

Effect of gamma irradiation, packaging and storage on the microbiological quality of garden eggs

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Abstract: Garden eggs are important economic vegetable crops grown in most tropical countries. The effect of gamma irradiation (1 – 3 kGy), packaging (polyethylene) and storage (5 weeks at 29±1°C) on the microbiological quality of three varieties of garden eggs (*Solanum aethiopicum* GH 8772 and *Solanum aethiopicum* GH 8773, and *Solanum torvum*) were studied. The population of aerobic mesophiles and yeasts and moulds were assessed by the method of serial dilution and pour plating. Irradiation dose of 3 kGy, significantly improved microbiological quality by reducing the population of aerobic mesophiles and yeasts and moulds in all varieties of the garden eggs. Storing for ≤ 3 weeks negatively affected the microbiological quality of the 2 varieties of *Solanum aethiopicum*. *Solanum torvum* was however very susceptible to spoilage at > 1 week. The use of polyethylene packaging did not improve the microbiological quality of the garden egg varieties during storage at ambient temperatures of 29±1°C.

Keywords: Gamma Irradiation, Microbiological Quality, Packaging, Garden Eggs

1. Introduction

Garden eggs (*Solanum spp.*) are one of the most consumed vegetable crops cultivated in the tropics and temperate parts of the world (1). It is a woody perennial herb that belongs to the Family *Solanaceae* together with potato, tomato and pepper. Mature fruits may be eaten raw or boiled, fried and sautéed for soup and stew (2, 3). The plant has rich sources of vitamins A, B and C, magnesium, calcium, potassium, sodium and phosphorus which are essential for the prevention or treatment of cholera, bronchitis, dysuria, and asthma (1, 4).

In 2010, garden eggs production exceeded 40 million tons on a global scale of which China, India, Egypt, Turkey and Indonesia were leading producers (5). Across Africa, fruits from different cultivars are sold in both rural and urban markets in Cameroon, Ghana, Kenya, Madagascar, Nigeria and South Africa, as well as in Guatemala, New Guinea and some Mediterranean regions (6). In Ghana, the total annual

national production of garden egg fruits is currently estimated to be around 4,200 tons (5).

Garden eggs are produced throughout the agro-ecological zone of Ghana, however commercial production is concentrated mostly in the forest zone. The plant is grown as a commercial crop for domestic consumption and also for export (7, 8).

Garden eggs have limited shelf-life for freshness as a result of higher rate of water loss. Poor post-harvest handling, shoot and fruit borers, and the lack of quality standards contribute greatly to yield losses in Ghana. Garden egg plants are also prone to infection by pests, fungi, bacteria and viruses at all stages of growth on field and after harvest (6).

In spite of the beneficial uses of garden eggs, research into extending the shelf-life of garden eggs using gamma irradiation has received little attention in Ghana. Refrigeration, modified atmosphere storage, freezing and drying represent the current methods employed in extending the shelf-life of garden eggs (9, 10, 11, 12). Irradiation is a

proven technology which has been endorsed by notable international health and food authorities. As a process it has the capacity to eliminate insect pests and microbial pathogens from fresh fruits and vegetables. It also delays ripening and senescence thereby extending the shelf - life of fresh fruits and vegetables without adverse effect on the quality. The country has the requisite capacity to use the technology to improve the quality of fresh produce such as garden eggs for the domestic and export markets (13).

The objective of the study was to determine the effect of gamma irradiation, packaging and storage on the microbiological quality of three types of garden eggs (*Solanum aethiopicum* L. [GH 8772 and GH 8773] and *S. torvum*).

2. Materials and Methods

2.1. Samples

Two varieties of *Solanum aethiopicum* (GH 8772 and GH8773) and *S. torvum* were obtained from a market in Accra, Ghana, two days after harvest. Bruised, pitted, ripped fruits and fruits without stalk (calyx) were discarded after sorting. Fruits of GH8772 and GH8773 were coded "A" and "C" while those of *S. torvum* were coded "B" for easy referencing.

2.2. Packaging

The surfaces of the fruits were sterilized with 70% alcohol. Ten fruits, approximately 400 – 500 g, of A and C and approximately 300 g of B were packaged in perforated polyethylene zip – lock pouches (density = 0.18 g/cm³) for gamma irradiation.

2.3. Irradiation

Irradiation of samples was carried using a Cobalt-60 source under ambient conditions at the Gamma Irradiation Facility (GIF) of the Radiation Technology Centre (RTC) in Ghana Atomic Energy Commission (GAEC) at irradiation doses 0, 1, 2 and 3 kGy before storage. The dose rate was 1.962 kGy/hr and ferrous sulphate (Fricke) dosimeter was used to measure absorbed dose.

2.4. Storage

The samples were stored under ambient conditions (29±1°C) under two packaging conditions. Packaged samples were stored in perforated zip – lock pouches of density, 0.18 g/cm³. Control samples were unpackaged.

2.5. Microbiological Analysis

At the beginning of each storage week, the microbiological quality of the samples were determined by estimating the counts of aerobic mesophiles and yeasts and moulds by the methods of serial dilution and pour plate. The total plate count using Plate Count Agar (Oxoid, UK) was used for aerobic mesophiles and growth on media was

recorded as colony forming unit per gram (CFU/g). Counts of yeasts and moulds were also determined using the Oxytetracycline 6-Glucose Yeast Extract Agar (Oxoid, UK) and growth was recorded as (CFU/g).

2.6. Statistical Analysis

The logarithm of counts of aerobic mesophiles as well as yeasts and moulds (log₁₀ CFU/g) obtained from the experiments were analyzed using Statgraphics Centurion software (XVI.I edition). One-way and two-way analyses of variance were used to determine significant differences at 95% confidence interval.

3. Results

3.1. Effect of Irradiation on the Microbiological Quality of Garden Eggs

In this study, the microbiological quality of the garden egg fruits was considered as the total population of aerobic mesophiles and yeasts and moulds. Tables 1 and 2 show the effect gamma irradiation had on the microbiological quality of the fruits during storage. Overall, the population of aerobic mesophiles as well as yeasts and moulds significantly (5%) declined with increase in irradiation dose. This trend was observed in all the three garden eggs. The population of microorganisms in samples of variety B was relatively lower compared to counts of microorganisms in varieties A and C. Low population of microorganisms in variety B represents samples analyzed after gamma radiation and first week of storage. Most fruits of variety B darkened after irradiation (with doses of 1, 2 and 3 kGy) and got spoiled as a result of fungal infestation, therefore, those samples were discarded. Irradiation dose of 3 kGy significantly reduced the population of aerobic mesophiles as well as yeasts and moulds in varieties A and C compared to doses of 1 and 2 kGy.

3.2. Effect of Storage on the Microbiological Quality of Garden Eggs

During the 5-week storage period, significant differences (P<0.05) were observed within the population of aerobic mesophiles of samples of varieties A and C (Table 3). The population of aerobic mesophiles increased significantly in samples of varieties A and C under the two packaging conditions. The increases appeared to be dependent on the storage period. There appeared to be an increase in the population of yeasts and moulds during the storage period in samples of varieties A and C (Table 4). The significant differences (P<0.05) in the population of yeasts and moulds were not dependent on the storage period.

3.3. Effect of Packaging on the Microbiological Quality of Garden Eggs

The effect of packaging on the population of aerobic mesophiles as well as yeasts and moulds on samples of all

three varieties of garden eggs is shown in Figs. 1 and 2. Variety B had the lowest counts of aerobic mesophiles as well as yeasts and moulds. Significant differences ($P < 0.05$) were observed in the population of aerobic mesophiles as well as yeasts and moulds between samples of all three varieties under each of the packaging conditions of storage. However, there were no significant differences ($P > 0.05$) in the population of the microbes in samples of all three varieties under the two packaging conditions of storage.

3.4. Interactive Effect of Irradiation and Storage on the Microbiological Quality of Garden Eggs

The interactive effect of irradiation and storage period on the population of aerobic mesophiles and yeasts and moulds in samples of the three varieties of garden eggs

under the 2 packaging conditions are shown in Tables 5 and 6. Significant differences ($P < 0.05$) in the population of aerobic mesophiles as well as yeasts and moulds were observed in all the varieties throughout the storage period as a result of the interaction of radiation dose and the storage period. Among the samples of varieties A and C, the population of aerobic mesophiles and yeasts and moulds generally increased throughout the storage period in most instances. Except in a few instances, populations of aerobic mesophiles as well as yeasts and moulds were significantly reduced by a combination of higher radiation dose (3 kGy) and longer storage period (> 4 weeks). In the case of variety B, although radiation dose of 3 kGy reduced the population of aerobic mesophiles and yeasts and moulds, all the samples were spoilt after 2 weeks.

Table 1. Effect of irradiation on counts of aerobic mesophiles of three varieties of garden eggs stored under two packaging conditions

Dose (kGy)	Varieties					
	<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
	Unpack	Pack	Unpack	Pack	Unpack	Pack
0	*2.80 ^b	4.48 ^b	0.81 ^a	0.75 ^a	4.67 ^d	4.23 ^b
1	3.40 ^b	2.16 ^a	0.95 ^a	0.89 ^a	2.69 ^{bc}	2.43 ^{ab}
2	3.50 ^b	2.60 ^{ab}	1.05 ^a	0.99 ^a	3.88 ^{cd}	3.88 ^b
3	0.62 ^a	1.79 ^a	0.37 ^a	0.13 ^a	0.92 ^a	1.53 ^a

*Log, CFU/g; Values are means of 15 determinations – (3 replicates by 5 storage weeks); Mean values in the same column with different letters (a - d) were significantly different ($P < 0.5$)

Table 2. Effect of irradiation on counts of yeasts and moulds of three varieties of garden eggs stored under two packaging conditions

Dose (kGy)	Varieties					
	<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
	Unpack	Pack	Unpack	Pack	Unpack	Pack
0	*5.37 ^b	4.16 ^b	0.49 ^a	1.46 ^a	4.31 ^b	5.47 ^b
1	3.90 ^{ab}	4.90 ^b	1.40 ^a	1.71 ^a	3.67 ^{ab}	4.99 ^b
2	3.51 ^{ab}	3.48 ^b	0.45 ^a	1.58 ^a	2.20 ^a	4.56 ^b
3	1.98 ^a	0.88 ^a	0.23 ^a	0.47 ^a	3.49 ^{ab}	0.96 ^a

*Log, CFU/g; Values are means of 15 determinations – (3 replicates by 5 storage weeks); Mean values in the same column with different letters (a - d) were significantly different ($P < 0.5$)

Table 3. Effect of storage on counts of aerobic mesophiles of three varieties of garden eggs stored under two packaging conditions

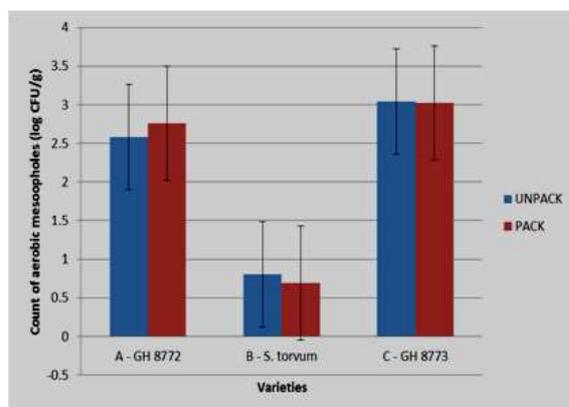
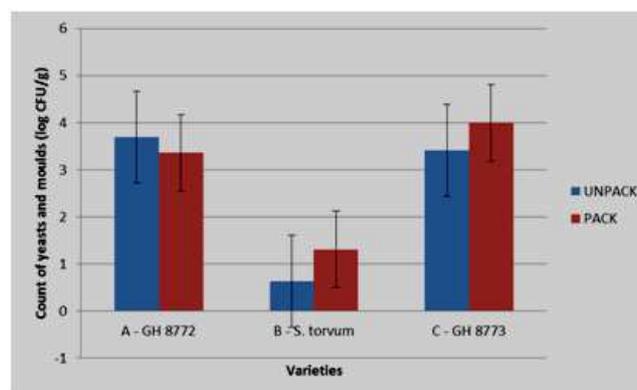
Storage Week	Varieties					
	<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
	Unpack	Pack	Unpack	Pack	Unpack	Pack
0	*0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
1	1.88 ^b	2.27 ^b	3.98 ^b	3.45 ^b	2.58 ^b	2.1 ^b
2	3.44 ^{bc}	1.59 ^{ab}	-	-	2.32 ^b	1.79 ^b
3	3.19 ^c	3.95 ^c	-	-	4.67 ^c	4.62 ^c
4	4.40 ^c	5.98 ^d	-	-	5.62 ^c	6.57 ^d

*Log, CFU/g; Values are means of 12 determinations – (3 replicates by 4 radiation doses); - = not available due to spoilage of samples; Mean values in the same column with different letters (a - d) were significantly different ($P < 0.5$)

Table 4. Effect of storage on counts of yeasts and moulds of three varieties of garden eggs stored under two packaging conditions

Storage Week	Varieties					
	<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
	Unpack	Pack	Unpack	Pack	Unpack	Pack
0	*0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
1	3.57 ^b	1.31 ^a	3.21 ^b	6.53 ^b	2.07 ^b	3.43 ^b
2	2.51 ^b	4.72 ^b	-	-	4.09 ^c	4.99 ^{bc}
3	7.36 ^c	6.12 ^b	-	-	5.43 ^c	5.68 ^c
4	5.01 ^d	4.63 ^b	-	-	5.50 ^c	5.88 ^c

*Log₁₀ CFU/g; Values are means of 12 determinations – (3 replicates by 4 radiation doses); - = not available due to spoilage of samples; Mean values in the same column with different letters (a - d) were significantly different (P<0.5)

**Figure 1.** Effect of two packaging conditions on the population of aerobic mesophiles for three varieties of garden eggs.**Figure 2.** Effect of two packaging conditions on the population of yeasts and moulds for three varieties of garden eggs.**Table 5.** Interactive effect of irradiation and storage on counts of aerobic mesophiles of three varieties of gardens eggs stored under two packaging conditions

Radiation Dose (kGy)	Storage Week	Varieties					
		<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
		Unpack	Pack	Unpack	Pack	Unpack	Pack
0	0	*0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	3.82 ^k _D	4.68 ^p _M	4.05 ^c _E	3.75 ^x _L	4.38 ⁱ _E	4.08 ^p _M
	2	1.26 ^e _B	2.34 ^f _M	-	-	3.27 ^e _C	2.34 ^s _M
	3	2.22 ⁱ _B	6.54 ^o _N	-	-	7.28 ^m _F	6.69 ⁿ _N
	4	6.69 ^o _D	8.83 ^l _O	-	-	8.43 ⁿ _F	8.05 ^k _O
1	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	2.09 ^h _C	0.55 ^x _K	4.74 ^d _E	4.45 ^w _M	0.96 ^c _{AB}	0.18 ^y _K
	2	5.62 ⁿ _E	1.76 ^s _L	-	-	0.99 ^c _{AB}	1.75 ^l _L
	3	5.64 ⁿ _D	1.38 ^u _L	-	-	4.32 ^h _C	3.66 ^f _M
	4	3.66 ^j _B	7.12 ^m _N	-	-	7.17 ^e _E	6.57 ⁿ _M
2	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	1.39 ^f _{BC}	3.58 ^q _L	5.27 ^f _F	4.94 ^v _M	2.78 ^f _C	3.71 ^q _L
	2	5.26 ^m _E	1.45 ^l _L	-	-	4.38 ⁱ _D	1.46 ^v _L
	3	4.22 ^l _C	6.62 ⁿ _N	-	-	6.41 ^j _E	7.58 ^l _O
	4	6.63 ^o _D	1.35 ^u _K	-	-	5.81 ^j _C	6.63 ^m _M
3	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	0.21 ^b _A	0.27 ^y _K	1.86 ^b _C	0.66 ^y _K	2.21 ^c _C	0.44 ^x _K
	2	1.60 ^g _B	0.81 ^w _K	-	-	0.65 ^b _A	1.62 ^u _L
	3	0.70 ^d _A	1.27 ^v _L	-	-	0.69 ^b _A	0.57 ^w _K
	4	0.60 ^c _A	6.63 ⁿ _M	-	-	1.08 ^d _A	5.03 ^o _L

* Log₁₀ CFU/g ; Values are means of 3 determinations ; - = not available due to spoilage; a-o (unpack), o-z (pack) = Mean values in the same column with different superscript letters were significantly different (P<0.05); A-F (unpack), K-O (pack) = Mean values in the same row with different superscript letters were significantly different (P<0.05) ; A = Solanum aethiopicum (GH 8772); B = Solanum torvum ; C = Solanum aethiopicum (GH 8773)

Table 6. Interactive effect of irradiation and storage on counts of yeasts and moulds of three varieties of garden eggs stored under two packaging conditions

Radiation Dose (kGy)	Storage Week	varieties					
		<i>S. aethiopicum</i> – [A]		<i>S. torvum</i> – [B]		<i>S. aethiopicum</i> – [C]	
		Unpack	Pack	Unpack	Pack	Unpack	Pack
0	0	*0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	4.31 ^h _E	0.79 ^x _{KL}	2.43 ^d _C	7.30 ^x _Q	0.73 ^d _{AB}	5.59 ^f _P
	2	6.15 ^k _E	7.30 ⁿ _N	-	-	5.75 ^k _D	6.92 ^o _M
	3	8.53 ^p _E	8.74 ^l _O	-	-	7.73 ^p _D	7.15 ⁿ _M
	4	7.86 ^o _D	3.98 ^l _L	-	-	7.34 ^o _D	7.70 ^l _O
1	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	3.80 ^s _{DE}	4.14 ^s _O	6.98 ^e _F	8.53 ^r _R	4.38 ^h _E	3.63 ^l _N
	2	1.99 ^d _B	5.27 ^q _L	-	-	0.47 ^c _A	6.53 ^q _M
	3	7.62 ⁿ _D	7.79 ^m _N	-	-	6.92 ⁿ _C	7.96 ^k _N
	4	6.07 ^j _C	7.30 ⁿ _{NO}	-	-	6.59 ^m _C	6.83 ^p _N
2	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	3.22 ^f _D	0.04 ^y _K	2.24 ^c _C	7.91 ^w _{QR}	2.88 ^g _{CD}	3.42 ^u _N
	2	1.47 ^c _B	5.13 ^f _L	-	-	5.16 ^j _D	5.16 ^s _L
	3	6.87 ^m _C	6.84 ^o _M	-	-	1.30 ^e _A	7.32 ^m _M
	4	5.99 ⁱ _C	5.40 ^p _M	-	-	1.68 ^f _B	6.87 ^p _N
3	0	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K	0.00 ^a _A	0.00 ^z _K
	1	2.94 ^c _C	0.28 ^y _K	1.18 ^b _B	2.39 ^m _M	0.28 ^b _A	1.06 ^s _L
	2	0.45 ^c _A	1.19 ^v _K	-	-	4.98 ⁱ _C	1.36 ^w _K
	3	6.41 ^l _C	1.11 ^w _L	-	-	5.77 ^k _B	0.27 ^y _K
	4	0.13 ^b _A	1.84 ^u _K	-	-	6.41 ^l _C	2.13 ^v _K

* Log₁₀ CFU/g ; Values are means of 3 determinations ; - = not available due to spoilage; a-p (unpack), o-z (pack) = Mean values in the same column with different superscript letters were significantly different (P<0.05); A-E (unpack), K-R (pack) = Mean values in the same row with different superscript letters were significantly different (P<0.05) ; A = *Solanum aethiopicum* (GH 8772); B = *Solanum torvum* ; C = *Solanum aethiopicum* (GH 8773)

4. Discussion

4.1. Effect of Irradiation on the Microbiological Quality of Garden Eggs

Microbiological quality, in this study, was considered as the total population of aerobic mesophiles and yeasts and moulds. Two weeks into the storage period, the population of aerobic mesophiles as well as yeasts and moulds for the three types of garden eggs, as shown in Tables 1 – 4, were within the recommended range of 10⁶ and 10³ CFU/g respectively for aerobic plate count and yeasts and moulds in fresh fruits and vegetable.

Discoloration of *S. torvum* (variety B) fruits radiated at doses of 1, 2 and 3 kGy occurred within the first week of storage under the two packaging conditions. This observation might be due to increased activity of polyphenoloxidase enzyme of the skin and pulp resulting in tissue-darkening. Studies in mangoes and banana have also reported tissue-darkening as a result of irradiation with doses of 1 to 2 kGy (14). Subsequent to this was infestation by *Alternaria sp.* and spoilage. Fungal spoilage of garden eggs by *Alternaria sp.* has been reported (15). Therefore the effect of gamma irradiation on the microbiological quality of *S. torvum* during storage under the two packaging conditions could not be determined. Tissue-darkening as a result of irradiation was however not observed in samples of varieties A and C in this study.

Microbiological counts were generally very low (< 10 CFU/g), possibly due to the sanitizing effect of 70% alcohol

treatment prior to irradiation and storage. A dose of 3 kGy significantly reduced the population of aerobic mesophiles as well as that of yeasts and moulds in all the garden eggs.

Irradiation has been used to control bacteria and other microorganisms in a variety of fresh fruits and vegetables. A dose of 2kGy was found to inhibit the growth of aerobic mesophiles and coliforms in shedded carrots (16). Recent research has also consistently shown irradiation as effective in killing bacterial pathogens (17). Irradiation has been used to control spoilage of vegetables caused by a variety of fungi (14). A study has also reported significant decrease in counts of *Salmonella* spp., lactic acid bacteria as well as yeasts and moulds in another vegetable (tomato) after irradiation at relatively low dose of < 1 kGy (18). The primary mechanism by which radiation kills microorganisms is by splitting water molecules into hydrogen (H⁺), hydroxyl (OH⁻) and oxygen (O⁻²) radicals. These radicals react with and destroy microbial DNA, proteins and other cell organelles [19].

4.2. Effect of Storage and Packaging on the Microbiological Quality of Garden Eggs

Vegetables require proper storage conditions (temperature and humidity) to lengthen storage life and maintain quality. In this study, the effect of storage on microbial quality was dependent on the variety. Samples of variety B were discarded after the first week of storage due to spoilage. Similarly, a study reported spoilage of other vegetables (cabbage, cauliflower, country bean, tomato, peas) after 9 days of storage at ambient temperatures of 24-15^oC [20].

Microorganisms can occur on raw and minimally processed vegetables at populations ranging from 10^3 to 10^9 cfu/g [21]. Results of this study indicates increases in population of aerobic mesophilic bacteria and yeasts and moulds were dependent on storage for samples of varieties A and B, however, that of yeasts and moulds in variety A was not definitive. A related study also reported increases in population of total viable count to 10^8 cfu/g in the natural microflora of tomatoes stored at an ambient temperature of 22 °C [22].

Good packaging protects food products from physical, microbiological and chemically induced changes. The choice of materials for packaging depends on the nature of product as well as the storage and handling conditions (temperature, humidity, risk of physical deterioration) and various other factors [23]. In this study, the use of perforated polyethylene zip – lock pouches did not improve the microbiological quality of the garden egg varieties. This observation might be due to the fact the ambient temperature of 29±1 °C was ideal for microbial growth and therefore possibly nullified the impact of the packaging material on the microbiological quality of the garden eggs.

4.3. Interactive Effect of Irradiation and Storage on the Microbiological Quality of Garden Eggs

The interactive effect of irradiation dose (1 to 3 kGy) and storage (up to 5 weeks) on microbial quality showed that 3 kGy was effective in reducing counts of aerobic bacteria and yeasts and moulds in varieties A and C. The higher counts observed in samples irradiated with 1 and 2 kGy in both unpackaged and packaged samples of varieties A and C may be due to their susceptibility to contamination and radiation sensitivity of the microorganisms. The effect of packaging on the microbial quality of the samples was not significant.

5. Conclusion

Gamma irradiation at a dose of 3 kGy significantly reduced the population of aerobic mesophiles as well as that of yeasts and moulds and therefore improved the microbiological quality of garden eggs during storage up to 5 weeks. Longer storage periods (4 weeks) negatively affected the microbiological quality of the 2 varieties of *Solanum aethiopicum*. *Solanum torvum* was however very susceptible to spoilage beyond the first week of storage. The use of polyethylene packaging did not improve the microbiological quality of the garden egg varieties during storage at ambient temperatures of 29±1°C.

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