards.

Food products have met rejections and even bans in markets such as the US, Vietnam, EU, Saudi Arabia, Japan and Bhutan due to disputes such as presence of more than chemical residues as well as pest and bacterial infestation beyond approved levels.

The gamma irradiation is a physical treatment comprising direct exposure of food to electron or electromagnetic rays, for their long time protection as well as upgrading of quality and safety (Mahindru, 2005). In this process, the isotope cobalt-60 (^{60}Co) produces electromagnetic γ-rays which have tremendous energy which swelling and break alongside the molecules DNA chain and preventing them from functioning normally.

As a result, the parasites and microorganisms that have been affected and losing their capabilities of reproducing themselves and they die (Lacroix and Ouattara, 2000). Consequently gamma irradiation provides food safety and enhancing the shelf life of peanut kernel due to its high effectiveness in deactivating pathogenic and spoilage microorganisms without affecting product quality (Ozden and Erkan, 2010).

The application of gamma radiation in food processing has been broadly accepted and is now legally endorsed as a safe and effective technique for maintaining food quality safety. Knowing the techno-economic benefits, about 100 different countries all over the globe have cleared radiation treatments for over more than 100 different food products.

Food irradiation has been recognized by key health organization such as the American Medical Association, Food and Agriculture Organization (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Codex Alimentarius Commission, the US Council of Agricultural Science and Technology. Considering the above facts in mind; research project was undertaken with the following objectives.

Materials and Methods

Sample preparations

Healthy and mature kernels of peanut variety GG-20 (Gujarat Groundnut-20) and TG-37A (Trombay Groundnut-37A), popular in sauratra region were procured in bulk from peanut processing industry namely Balikrut Peanut Industries, Junagadh (Gujarat). Since initial moisture content of the two varieties of peanut (GG-20 and TG-37A) kernels was high ~11% w.b., therefor be tone packaging, the peanut samples were dried at 50±2 °C to attain the safe storage moisture level of ~6.0 % in a laboratory tray dryer (Gojiya*et al.*, 2020). After getting initial moisture content, the samples have been conditioned to 6.0, 8.5 and 11.0 % moisture content (w.b.) by adding pre-determined amount of distilled water to the fixed quantity of kernels. The following equation was used to estimate the quantity of distilled water to be added to the peanut kernels (Obi *et al.*, 2014).

$$Q = \frac{M(x-y)}{(100-x)} \qquad \dots (1)$$

Where,

Q = Amount of water to be added (kg)
M = Initial weight of sample (kg)
y = Initial moisture content of the sample (% w.b.)
x = Final (desired) moisture content of the sample (% w.b.)

After addition of calculated amount of distilled water, the samples were packed in the peanut kernels sample of ~650 Grams were packed in virgin Poly Propylene (PP) bags of 50µ thickness having size: 260 x 200 mm and sealed by hand sealing machine (Plastic Impulse Hand Sealer, model: POLY SEAL-300mm. After that these samples were stored in incubator at 25 ^{0} C for 24 h and during the storage, the samples were stirred at every 2 h during the day time to ensure uniform moisture.

Application of irradiation treatment

The given irradiation doses in this study were 2.5, 5.0 7.5 and 10.0 kGywith an accuracy of ± 5.0 %. The absorbed dose was monitored by Ceric- Cerous 100 X 2 ml ampoules, type dosimeters (Kilo gray Gamma Dosimeter, Batch No: DS-(3)10, Range 1-30 kGy, Bhaba Atomic Research Centre (BARC), Navi-Mumbai). The dosimeters were calibrated to ISO/ASTM 51205 (E) Standard Practice for use of a Ceric-Cerous Sulfate Dosimetry System, an "International Organization for Standardization" approved by "American Society for Testing and Materials standard". Dosimeters were positioned a box in front, middle and rear side and total dosimeters was 4 per run. Peanut samples were maintained at 20±2 °C during irradiation.

Storage of Peanuts Samples

The peanut variety GG-20 and TG-37A samples were packed in PP bags and irradiated with gamma irradiation @ 2.5, 5.0, 7.5 and 10.0 kGy using ^{60}Co. After applying gamma irradiation, Irradiated and non-irradiated (control) samples were stored at ambient temperature.

These irradiated peanut kernel samples of both the varieties were evaluated at one month interval during storage besides just after irradiation, for its microbiological characteristics *viz.* Total plate count (log (CFU/g)), Yeast and mould (log (CFU/g)), *E. coli* (log (CFU/g)) and S*almonella* (log (CFU/g)).

The radiation resistance (D_{10}value) of the tested microbial load (total plate count (TPC), yeast and mold count (YMC), *salmonella* and *Escherichia Coli.*) were determined. All the obtained data were statistically analysed.

Statistical Analysis

By using factorial complete randomized design (F-CRD) with 15 treatment combinations (3 moisture levels X 5 irradiation doses level) were obtained for each variety of peanut. Treatments having 0.0 kGy gamma irradiation dose (no irradiation) served as control for each variety and each moisture content level. All the 30 treatments were examined for all the biochemical, microbiological as well as sensory evaluation of the sample. The mean values generated from the analysis of each of quality attributes obtained from three replications during the research were subjected to statistical analysis using factorial completely randomized design (F-CRD) and Microsoft Excel as per the procedure suggested by Panse and Sukhatme (1985).

Natural microbial contamination

Natural microbial inoculation of peanut kernels was most commonly achieved by submersion in aqueous spore suspensions (Prado *et al.*, 1999). 100 gram microbiologically contaminated peanut seed were grinded and submerged in 1 liter distilled water. Filtrates of this solution add into distilled water and mixed thoroughly with peanut sample and shake it at low speed until all of the added water was uniformly absorbed to final moisture content of 11 % (w.b). These contaminated peanut samples incubated at 35±2 °C temperature and 80% relative humidity in incubator for seven days. Samples were analyzed for microbial load after 7 days storage and used for further experiments.

Determination of Microbiological Characteristics of Peanut Kernels

The microbiological characteristic of peanut was carried out according to standard method recommended by AOAC (2006). All microbiological media were procured from Hi-Media laboratories, Mumbai, India. First of all, the media and all the glass accessories to be used in the analysis were sterilized by autoclaving at 121 °C for 20 min. The peanut sample was diluted to 1:10 (peanut: distilled water) and was thoroughly shaken for 10-15 min to obtain homogenized suspension of micro-organisms. For preparation of 1:100 dilution, the 1 ml sample from 1:10 dilution was taken with the help of pipette and 9 ml distilled water was added into the prepared solution. The process was repeated up to 1:1000 dilutions with the distilled water. The test tubes containing the diluted samples were immediately closed by cotton plugging. The whole procedure for sample preparation was carried out in laminar air flow (Nova Instruments Pvt. Ltd., Ahmedabad) under aseptic condition.

Radiation-decimal reduction dose (D_{10}-value)

The radiation resistance (D_{10}-value) of the tested microbial load *(*Total plate count (TPC), Yeast and mold count (YMC), *Salmonella* and *Escherichia Coli.)* were determined as follow**.** D_{10} value for each tested microbial load was determined for each radiation dose as per standard method suggested by Khalek (2008). The number of viable cells after radiation was divided with the initial viable cell number. For all the strains, survival curves relating to log (survival) were obtained with irradiation dose. Finally, the D_{10} values or the doses which can reduce the microbial population by 90% were calculated by using the equation

$$D_{10}\text{ value} = \frac{D}{(\log No - \log N)} \dots (2)$$

Where,

D is the radiation dose,

No is the untreated bioburden, and
N is the irradiated bioburden.

Graphically D_{10} values were calculated by using Excel's linear regression analysis based on the linearity of the survivor curves in which D_{10} values were taken as the negative reciprocal of the slope of the regression line from dose-response curve, which was constructed by plotting log survival counts against irradiation doses.

Results and Discussion

Effect of Gamma Irradiation on Total plate count (TPC) of Peanut Kernels

Total bacterial count is widely used as an indication of the microbiological quality of food. It can be observed that Total plate count of tested microorganism increases in non-irradiated samples whereas, irradiated samples showed decrease in population as storage time progressed. Irradiation treatments induced ionization for the cell of bacteria and direct effects on DNA of nucleus cells (Shea *et al.*, 2000; Temur and Tiryaki, 2013) resulting in death of cell complete elimination was observed at and above the irradiation dose of 7.5 kGy. As gamma irradiation dose was enlarged from 0.0 to 10.0 kGy, Total plate count of the peanut kernels reduced significantly. This was true for both peanut kernels varieties and at all the three moisture contents. These results indicate that the higher doses of gamma radiation have vigorously effect on Total plate count.

At zero time after irradiation, it was noted as dose was increased from 0.0 to 10.0 kGy, the Total plate count (TPC) in GG-20 peanut kernels severely reduced from 5.81 log (CFU/g) to <1 log (CFU/g) and 6.72 to 7.72 log (CFU/g) to <1 log for the kernels with initial moisture content of 6, 8.5 and 11 % (w.b.), respectively. It was noticed once microbial population reduced due to gamma irradiation it could not be revived again during the subsequent storage period of three month.

For TG-37A variety, as irradiation dose was amplified from 0.0 to 10.0 kGy, the Total plate count (TPC) in TG-37A peanut kernels sharply reduced from peanut 5.89 log (CFU/g) to < 1 log, 6.73 log (CFU/g) to <1 log and 6.53 log (CFU/g) to <1 log for the kernels with initial moisture content of 6, 8.5 and 11 % (w.b.), respectively. It was also reported that the reduction in Total plate count due to gamma irradiation it could not be recovered again during the subsequent storage period. These findings were in accordance with results noted by Farkas (1983) for (garlic powder, cumin, coriander, turmeric and rosemary), Sharma *et al.*, (1989), Hammad*et al.*, (1987) and El-Zawahry *et al.*, (1985) for different spices and its storage. Hilmy and Suryasaputra, (1981) also reported similar results that the microbes' populations decreased as much as 2-3 log cycles after irradiation with 5 kGy in medicinal herbs. After 6 months of storage, microbial load of irradiated samples further decreased and in the case of microorganisms that survive during irradiation treatment may be more sensitive to environmental conditions.

The statistical interpretation of Total plate count of both peanut variety were presented in the Table 1 indicated that during entire storage of GG-20 and TG-37A peanut kernels, the individual effect of gamma irradiation dose (D) and its interaction effect with peanut kernels initial moisture content (D X M) had statistically significant difference at 5% level of significance. The individual effect of initial moisture content (M) had found significant ($P<0.05$) in GG-20 whereas in TG-37A it noted non-significant ($P\geq0.05$) during entire storage.

Table.1 Effect of gamma irradiation on Total plate count (log (CFU/g)) of peanut kernels conditioned at different initial moisture content during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1st month	2nd month	3rd month	0 month (Initial value)	1st month	2nd month	3rd month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Gamma radiation doses (D)								
D1 (0 kGy)	6.75	6.96	7.18	6.86	6.50	6.60	6.81	7.03
D2 (2.5 kGy)	3.76	3.98	4.19	3.87	3.70	3.91	4.12	4.34
D3 (5.0 kGy)	1.69	1.89	2.12	1.79	1.04	1.10	1.15	1.47
D4 (7.5 kGy)	0.32	0.38	0.43	0.35	0.83	0.90	0.85	0.99
D5 (10 kGy)	0	0	0	0	0.11	0.14	0.14	0.18
S.Em±	0.13	0.15	0.16	0.16	0.11	0.12	0.14	0.16
CD at 5%	0.37	0.42	0.46	0.45	0.32	0.35	0.40	0.46
Moisture Content (M)								
M1 (6.0 %, w.b)	2.82	2.98	3.14	3.30	2.31	2.50	2.64	2.82
M1 (8.5 %, w.b)	3.12	3.30	3.47	3.65	2.52	2.61	2.70	2.95
M1 (11.0 %, w.b)	3.46	3.63	3.82	3.96	2.47	2.48	2.52	2.65
S.Em±	0.10	0.11	0.12	0.12	0.09	0.10	0.11	0.12
CD at 5%	0.28	0.33	0.36	0.35	NS	NS	NS	NS
Interaction (D x M)								
S.Em±	0.22	0.25	0.28	0.27	0.19	0.21	0.24	0.28
CD at 5%	0.64	0.73	0.80	0.78	0.56	0.61	0.68	0.80
C.V.%	15.23	16.53	17.54	15.98	13.83	14.58	15.68	17.04

Table.2 Effect of gamma irradiation on Yeast and Mold count (log(CFU/g)) in peanut kernels conditioned at different initial moisture content during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1st month	2nd month	3rd month	0 month (Initial value)	1st month	2nd month	3rd month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Gamma radiation doses (D)								
D1 (0 kGy)	5.77	6.02	6.24	6.45	5.97	5.98	6.00	6.01
D2 (2.5 kGy)	3.30	3.51	3.73	3.94	3.28	3.30	3.31	3.33
D3 (5.0 kGy)	1.80	2.01	2.22	2.44	1.16	1.17	1.18	1.20
D4 (7.5 kGy)	0.21	0.26	0.35	0.42	0.41	0.41	0.42	0.42
D5 (10 kGy)	0	0	0	0	0.18	0.20	0.21	0.22
S.Em±	0.05	0.07	0.08	0.09	0.08	0.08	0.08	0.08
CD at 5%	0.15	0.19	0.23	0.26	0.24	0.24	0.23	0.24
Moisture Content (M)								
M1 (6.0 %, w.b)	2.48	2.68	2.87	2.87	2.00	2.01	2.02	2.03
M1 (8.5 %, w.b)	2.89	3.05	3.24	3.24	2.76	2.77	2.78	2.80
M1 (11.0 %, w.b)	3.85	4.07	4.28	4.28	3.48	3.49	3.51	3.52
S.Em±	0.04	0.05	0.06	0.07	0.06	0.06	0.06	0.06
CD at 5%	0.11	0.15	0.18	0.20	NS	NS	NS	NS
Interaction (D x M)								
S.Em±	0.09	0.12	0.14	0.16	0.14	0.14	0.14	0.14
CD at 5%	0.25	0.33	0.40	0.46	0.42	0.41	0.40	0.41
C.V.%	6.86	8.50	9.64	10.45	11.93	11.60	11.46	11.61

Table.3 Effect of gamma irradiation on *E. Coli*. (log(CFU/g)) in peanut kernels conditioned at different initial moisture content during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 days (Initial value)	30	60	90	0 days (Initial value)	30	60	90
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Gamma radiation doses (D)								
D1 (0 kGy)	2.29	2.28	2.28	2.29	2.10	2.09	2.08	2.08
D2 (2.5 kGy)	1.21	1.21	1.22	1.22	1.41	1.42	1.43	1.43
D3 (5.0 kGy)	0	0	0	0	0	0	0	0
D4 (7.5 kGy)	0	0	0	0	0	0	0	0
D5 (10 kGy)	0	0	0	0	0	0	0	0
S.Em±	0.01	0.02	0.02	0.01	0.03	0.03	0.03	0.03
CD at 5%	0.04	0.05	0.06	0.04	0.07	0.07	0.09	0.07
Moisture Content (M)								
M1 (6.0 %, w.b)	1.66	1.66	1.66	1.67	1.643	1.633	1.630	1.634
M1 (8.5 %, w.b)	1.68	1.69	1.69	1.69	1.741	1.741	1.744	1.745
M1 (11.0 %, w.b)	1.90	1.90	1.90	1.91	1.877	1.883	1.882	1.880
S.Em ±	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02
CD at 5%	0.03	0.04	0.05	0.03	0.06	0.06	0.07	0.06
Interaction (D x M)								
S.Em ±	0.02	0.03	0.04	0.02	0.04	0.04	0.05	0.04
CD at 5%	0.06	0.09	0.10	0.06	0.13	0.13	0.15	0.13
C.V.%	5.47	7.63	8.90	5.28	10.87	10.78	13.04	10.78

Table.4 Effect of gamma irradiation on *Salmonella* (log (CFU/g)) in peanut kernels conditioned at different initial moisture content during storage

Effect	**GG-20**				**TG-37A**			
	Storage period (month)				**Storage period (month)**			
	0 month (Initial value)	**1st month**	**2nd month**	**3rd month**	**0 month (Initial value)**	**1st month**	**2nd month**	**3rd month**
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Gamma radiation doses (D)								
D1 (0 kGy)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
D2 (2.5 kGy)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
D3 (5.0 kGy)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
D4 (7.5 kGy)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
D5 (10 kGy)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.Em ±	-	-	-	-	-	-	-	-
CD at 5%	-	-	-	-	-	-	-	-
Moisture Content (M)								
M1 (6.0 %, w.b)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
M1 (8.5 %, w.b)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
M1 (11.0 %, w.b)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.Em ±	-	-	-	-	-	-	-	-
CD at 5%	-	-	-	-	-	-	-	-
Interaction (D x M)								
S.Em ±	-	-	-	-	-	-	-	-
CD at 5%	-	-	-	-	-	-	-	-
C.V.%	-	-	-	-	-	-	-	-

Table.5 Radiation-decimal reduction dose (D_{10}-value)

SSr. No	Microbial Parameter, (log CFU/g)	Moisture content, w.b (%)	D_{10} values in kGy	
			GG-20	TG-37A
(1)	(2)	(3)	(4)	(5)
11	Total Plate Count	M1=6.0 %	0.83	1.09
		M2=8.5 %	0.97	1.02
		M3=11 %	1.26	1.09
22	Yeast and Mold Count	M1=6.0 %	1.21	1.07
		M2=8.5 %	1.06	0.96
		M3=11 %	1.57	0.92
3	*E. Coli.*	M1=6.0 %	2.18	4.40
		M2=8.5 %	2.19	3.84
		M3=11 %	2.61	3.11

Effect of Gamma Irradiation on Yeast and mold counts of Peanut Kernels

The results pertaining to effect of gamma irradiation dose on Yeast and mold counts of both peanut varieties at different initial moisture content during whole storage period reported in Table 2. It can be evidently seen from data that Yeast and mold count decrease with increase in gamma irradiation dose, at and above 7.5 kGy gamma irradiation dose, Yeast and mold in both the peanut kernels varieties reduced below 1 log (CFU/g).

It was noted that on initial day after gamma irradiation, as dose increased from 0.0 to 10.0 kGy, Yeast and mold counts in GG-20 peanut kernels significantly ($P<0.05$) declined from 5.95 to <1 log (CFU/g), 6.18 to <1 log (CFU/g) and 7.26 to <1 log (CFU/g) for the kernels with initial moisture content of 6, 8.5 and 11 % (w.b.), respectively.

The data shows that the reduction in yeast and mold counts could not be recovered again during the consequent storage period. Yeast and mold reportedmaximum in the treatment F1D1M3 (7.81 log (CFU/g)) followed by F1D1M2 ((7.44 log (CFU/g)) and F1D1M1 (7.09 log (CFU/g)) after three month of storage.

It was also noticed that, Yeast and mold counts in TG-37A peanut kernels significantly decrease from 5.95 to <1 log 7.09 (CFU/g), 6.18 to <1 log (CFU/g) and 7.26 to <1 log (CFU/g) with increase in gamma irradiation dose level 0, 2.5, 5.0, 7.5 and 10.0 kGy at different initial moisture content (6.0 to 11 % (w.b.)). The maximum rise in yeast and mold counts was found in the treatment F2D1M3 (7.45 log (CFU/g)) followed by F2D1M2 ((6.96 log (CFU/g)) and F2D1M1 (6.31 log (CFU/g)) samples after three month of storage (Table 2). The similar results were reported by Silberstein *et al.*, (1979) for onion powder, Farkas (1983) and Sharma *et al.*, (1984) for spices, Ramamurthy *et al.*, (2004) for capsicum, Yun *et al.*, (2012) for soybeans (*Glycine max* L. *Merrill*) and Ashtari*et al.*, (2019) for pomegranate cv. *Malas-e-Saveh* that gamma irradiation significantly reduced the population of bacteria, fungi and yeasts in all this product.

The statistical analysis of Yeast and mold counts of both peanut variety were the indicated that during entire storage of GG-20 and TG-37A peanut kernels, the individual effect of gamma irradiation dose (D) and its interaction effect with peanut kernels' initial moisture content (D X M) had statistically significant difference at 5% level of significance. The individual effect of initial moisture content (M) had found significant ($P<0.05$) in GG-20 peanut kernels while in TG-37A it noted non-significant ($P\geq0.05$) during entire storage.

Escherichia coli (E. coli)

The *Escherichia coli (E.coli)* of both peanut varieties irradiated with different doses level and different initial moisture content and stored for three month period are reported in Table 3. It can be observed from data that *E.coli* decrease with increase in gamma irradiation dose and at and above gamma irradiation dose 5.0 kGy, *E.coli*in both the peanut kernels varieties reduced below 1 log (CFU/g).

During initial day of storage it was noticed that as gamma irradiation dose increased from 0.0 to 10.0 kGy, *E.coli* in GG-20 peanut kernels significantly decline from 2.23 to <1 log (CFU/g), 2.25 to <1 log (CFU/g) and 2.38 to <1 log (CFU/g) for the kernels having initial moisture content of 6, 8.5 and 11 % (w.b.), respectively. The maximum survival of *E.coli* was found in the treatment F1D1M3 (2.32 log (CFU/g)) followed by F1D1M2 (2.25 log (CFU/g)) and F1D1M1 (2.23 log (CFU/g)) after three month of storage.
It was noted, as gamma irradiation dose was increased from 0.0 to 10.0 kGy, *E.coli* in TG-37A peanut kernels significantly reduced from 1.91 to <1 log (CFU/g), 2.07 to <1 log (CFU/g) and 2.28 to <1 log (CFU/g) for the kernels having initial moisture content of 6, 8.5 and 11 % (w.b.), respectively. The end of three month storage, the maximum survival of *E.coli* was reported in non- irradiated treatment F2D1M3 (2.27 log (CFU/g)) followed by F2D1M2 (2.06 log (CFU/g)) and F2D1M1 (2.27 log (CFU/g)).

These results are in agreement with previous findings of Farkas (1988) for artificially contaminated coconut, Singh *et al.*, (1988) in spices (Turmeric, black pepper, chilli), Swailam and Abdullah (2002) for spices and herbs found same results.

The statistical interpretation of noted *E.coli*data of both peanut varieties was presented in the Table 3. It indicates that the individual effect of initial moisture content (M) and gamma irradiation dose levels (D) giving significant values ($P<0.05$) while its interaction effect (D X M) also found statistically significant at 5% level of significance for both peanut variety during entire storage periods.

Salmonella

The *Salmonella* in both peanut varieties irradiated with different gamma irradiation doses with different initial moisture content, packed in poly propylene bag (PP) and stored at room temperature for three month storage was not noticed in any of the samples during whole storage period.

Radiation-decimal reduction dose (D_{10}-value)

Microorganisms vary significantly in their resistance to gamma radiation and differences in radiation resistances from species to another and even among strains of the same species with different in condition. The microorganism's resistance against radiation is measured in terms of decimal reduction dose (D_{10}-value). D_{10}-value can be defined, as the radiation dose (kGy) essential for killing 90 % of the load of a microbe (Ingram and Roberts, 1980). Therefore, D_{10}-values of potentially microbial load (Total plate count (TPC), yeast and mold count (YMC) and *Escherichia Coli.*) in peanut kernels varieties namely GG-20 and TG-37A at three different (6.0, 8.5 and 11 %, w.b) initial moisture content were determined. D_{10}-value of a microbe can be determined from dose response curve of the microbe, which is obtained by plotting the number of surviving cells against radiation dose (kGy). The effect of increment doses of gamma irradiation on the Total plate count (TPC), Yeast and mold count (YMC) and *Escherichia Coli.* noted in Table 4.

From the radiation survival curves; it was found that the D_{10} value (calculated from linear regression and equation) of *E. coli* (it had the highest radiation resistant among other tested microorganism) was 4.40 kGy, 4.40 kGy and 3.84 kGy in TG-37A and 2.18 kGy, 2.19 kGy and 2.61 kGy in GG-20 peanut kernels at 6, 8.5 and 11 %, w.b moisture content respectively. The D_{10} value of Total plate count in GG-20 was noted 0.83 kGy, 0.97 kGy and 1.26 kGy, while in TG-37A peanut kernels it was found 1.09 kGy, 1.02 kGy and 1.09 kGy at 6, 8.5 and 11 %, w.b moisture content respectively. In case of GG-20 peanut kernels, D_{10} value of yeast and mold count was revealed 1.21 kGy, 1.06 kGy and 1.57 kGy whereas it observed 1.07 kGy, 0.96 kGy and 0.92 kGy in TG-37A kernels at 6, 8.5 and 11 %, w.b moisture content respectively. There were no steady patterns of D_{10} values change with respect to initial moisture were observed in both peanut varieties.

Knowledge of the D_{10}-value of different microorganisms from survival curves (dose-response curves) is very needful in calculating the lethal and sub-lethal dose of microorganisms. It is also useful in providing information about the relative resistance of particular microorganisms to ionizing radiation and calculating the exact radiation dose required for 5-log cycle's reduction of pathogenic bacteria and mycotoxin production fungi, which is very important for the practical application of ionizing radiation. By knowing the D_{10}-value of any microorganisms and its level in a product (food, feed, medicinal or pharmaceutical products) one can calculate the exact irradiation dose required to eliminate that microorganisms from that product.

Many researchers have reported the similar variations in radio sensitivity of different microbial species. Generally spore-forming bacteria are more resistant (Aziz and Moussa, 2004). Relatively similar result also reported by Hammad and El-Bazza (1988) and Thayer and Boyd (1993) in D_{10}-value of *Escherichia coli* isolated from dry foods. Youssef *et al.,* (1999) also observed complete inhibition of fungi by gamma irradiation doses from 4 to 6 kGy in different food and feed products. It can be said that irradiation dose of about 7.2 kGy (considering max D_{10} value 1.2 kGy) required for the acceptable 6 (six) log reductions of the microbial load. Al-Bachir*et al.,* (2004) reported D_{10} value of total count was about 1.4 kGy in licorice root products.

In conclusion the research data discovered from this research suggest that the gamma irradiation is proficient technology to control microbial load including total plate count, yeast and mold count, *E.coli* with respect to initial moisture of peanut kernels. The report also advocates that the gamma irradiation can control the microbial load of both the varieties of peanut kernels, just after irradiation and during storage. Gamma irradiation dose of 7.5 kGy is the sufficient dose for complete elimination of total plate count as well as yeast and mold; whereas the 5.0 kGy is sufficient for complete killing of *E. Coli.*in kernels of both peanut varieties.

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