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Irradiation to Ensure Safety and Quality of Fruit Salads Consumed in Bangladesh

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Abstract Food was, is and perhaps will be the greatest concern for humankind due to outbreak of foodborne diseases and for sake of good health. Food suppliers and/or industries follow various techniques for ensuring their food safety. One way to overcome this situation is the use of ionizing radiation applied to food for assuring microbial as well as nutritional safety concern. Here apple, grape, guava, pear and plum sample were treated with 0.5, 1 and 1.5 kGy radiation from a ^{60}Co gamma irradiator. Changes of the native microflora and some specific nutritional and physico-chemical properties of irradiated fruit were determined. It was observed that 0.5 kGy irradiation dose reduce a significant amount of microbial load compared to control and 1 kGy irradiation reduce microbial load under the sanitary level recommended by International Atomic Energy Agency (IAEA).Samples treated with 0.5kGy were healthier acceptance than samples treated by 1kGy and 1.5kGy during the six successive days of storage. Moisture content more than 90%were found in apple, plum and pear and statistically no significant changes ($p<0.05$) were observed in irradiated samples compared to the non-irradiated samples. At 1.5 kGy carotenoid content was increased 45.7926% in apple. Statistically significant increase of total carotenoid was observed in plum at 0.5, 1 and 1.5 kGy but the decrease in apple, pear and guava at dose 1 and 1.5 kGy. Thus, insignificant variation of ascorbic acid content was observed at radiation dose 1±0.5kGyin fresh cut fruit produce.

Keywords: *radiation, moisture, carotenoid, ascorbic acid, IAEA, microbes*

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1. Introduction

Fruit and vegetable play a significant role in human nutrition and its consumption on a daily basis is highly recommended because of associated health benefit [1,2]. So, safety and quality of foods is an immense concern in the people of the world due to overcoming of many foods borne illness. It can become contaminated with pathogenic microorganisms and chemicals while growing in fields or orchard, or during harvesting, postharvest handling, processing, and distribution [3] which increase the risk of diseases [4]. World health organization [5] reported that everyone is susceptible to foodborne illness, particularly of young children, infants, the elderly, pregnant women, immunocompromised patients and travelers, astronauts, post-operative patients [6]. Center for Diseases Control and Prevention estimated that each year roughly 1 in 6 Americans get sick, 128,000 are hospitalized and 3000 dies to due to foodborn diseases [7]. Nevertheless, consumption of raw and minimally processed foods without ensuring its safety and quality is the common feature in many residences of the world. In addition, immunocompromised people are at increased risk of complications such as septicemia, arthritis, meningitis, pneumonia etc [8]. Radiation has been proving effective in controlling the risk of pathogen and/or chemicals to protect the consumers and benefits the producer owing to self-life extension [9,10]. Nutritional stability at this type of treatment was considered to be well established [11,12] and especially important for the immunocompromised people [13,14]. In Bangladesh, most fruits and vegetables are sold from local markets to a wholesaler who transports the produce to city market i.e. Dhaka [15]. In Dhaka, most of the foodstuffs manufactured or processed their foods are unsafe for consumption or adulterated in varying degrees. This problem persists at every level of food from preparation to consumption [16] which is a great issue in the food sector in Bangladesh. The aim of this study is to the evaluation of safety and quality of irradiated food salads to consumers and/or suppliers in Bangladesh.

2. Materials and Methods

2.1. Collection and Preparation of Sample

The study was conducted on five commonly consumed fruits in Bangladesh: Apple (*Malus domestica*), Grape (*Vitis vinifera*), Pear (*Pyrus x bretschneideri*), Plum (*Ziziphus mauritiana*), Guava (*Psidium guajava*) were collected from the new market area, Agargaon, Dhaka, Bangladesh. The samples were washed with running tap water, as usually practiced in domestic kitchens. After washing, the sample were peeled with a sterile peeler then uniformly sliced with a sterile knife on a clean sterile chopping board and cut into uniform slices.

2.2. Irradiation Treatment

The samples were packed into sterilized (with 25 kGy radiation dose by ^{60}Co gamma irradiator) food grade transparent low-density polyethylene (LDPE) and then sealed with a heat sealer (Impulse Sealer, TEW Electronic Heating Equipment CO. Ltd., Taiwan). Four packed of samples each containing 50g were irradiated with three different irradiation doses 0.5, 1.0 and 1.5 kGy and four sets (day-0, day-2, day-4 and day-6) of samples were irradiated at the same doses for evaluation of microbiological, biochemical and organoleptic analysis, respectively. The samples were reserved at 4±1°C in the refrigerator for further evaluation. A non-irradiated sample of each type of fruit was kept as a control sample. All the procedures were done inside laminar airflow cabinet. Doses were applied to the samples at room temperature from the Co-60 gamma irradiator source (Located at Atomic Energy Research Establishment, Institute of Food and Radiation Biology, Dhaka, Bangladesh) by calibrating with dose and time basis on central distance from source to sample where these were placed.

2.3. Microbiological Analysis

Determination of microbial population in the sample decimal dilution technique followed by pour plating method was used [17] on the day of irradiation. Stock solution were prepared by taking 5 g (25 g for *Listeria* spp) of homogenized and filtered sample in 50 ml of sterile saline (0.9% NaCl wate) by a autoclaved mortar and pestle and filtered through a sterile muslin cloth to a conical flask with 50 ml sterilized saline (0.9% NaCl) water to prepare the stock sample under a laminar air flow cabinet. For total aerobic spore count, these suspensions were heated at 80°C for 10 min in a water bath. 1 ml sample from conical flask was taken in a test tube containing 9 ml of previously sterilized saline water. Thus, 10^{-1} dilution was got. This procedure was repeated where further dilution was required. With the help of micropipette, 1 ml of the sample from the test tube was poured into Petri dishes then sterilized specific media was poured into Petri dishes and shaken horizontally to spread out the sample uniformly over the media. After solidification of the media, the Petri dishes were covered with lids. Then, the Petri dishes were placed in upturned position in incubator at 37°C (30°C for yeasts and molds) for 24-48 hr. The analyses were the enumeration of total aerobic flora, total anaerobic bacteria, total aerobic spore, total yeast and mold, total coliform, *Listeria* spp. and *Staphylococcus aureus*. For microbiological purposes Nutrient Agar, Thioglycollate media, Potato Dextrose Agar, MacConkey Agar, Mannitol Salt Agar were purchased from Scharlau Chemie S.A. (Spain). *Listeria* Selective Agar Base (Oxford formulation) was purchased from Oxoid LTD (England). For anaerobic bacteria, Thioglycollate media was used. After spreading, plates were kept in an anaerobic jar. The lid of the jar was closed. After that, a vacuum pump was attached to one port of the jar, and a nitrogen source was attached to another port of the jar. Then the air inside the jar was sucked out with a vacuum pump and the jar was filled with nitrogen gas to maintain anaerobic condition inside the jar. Then the jar was put inside the incubator.

2.4. Biochemical and Nutritional Analysis

2.4.1. Determination of Ascorbic Acid and Total Carotenoid

The estimation of ascorbic acid content was carried out by the titration result of the sample extract with 2, 6-Dichlorophenol-Indophenol dye (Oxford formulation, Oxoid LTD, England) which is reduced by ascorbic acid to colorless form in pH range 1-3.5 [18,19]. For total carotenoid estimation 4 g of homogenized sample was transferred to a mortar and mixed 0.3 g of $MgCO_3$ and 25 ml of cold acetone (Refrigerated about 2 Hours) to prepare acetone extract by filtration [20]. Twenty (20 ml) of petroleum ether was taken in separating funnel and 15 ml of acetone extract were added to allowed 15 min. After that, 150 ml of distilled water was added and allowed to separate two phase. After discarding aqueous phase the ether phase was collected in a 25 ml of volumetric flask containing 7.5 g of anhydrous sodium sulfate to remove residual water. The flask was filled up to volume with petroleum ether and total carotenoid was determined from the molar absorptivity β-carotene E1% = 2590 at λmax450 nm and lycopene E1% = 3450 at λmax 472 nm derived from the standard plots [21,22].

2.4.2. Determination of Moisture Content

The moisture content was determined by drying the sample at the same elevated temperature and reporting the loss in weight as moisture [23].

2.5. Organoleptic Analysis

The organoleptic analysis was carried out followed by the method described by Miyauchi *et al.*, 1964 [24]. Following nine points of the hedonic scale was used for sensory evaluation by twenty trained judge's age between 20 – 39.

9= Like extremely
8= Like very much
7= Like
6= Like slightly
5= Neither like nor dislike
4= Dislike slightly
3= Dislike
2= Dislike very much
1= Dislike extremely

Average sensory score 5 (neither like nor dislike) is usually acceptable in an organoleptic evaluation. So, the acceptability threshold we considered was around 7, which means "like" in hedonic scale. Sensory quality attributes including colour, flavour, taste and texture of samples were evaluated immediately after irradiation and during refrigeration (4 ± 1°C) storage.

2.6. Statistical Analysis

Results were expressed as mean ± SD (Standard deviation). One way ANOVA was performed for data analysis. ANOVA was followed by Fisher's Least Square Differences (LSD) for post hoc comparisons. The statistical program used was Microsoft Office Excel 2010 and its add-in DSAASTAT (Andera Onofri, Dipartimento di scienze Agrarie Ambientali, Borgo xx Giugno, 7406121 Perugia, Italy). $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Microbiological Analysis

The extent of contamination by microorganisms in fruit salads (apple, grape, guava, pear, and plum) and the effect of different doses of irradiation on it were determined. Microbial contaminations for both control and irradiated salads are shown in Table 1. The aerobic bacterial cells bacterial cell can survive in the presence of free radicals [25]. So, elimination of aerobic microbe could take a high dose of irradiation because of it produce free radical in cell. The Initial total aerobic plate counts was 1.5×10^4, 1.1×10^4, 3×10^4, 8×10^3, and 4.8×10^4 in apple, grape, plum, pear, and guava respectively. Total anaerobic plate counts decreased average 4.34 log cfu/g in guava, plum, grape, apple, and 3.90 log cfu/g in pear at 1kGy. Reduction around 3log cfu/g was found in apple, plum, and guava but not in grape and pear at irradiation dose 0.5 kGy. Similar result reported in carrots and peppers [26,27,28,29]. Total anaerobic plate counts were (TAPC) found in all samples where 4.54 log cfu/g reduced to 3.07 log cfu/g and 4.67 log cfu/g reduce to 2.72 log cfu/g in apple and plum at 0.5 kGy irradiation respectively. Aerobic spores were most prevalent in plum and pear where contaminated at the level of 3 log cfu/g and 2.11 log cfu/g respectively and eliminated at 0.5 kGy. Yeasts and molds were grown at a rapid rate and created infections in humans especially in children due to immune incapacity [30]. In our study, yeasts and molds were found minimal and very sensitive to irradiation than other microorganisms present in experimental fruit. Yeasts and molds were found in plum, pear, and guave at the level of 2.90 log cfu/g, 2.69 log cfu/g, and 1.47 log cfu/g and eliminated at 0.5 kGy irradiation, respectively. Rodriguez reported that total yeast and mold counts (YMC) reduce to 3-4 log cfu/g at 0.5 kGy of irradiation [31] and 2 kGy was effective in tomato sample [32] even after 14 days of storage. Total coliform count in guava was 2.30 log cfu/g but varies in other samples like 1.77 log cfu/g in pear, 1.30 log cfu/g in plum, 1.47 log cfu/g in grape, 2 log cfu/g in apple, and all counts were eliminated at 0.5 kGy of irradiation. No *Listeria* spp. contamination was found in our sample although it is potential to be present in all raw foods and cause listeriosis [33]. In the present study, *Staphylococcus aureus* was detected at the level of 1.95 log cfu/25g and completely eliminated at 0.5kGy of irradiation. The 5 log reduction of *S. aureus* in fresh processed fruits and vegetables was achieved by about 2.1 – 2.7 kGy [34].

Interestingly, our study revealed that 0.5 kGy irradiation dose reduces significant amount of microbial load in prepared salad samples compared to control and 1 kGy irradiation dose reduces microbial load under the sanitary level recommended by International Atomic Energy Agency (IAEA, 2010) and International Commission on Microbiological Specifications. So we can say that 1 kGy irradiation dose is used for the preparation of these types of fruit salad. Niemira and Sommers [35] found that irradiation dose 0.2-0.8 kGy is sufficient to achieve a 1-log reduction for surface contaminating bacterial pathogens and often 1-3 kGy of irradiation required for viruses and fungi to achieve the same level of reduction. Edward Groth [36] a scientist reported in his study that doses around 1 kGy are typical for fresh food produce, although the different doses of irradiation uses unquestionable for processing many fruit and vegetable. For food processing, there is no specific dose of irradiation approved by US Food and Drug Administration (FDA) yet but specific radiation uses must be permitted by the FDA as safe and effective before commercially applied.

Table 1. Microbial counts of different samples at different radiation doses

Fruit Salad	Dose (kGy)	Microbiological Analysis (cfu/g)						
		TAPC	AnPC	ASC	YMC	TCC	*Listeria* spp. (in 25gm)	*S. aureus* (in 25gm)
Apple	0	1.5×10^4	3.5×10^4	0.5×10^3	-	0.1×10^3	-	-
	0.5	0.5×10^2	0.3×10^2	-	-	-	-	-
	1	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-
Grape	0	1.1×10^4	2×10^3	-	-	0.3×10^2	-	-
	0.5	0.3×10^2	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-
Plum	0	3×10^4	4.7×10^4	10^3	0.8×10^3	0.2×10^2	-	-
	0.5	0.6×10^2	0.7×10^2	-	-	-	-	-
	1	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-
Pear	0	8×10^3	2.3×10^2	1.3×10^2	0.5×10^3	0.6×10^2	-	-
	0.5	4×10^2	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-
Guava	0	4.8×10^4	1×10^3	0.3×10^2	0.3×10^2	2×10^2	-	0.9×10^2
	0.5	0.2×10^2	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-

Data represents (CFU) Colony Forming Unit per gram of irradiated samples. [TAPC= Total aerobic plate count, AnPC= Total anaerobic plate count, ASC= Aerobic spore count, YMC= Yeast and Mould count, TCC= Total coliform count].

3.2. Effect of Irradiation on Moisture Content

Moisture is essential to life itself, playing the crucial rule in the physical and chemical functions of our bodies, the food we eat and materials surround us. Determination of moisture is often the first interest of many foodstuffs as excess moisture can promote microbial growth, which quickly depreciates the value of food. In our study, we found that samples had the moisture content of more than 90%, favorable of microbial growth and stable of other chemicals presents in fruit salads. No statistically significant changes ($p<0.05$) were observed in irradiated samples compared to the non-irradiated samples (Table 2).

Table 2. Effect of irradiation treatment on moisture contents in fruit salad

Radiation Dose(kGy)	Samples (g/100g of fresh weight of edible fruit salads)				
	Apple	Grape	Plum	Pear	Guava
0	92.52±0.297 [a]	87.28±0.311 [a]	92.449±0.788 [a]	89.222±0.556[a]	80.585±1.027[a]
0.5	93.027±0.312[a]	86.465±2.32 [a]	92.459±0.335 [a]	90.193±0.117[a]	77.083±2.315[a]
1	92.29±0.891[ab]	87.085±1.70 [a]	93.368±0.24 [a]	90.835±0.355[a]	78.607±3.925[a]
1.5	90.940±0.397 [b]	86.24±0.070 [a]	93.423±0.381 [a]	90.259±1.149[a]	74.249±4.92[a]

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$.

3.3. Effect of Irradiation On Ascorbic Acid and Total Carotenoid Contents

The effect of irradiation on the ascorbic acid content of normal packed apple, grape, plum, pear, and guava were shown on Table 3. In our study, it was found that ascorbic acid content was increased 14.99-32.53% in apple, 9.34-10.67% in grape except for dose 0.5 kGy, 8.944-30.54% in guava. The decrease of ascorbic acid content was found in plum and pear were 12.659- 35.16% and 14.78-42.96%, respectively.

Table 3. Effect of irradiation on ascorbic acid and total carotenoid contents in fruit salads

Radiation dose(kGy)	Salad samples				
	Apple	Grape	Plum	Pear	Guava
			Ascorbic Acid Content (mg/100g)		
0	8.284±0.0 [a]	13.861±0.0[a]	53.416±1.842 [a]	16.763±1.824[a]	475.653±1.8 [d]
0.5	9.703±1.960 [a]	13.670±0.0 [a]	39.519±1.967 [c]	11.725±1.82[a]	595.563±1.9 [b]
1	10.979±0.0 [a]	15.340±1.972[a]	44.268±1.89 [b]	11.768±1.846[a]	684.233±3.82[a]
1.5	9.683±1.956 [a]	15.156±1.949[a]	37.414±1.91 [b]	14.604±1.878[a]	522.372±2.48 [c]
			Total Carotenoid (µg/g)		
0	0.0277±0.003 [a]	0.0361±0.010[a]	0.0392±0.04 [b]	0.0918±0.000[a]	0.075±0.0014[a]
0.5	0.0245±0.006 [a]	0.0258±0.00 [a]	0.1396±0.00 [a]	0.0885±0.000[a]	0.0139±0.00[a]
1	0.0250±0.002 [a]	0.0287±0.000[a]	0.1393±0.001 [a]	0.0504±0.00 [b]	0.0137±0.000[a]
1.5	0.0511±0.019 [a]	0.0293±0.00 [a]	0.1109±0.000 [a]	0.0866±0.00[a]	0.0110±0.001[a]

[Results are expressed as mean ± SD (Standard deviation), n=3eplicates of each sample for each radiation dose. Values in the same row with different superscripts are significantly different at $P<0.05$ One-way ANOVA followed by Fisher's LSD for post hoc comparisons].

No statistically significant change in ascorbic acid content was observed in apple, grape, and pear due to irradiation. Total carotinoid content was decreased by 13.06-20.43% in apple except dose 1.5 kGy, from 23.20-39.92% in grape, from 6.004-82.14% in pear and from 15.894-25.89% in guava due to irradiation. In plum carotenoid content was increased from 0.07-71.91%. At 1.5 kGy carotenoid content was increased 45.79% in apple. Statistically significant increase of total carotenoid was observed in plum at 0.5, 1 and 1.5 kGy but decrease in apple, pear and guava at dose 1 and 1.5 kGy. A significant increase of total carotenoid was observed in pear and guava but decrease in pear at irradiation dose 0.5 kGy. In a previous study, it was shown that irradiation dose ≤1 kGy can reduce ascorbic acid in some vegetables but insignificant variation observed in fresh produce [37]. Irradiation can cause a partial conversion of ascorbic acid to dehydroascorbic acid which could account for the losses of ascorbic acid observed in another study [38,39,40,41] which exhibit biological activity and are readily interconvertible [42,43,44].

3.4. Effect of Irradiation on Sensory Quality

Historically, the high radiation doses used in attempts to produce a sterile or shelf-stable fruit or vegetable commodity have resulted in unpalatable products. Irradiation may induce the loss of firmness (softening) in some fruits [45,46]. Analyses of irradiated fruit salad during the six consecutive days of storage at 4°C are shown in Figure 1. Data represents overall (colour, flavour, taste and texure) sensory score from zero to six day of storage. At seven days all salad sample drives below acceptability threshold. Samples were better acceptability threshold at zero days of storage but with the progress of storage period overall acceptance was decreased. From our result, interestingly it was found that salad samples (Apple, Grape, Guava, plum & Pear) treated with 0.5kGy of irradiation were healthier acceptance than samples treated by 1kGy and 1.5kGy during the six successive days of storage. Plum was more sensitive to irradiation than other salad samples especially to high dose above 0.5kGy. Thus, it can be said that high dose of irradiation affects the organoleptic quality of fruits (apple, grape, guava, plum, and pear) salad. In previous studies, it was seen that higher doses (above 1kGy) often cause softening, electrolyte leakage of many fruit and vegetables, cell membrane damage which may result unwanted appearance of fruit and vegetable [47,48].

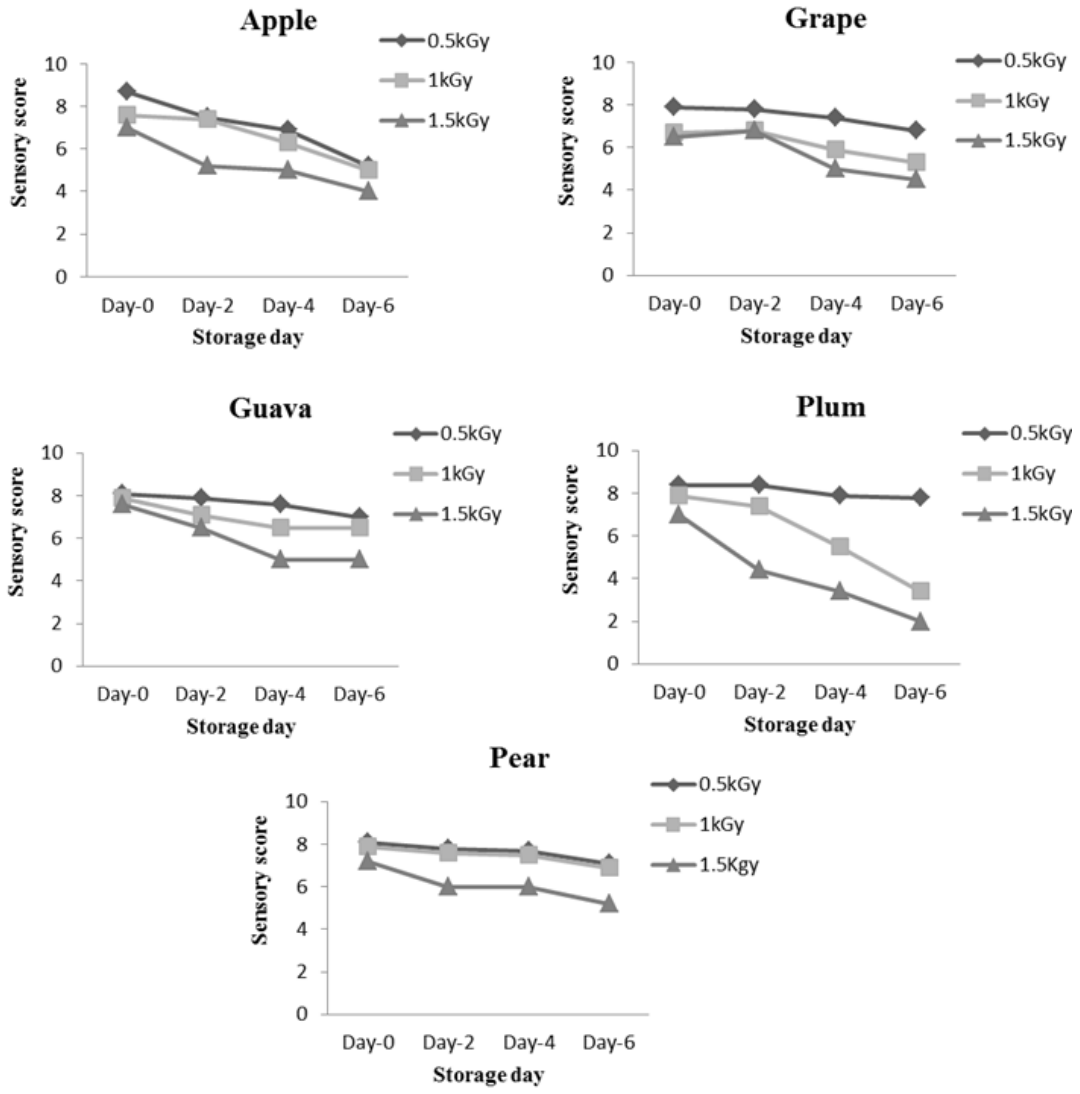

Figure 1. Changes of overall acceptance of polythene packed guava grape, pear, plum, and apple during six consecutive days of storage at 4°C. Results are expressed as average of sensory scores (color, flavor, taste and texture)

4. Conclusion

There is no specific dose of radiation recommended by FDA (Food and Drug Administration, USA) applied to fruits and vegetables. From our study, it can be said that 1kGy of radiation notably reduce the microbial loads and moisture content more than 90%, favorable of microbial growth and stable of other chemicals presents in fruit salads at six consecutive days of storage at 4°C refrigeration temperature which ensures the safety of these fruits. Therefore, further studies are needed.

Conflict of Interest

No conflicts of interest have been disclosed with the submission of this paper.

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