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QUALITY IMPROVEMENT OF FRESH-CUT-FRUITS BY GAMMA RADIATION FOR IMMUNE-COMPROMISED PATIENTS

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ABSTRACT: Objective: Immune-compromised patients are vulnerable to microbes, which are usually safe and sound for healthy individuals. The aim of this study is to provide a quality fresh fruits such as apple, grape, guava, pear and plum for immune-compromised personnel sing different doses of gamma radiation. **Methods:** Irradiation impacts on microbial number, in treated fruits were assessed and compared to sanitary microbial safety criteria for immune-compromised patients as suggested by IAEA. Fresh-cut fruits were exposed to various doses of gamma radiation (0, 0.5, 1.0 and 1.5 kGy) and microbial load was analyzed. **Results:** Microbiological analysis showed that aerobic plate counts in case of guava, grape and pearwere4.53, 3.42 and 3.24 log CFU/g respectively which were eliminated at 1.0 kGy. Aerobic spores, except apple, were totally eliminated just at 0.5 kGy. Similarly, at 0.5 kGy, total Coli form in plum, pear, guava and apple, which were 4.43, 2.25, 2.0 and 1.04 CFU/g respectively, as well as pathogenic Listeria spp. 3.3 log CFU/gin guava were also eradicated. *Staphylococcus aureus* were detected only in fresh-cut guava in the level of 3.77 log CFU/g that was eliminated at 1.0 kGy. Yeast and mold found in processed plum and pear were about 4.0 and 1.47 log CFU/g respectively, were eliminated at 0.5 kGy. **Conclusion:** We found that radiation dose of 1.0 kGy fulfilled the microbial safety criteria for immune-compromised patients.

INTRODUCTION: Food borne disease is prevalent among world population, and certain groups are particularly at high risk of contracting a food borne illness, namely the young children, older adults, pregnant women, immune compromised persons and travellers [1].

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A person who has one or more defects in the normal defense mechanisms that protect host from infectious agents predisposing the individual to an increased risk of severe life threatening infections can be defined as immune-compromised patients. These defects may be malignancy or acquired immunodeficiency syndrome [2].

Noticeably, immunocompromised patients are more vulnerable to infections including infection with organisms that do not normally cause disease[3]. The diet for immunocompromised patients should be free of microorganisms. International Atomic

Energy Agency[4] suggested sanitary microbiological levels for foods intended for immunocompromised people and other potential target groups in which Aerobic Plate Counts (< 500CFU/g), Aerobic spore count (< 10 CFU/g), Yeast and Mold (< 10 CFU/g), Total Coliforms (<10CFU/g), *Staphylococcus aureus*(<1 CFU/g), *Listeria spp*. (not be detected in 25g) should not excel the limit. Different physical and chemical techniques such as: disinfection [5]; edible coatings [6]; C_2H_4 absorbents [7]; natural plant products [8]; non-thermal physical treatments [9]; heat treatments or heat shock[10] microbial competition[11]; pulsed-microwave irradiation[12]; gamma irradiation[13] have been studied especially for fresh-cuts.

Predominantly, irradiation has gained attention as an effective tool for assuring food safety due to cost effectiveness and protection of heat sensitive food compounds as well as microbial degradation [14]. World Health Organization judges ionizing radiation an important process toward ensuring food safety[15]. It can be a useful control measure in the production of several types of raw or minimally processed foods such as poultry, meat and meat products, fish, seafood, and fruits and vegetables [16]. Several studies showed the effectiveness of low-dose irradiation for microbiological safety of fresh-fruits such as mango (*Mangifera indica*), watermelon (*Citrullus vulgaris*) and pineapple (*Ananasco mosus*)[17]; pears (*Pyruscom munis*) and fresh cut apples (*Malusdomestica*)[18]; watermelon (*Citrulluslanatus)*[19]; pineapple (*Ananascomosus)*[20]; pineapple (*Ananascomosus),* jackfruit (*Artocarpus* heterophyllus), pomelo (*Citrus maxima)* and mixed fruits pineapple and guava (*Psidiumguajava*)[21]; apples *(Pyrusmalus)* and melon (*Cucumismelo)* [22].

Our present study emphasizes on the determination and elimination of total aerobic plate count, anaerobic plate count, aerobic spore count, total coliform count, total yeast and mold count, *Listeria spp*. and *Staphylococcus aureus* from fresh apple, grape, guava, plum, pear by using low-dose cobalt-60 gamma irradiation and our findings were compared with sanitary microbiological level which was suggested by International Atomic Energy Agency [4]. This study leads us a radiation dose of 1.0kGy that fulfils the microbial safety criteria for all of our studied fruits.

MATERIAL AND METHODS:

Preparation of fruit samples:

Fresh fruits free of any type of injury or deterioration like apple (*Malusdomestica*), grape (*Vitisvinifera*), guava (*Psidiumguajava*), pear (*Pyruspyrifolia*) and plum (*Prunusdomestica*) were purchased from the local market of Dhaka city, Bangladesh. Collected fruits were washed with running tap water, then cut into uniform slices with sterile knife on a chopping board and packed into sterilized (15 kGy irradiation) LDPE (200 gauge) plastic pouches and finally sealed with a electric sealer. All of the fruit processing was done inside the laminar airflow cabinet.

Irradiation treatment for decontamination of fruits:

For microbiological analysis, there were four packets for each type of fruits that contained 25 g sample and marked by 0, 0.5, 1.0 and 1.5 kGy. Packed samples were then transferred to Gamma Source Unit (GSU) of Institute of food and Radiation Biology, Savar, Dhaka, Bangladesh. As the radiation source, 50kCi Cobalt-60 (1850 TBq) Gamma Irradiator was used.

Detection of microbes from fruits:

Microbiological analysis was done from the day of irradiation. 10g of sample was mixed with 100 ml sterile saline water and homogenized by stomacher and filtered through a sterile filter paper to a conical flask to prepare the stock sample (10%*)*.Total aerobic plate count was done by using LB agar plate (Scharlau Chemie S.A., Spain) and total anaerobic plate count was determined by decimal dilution technique followed by pour plating. Each assay in this experiment was replicated three times[23].

For total anaerobic plate count, 0.1 ml of the sample from each of the neat was taken and dilution was done in petridish and then sterilized Thioglycollate media (Scharlau Chemie S.A., Spain) was poured. A candle was lightened inside the jar and then closed. The jar containing the plates was filled with nitrogen gas and thus maintaining anaerobic condition, incubated at 37°C for 24-48 hours. Total yeast and mold count were determined using Potato Dextrose Agar (Scharlau Chemie S.A., Spain) with incubation at 30°C, total coliform count using Mac Conkey Agar (Scharlau Chemie S.A., Spain) with incubation at 37°C, total *Listeria* count using *Listeria* Selective Agar Base (Oxford formulation, Oxoid LTD, England) with incubation at 37°C, *Staphylococcus aureus* count using Mannitol Salt Agar (Scharlau Chemie S.A., Spain) with incubation at 37°C for 24h and total aerobic spore count by heating the sample solution at 80°C for 10 minutes followed by the method as for total aerobic plate count[24].

RESULTS AND DISCUSSION

Food borne illness caused by microorganisms is a vital public health problem. Centers for Disease Control and Prevention[25] estimated that each year roughly 1 in 6 Americans gets sick, 128,000 are hospitalized, and 3,000 die of food borne diseases. This pattern is also severe in developing countries. So, proper method for eliumination of pathogenic microbes from ready-to-eat fruits is crucial. It has been found that irradiation reduces the number of disease-causing bacteria such as *Listeria monocytogene* [26], *Escherichia coli* O157:H7 [27], *Salmonella* [28], *Clostridium botulinum*[29], *Vibrio parahaemolyticus* [30] *etc*. in various food commodities which suggests that food should be irradiated in its final packaging stage.

We reported that irradiation treatment of fresh-cut-fruits was significant to eliminate microbial load. It has also been implicated in the elimination of pathogenic *Listeria spp.*, *S. aureus*, and total coliforms at a minimum dose as well. Survival and elimination curves of microbes in different fruit samples were shown in the Figures (**Fig 1-7**).

TABLE 1: MICROBIAL COUNT OF DIFFERENT SAMPLES AND THEIR ELIMINATION THROUGH DIFFERENT DOSES OF RADIATION

Sample	Microbiological analysis (Log CFU/g)							Dose (kGy)
	APC	AnPC	ASC	TYMC	TCC	*Listeria spp.*	*Staphylococcus aureus*	
Apple	2.88	3.68	2.72	0	1.04	0	0	0
	0	1.23	1.02	0	0	0	0	0.5
	0	0	0	0	0	0	0	1.0
	0	0	0	0	0	0	0	1.5
Grape	3.42	1.54	1.54	0	0	0	0	0
	2.64	0	0	0	0	0	0	0.5
	0	0	0	0	0	0	0	1.0
	0	0	0	0	0	0	0	1.5
Guava	4.53	4.23	1.87	0	2.00	3.3	3.77	0
	2.39	2.77	0	0	0	0	0	0.5
	0	0	0	0	0	0	0	1.0
	0	0	0	0	0	0	0	1.5
Pear	3.24	2.89	1.47	1.47	2.25	0	0	0
	1.47	0	0	0	0	0	0	0.5
	0	0	0	0	0	0	0	1.0
	0	0	0	0	0	0	0	1.5
Plum	3.47	3.77	3.65	4.00	4.43	0	0	0
	0	3.17	0	0	0	0	0	0.5
	0	0	0	0	0	0	0	1.0
	0	0	0	0	0	0	0	1.5

(0) means nil, Log CFU/g= Logarithm Colony Forming Unit/gram, kGy= Kilo Gray, TAPC = Total Aerobic Plate Count, AnPC = Anaerobic Plate Count, ASC= Aerobic Spore Count, TYMC = Total Yeast and Mold Count, TCC = Total Coliform Count, spp. = species

Aerobic bacterial cells can survive in the presence of free radicals (by product of oxygen metabolism) using superoxide dismutase in their elaborate system of defenses[31]. So, it can be assumed that elimination of aerobic microbes could take high dose of radiation as radiation also produce free radicals in cells. Present research revealed that TAPC decreased maximum 4.53log CFU/g in fresh-cut guava at 1.0 kGy irradiation (**Fig. 1**). Farkas *et al.*, reported the similar result in bell peppers that 1 kGy dose reduce 4-log TAPC[32]and also in peeled ready-to-use carrots[33]. Our findings depicted that approximately 3.47 log CFU/g TAPC decreased in minimally processed plum only at 0.5

kGy irradiation (**Table 1, Fig. 1**). Lacroix *et al.*[34] mentioned similar result for uncoated carrots; a dose of 0.5 and 1 kGy reduced aerobic plate count by 3.5 and 4.0 log CFU/g respectively.

Life threatening infections caused by anaerobic microbes in human organs such as in intra-abdomen, Lung, brain, pelvic, skin and soft tissues, oral and dental[31].Anaerobic microbes are found in plum and guava where3.77 log CFU/g reduced to 3.17 log CFU/g and 4.23 log CFU/g to 2.77 log CFU/g at 0.5 kGy irradiation respectively (**Table 1, Fig.2**). Remarkably, maximum 4.23 log CFU/g anaerobic microbes were decreased at 1 kGy of irradiation in guava (**Table 1**). Anaerobic and facultative anaerobic bacteria in beef patties were killed by 1.0 kGy irradiation and in some cases survived [35].

Various aerobic spore-forming microbes that cause spoilage were identified in minimally processed dairy milk[36]. Spore forming microbes are very resistant to heat treatment. Aerobic spores were most prevalent in plum, which were contaminated at a level of 3.65 log.

CFU/g and eliminated at 0.5 kGy (**Table 1, Fig. 3**). In contrast, for unknown reason, aerobic spore at a level of 2.72 log CFU/g in apple sample required 1.0 kGy for total elimination.

Barata *et al.* found that when irradiation dose was increased from 1.05 to 3.99 kGy, mesophilic aerobic spore formers in Brazilian Comun Cocoa beans considerably decreased[37].

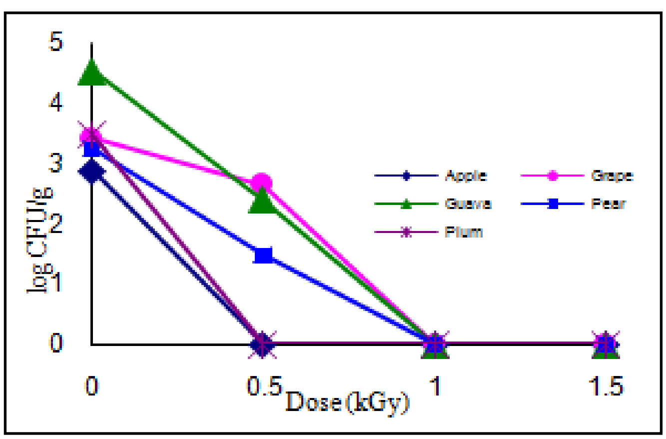

FIG. 1: EFFECT OF IRRADIATION ON TOTAL AEROBIC PLATE COUNT IN APPLE, GRAPE, GUAVA, PEAR, PLUM

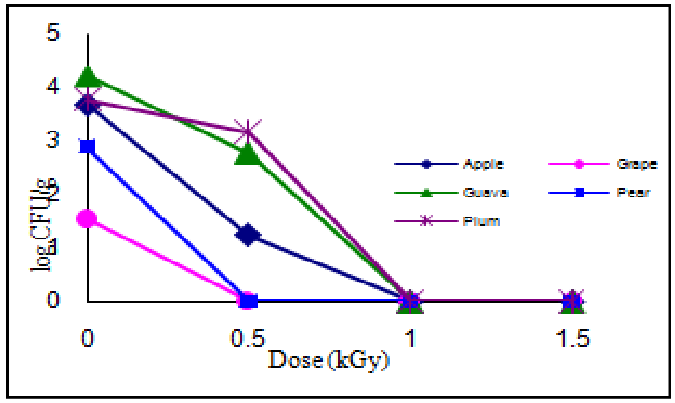

FIG. 2: EFFECT OF IRRADIATION ON ANAEROBIC PLATE COUNT IN APPLE, GRAPE, GUAVA, PEAR, PLUM

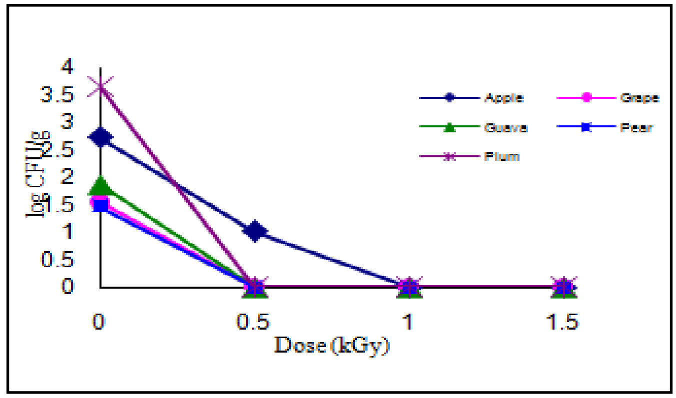

FIG. 3: EFFECT OF IRRADIATION ON AEROBIC SPORE COUNT IN APPLE, GRAPE, GUAVA, PEAR & PLUM

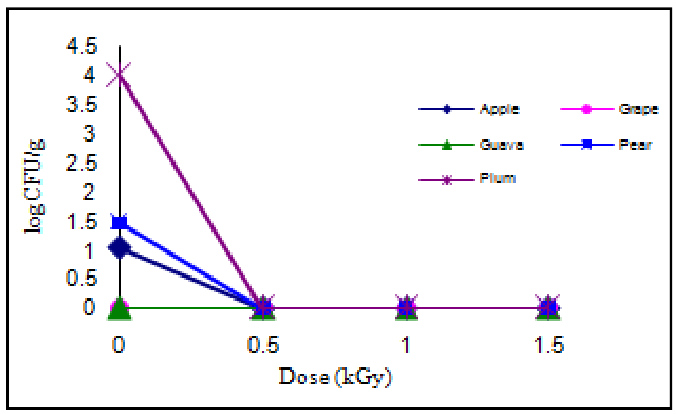

FIG. 4: EFFECT OF IRRADIATION ON TOTAL YEAST AND MOULD COUNT IN APPLE, GRAPE, GUAVA, PEAR, PLUM

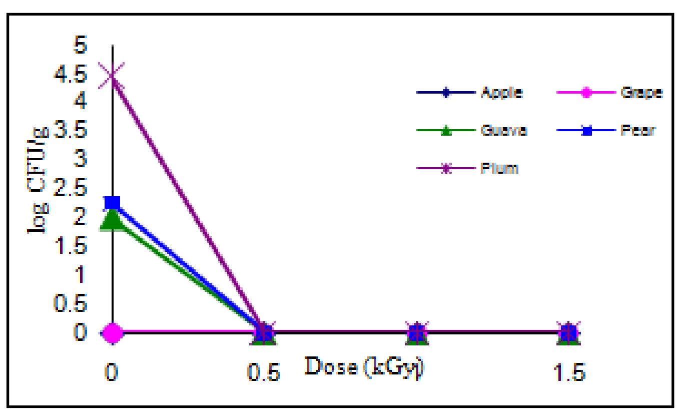

FIG 5: EFFECT OF IRRADIATION ON TOTAL COLIFORM COUNT IN APPLE, GRAPE, GUAVA, PEAR & PLUM

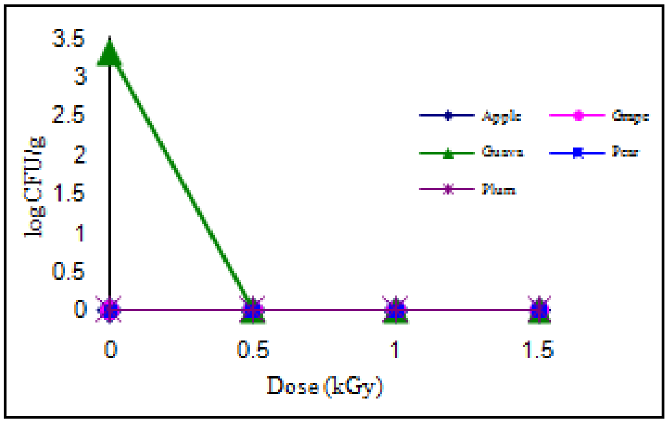

FIG 6: EFFECT OF IRRADIATION ON TOTAL *LISTERIA SPP.* COUNT IN APPLE, GRAPE, GUAVA, PEAR, PLUM

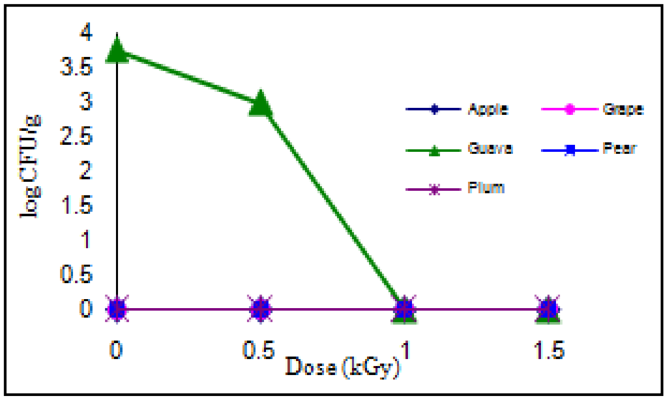

FIG 7: EFFECT OF IRRADIATION ON *STAPHYLOCOCCUS AUREUS* COUNT IN APPLE, GRAPE, GUAVA, PEAR, PLUM

CFU/g and eliminated at 0.5 kGy (**Table 1, Fig. 3**). In contrast, for unknown reason, aerobic spore at a level of 2.72 log CFU/g in apple sample required 1.0 kGy for total elimination. Barata *et al.* found that when irradiation dose was increased from 1.05 to 3.99 kGy, mesophilic aerobic spore formers in Brazilian Comun Cocoa beans considerably decreased[37].

Fungal infections are increasingly growing at a rapid rate among children due to immune incapacity[38]. National Institute of Allergy and Infectious Diseases[39]mentioned that various fungi are capable of causing infections in our lungs, skin and nails. Our findings revealed that Yeast and mold were found minimum and most sensitive to irradiation than others microbes present in the experimented fruits. Only plum and pear were contaminated at a level of 4.0 log CFU/g and 1.47 log CFU/g of TYMC respectively and eliminated at 0.5 kGy (**Table1, Fig.4**). Rodriguez *et al.* determined that irradiation at 0.59 kGy was effective in reducing yeast and mold counts by 3-4 log CFU/g [40]. Radiation dose 2 kGy was effective in lowering the total bacterial count and fungal colony in tomato sample and kept in safe limit even after 14 days of storage[22].

Coli form bacteria are common in water sample causing intestinal disturbances in human such as cramps, diarrhea, and in very rare cases hemolytic uremic syndrome (Vermont department of health). The spread of coli form in food is a severe threat for public health. Our studies depicted that, total coli form counts in plum were 4.43 log CFU/g (**Table 1**) but varied in others sample like pear 2.25 log CFU/g, guava 2 log CFU/g, apple 1.04 log CFU/g respectively. All counts were eliminated just at 0.5 kGy irradiation (**Fig.5**). Harewood *et al.*, observed that 1.02 to 1.75 kGy is required to kill 90% of the total number of coli forms in hard-shelled clams [41]. Bibi *et al.* unveiled that control cucumber samples had coliform count of 8.0 x 10^3 (TCC/g) after 14 days of storage while the 2.0 kGy treated samples had no coliform after the same storage period and 1.0 log CFU/g TCC reduces at 0.5 kGy[22].

Listeria monocytogenes has the potential to be present in all raw foods and causes listeriosis[42]. Listeriosis is the third leading cause of death among major pathogens transmitted usually by food [43]. People of older ages and persons with immunocompromising conditions are at higher risk for *Listeria* bacteremia and meningitis[44]. In our study, *Listeria spp.* contamination was found only in case of guava at a level of 3.3 log CFU/g which was eradicated at 0.5 kGy (**Table 1, Fig.6**). Hammad *et al.*, found that radiation doses of about 2.6–3.3 kGy inactivate 5 log cycles of *Listeria monocytogenes* [18]. Low dose (0.8 kGy) radiation and heat treatment is also effective in elimination of *L. monocytogenes* from cook-chill (beef roast) products [45]. Miniter and Foley found that 0.56 kGy required to eliminate 2.91, 2.62, and 2.66 log CFU/g for three different *Listeria monocytogenes* strains from lettuce [26].

Centers for Disease Control and Prevention[46] described that Staphylococcus is the cruel risk for human health, contains different types of toxins which are fast acting and responsible for nausea, vomiting, stomach cramps, and diarrhea. This food poisoning, due to poor handling specially contact of contaminated water, found in sliced meat, puddings, some pastries and sandwiches. In our present research, *Staphylococcus aureus* detected at a level of 3.77 log CFU/g in guava and remained 3.0 log CFU/g at 0.5 kGy dose but totally eliminated at 1.0 kGy (**Fig.7**).

The 5-log reduction of *S. aureus* in fresh produce was achieved by about 2.1 – 2.7 kGy[18]. Bandekar *et al.* observed that samples treated with 1 kGy or 2 kGy doses were free from *Salmonella*, *E. coli*, coagulase positive *S. aureus* and *L. monocytogenes* on 0 day and up to 12 days of storage at 4° and 10ºC[20].

FDA approved the use of irradiation up to 4.0 kGy on fresh lettuce and fresh spinach to improve food safety and extend shelf life [47]. Many studies have demonstrated that most fresh-cut fruits and vegetables irradiated at a dose of 1 kGy did not exhibit any significant change in appearance, texture, flavor, or nutrient quality [48, 49, 50, 51, 52, 53, 54]. Present research is consistence with the previous research for safety of fresh fruits radiation acceptability at 1kGy. Niemira and Fan [55] concluded the studies conducted in the last decade demonstrated that most fresh-cut fruits and vegetables could tolerate up to 1kGy radiation without deleterious sensory impact.

CONCLUSION: Our findings showed that radiation dose of 1.0 kGy fulfilled the microbial safety criteria for immune-compromise patients which suggests that fresh-cut packed fruits irradiated at 1.0 kGy is safe and it would be considered as safe for the immune-compromised patients.

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