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Effects of irradiation on the cowpea weevil (*Callosobruchus maculatus* F.) and moisture sorption isotherm of cowpea seed (*Vigna unguiculata* L. Walp)

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Abstract

Cowpeas during storage may be attacked by a number of biological agents (microorganisms, rodents, and insects) which results in losses in the quality and quantity of the stored seeds. One of the means of reducing these losses is through the application of radiation processing. The aim of this study was to determine the effects of gamma irradiation on a major storage insect pest, *Callosobruchus maculatus* F. and on moisture sorption isotherms of cowpea seeds in storage. The cowpeas were infested with adults *C. maculatus* and then irradiated at doses of 0.0, 0.25, 0.5, 0.75, 1.0 and 1.5 (kGy) at a dose rate of 1.074 kGyhr⁻¹. Samples were stored for 1 month under controlled temperature (27.3-30 °C) and humidity (70-85 %) during which counting of the insects was done every 48 hours and those alive or dead noted. Moisture sorption isotherms of the cowpea samples were equally determined by establishing equilibrium relative humidity (ERH) of 55, 65, 75, 85 and 95 % using a formulation of glycerol-water mixture at temperature of 30 ± 1 and the weight (loss or gain) of the samples was determined every 2 days. Irradiation at a dose of 0.25 kGy killed the *C. maculatus* within eight days and therefore 0.25 kGy would be economically beneficial as a control dose. There was significant difference ($p < 0.05$) in the percent mortality between the irradiated and the non-irradiated weevils, and the percent mortality increased with increase in the radiation dose. At moisture content of 14 % the irradiated cowpea samples were safely stored for one month at an equilibrium relative humidity of 60-75 % at a temperature of 29 ± 2 °C.

Keywords Irradiated cowpea; radiosensitivity; *Callosobruchus maculatus*; moisture sorption isotherms; percent mortality.

1 Introduction

In Africa and the rest of the developing world, where malnutrition due to inadequate protein in the diet is a prevalent problem, there is an urgent need to explore the utilization of plant proteins in the formulation of new food products or in conventional food (Khalid et al., 2003). This is so because animal protein such as meat, milk and eggs are expensive and thus relatively difficult to acquire (Chel-Guerrero et al., 2002).

Cowpeas are an important tropical legume crop of African origin and have become an integral part of the traditional cropping system, particularly in the semi-arid West African and Savanna (Steele, 1972). Cowpeas are produced countrywide in Ghana as a monocrop or an intercrop with maize. Unlike animal sources, which

are scarce and expensive, cowpea is an inexpensive source of protein, vitamins and minerals (Singh and Rachie, 1985; Oyefeso, 1980). Inadequate intake of protein in the diet is one of the factors that contribute to such high prevalence of malnutrition in developing countries. Therefore wider utilization of cowpea in the diet presents a source of protein that is within the means of most households of Ghana (GNA, 2008).

During storage, cowpea seeds may be attacked by a number of biological agents which result in losses in the quality and physical losses in the quantity of the stored seeds. The main reason explaining the disinterest of the African peasant farmer towards the cultivation of cowpea is certainly the infestation of the seeds often by *Callosobruchus maculatus* making the marketing of cowpeas unattractive and unprofitable (Diop, 1997). Although losses can be reduced by using chemicals, the resulting chemical residue can be harmful (Lan et al., 1991) and since irradiation is a physical method it has no residual effect on the food items but can effectively disinfect them. Disinfestation by irradiation is a promising method that has been studied extensively in many countries (Begum et al., 1980; Ahmed et al., 1977).

Moisture content had been identified as the most crucial storage, preservation or processing parameter, therefore one set of useful fundamental data is the moisture sorption characteristics of a given food material (Labuza, 1975). There had been also an indication that equilibrium relative humidity (ERH) is the most important property for the prediction of food stability in storage (Onayemi and Oluwamukomi, 1987). Apart from its importance in the storability of foods and hence their stability, the moisture content of food material is an index of sensory and process quality (Joslyn, 1970). Findings on the effects of gamma irradiation on moisture sorption isotherms of cowpea in literature are scanty. In addition, there is no data on storage stability of irradiated cowpea in Ghana, hence the need for this study.

The objective of this study was to determine the effects of irradiation on a major storage pest, *C. maculatus* and moisture sorptions isotherm of cowpea (*Vigna unguiculata* L. Walp) seeds in storage.

2 Materials and Methods

2.1 Sample collection, preparation and treatment

Two cultivars of cowpea “Asontem” and “Nhyira” were bought from the Crop Research Institute of CSIR, Fumasua-Kumasi, Ghana and two other cultivars of cowpea “Togo” and “Nigeria” were bought from a local market in Accra, Ghana. The irradiation was done at the Radiation Technology Centre, Ghana Atomic Energy Commission (GAEC) using the cobalt 60 source. The radiation doses used were 0.0, 0.25, 0.50, 0.75, 1.0 and 1.5 kGy at dose rates of 46.813 Gyhr⁻¹ (0.50 and 0.75 kGy) and 45.374 Gyhr⁻¹ (0.25, 1.00 and 1.50 kGy) at 100/70 cm positions in air and the absorbed dose confirmed by Fricke’s dosimetry.

2.2 Effects of irradiation on *Callosobruchus maculatus* in stored cowpea

Thirty grams of each of the insect-free cowpea cultivars were placed in specially cut and mesh-sealed plastic containers. The cowpeas in the containers were infested with 7 adults of *C. maculatus* each. The insects were then irradiated at doses of 0.0, 0.25, 0.5, 0.75, 1.0 and 1.5 (kGy) at a dose rate of 1.0744 kGyhr⁻¹ after which they were stored for 1 month under controlled temperature and humidity (temperature between 27.3 and 30 °C and humidity between 70 and 85 %). Counting of the insects was done every 48 hours to determine the number of those alive or dead. The levels of infestation, extent of damage and percent mortality were then related to the radiation dose.

2.3 Determination of moisture sorption isotherms of irradiated cowpea seed cultivars

The equilibrium relative humidity for the samples was determined according to the method described by Banu et al. (2008). Two grams of each of the seeds (*V. unguiculata*) were weighed and placed in desiccators having specific equilibrium relative humidities at an ambient temperature of 30 ± 1 °C. Duplicates of each sample were placed in the upper section of each glass desiccator on wire/plastic mesh while the lower section

contained the formulation of the glycerol-water mixture (Table 1). The relative humidity values in the desiccators and the temperatures were monitored using a thermo-hygrometer. The interior of the desiccators had a temperature of 29 ± 1 °C. The weight (loss or gain) of the samples was determined every 2 days by weighing the samples and graphs were drawn for the values obtained.

Table 1 shows how the various equilibrium relative humidity were established for the moisture sorption isotherms.

Table 1 Formulation of glycerin-water ratio used to establish the prescribed equilibrium relative humidity (Banu et al., 2008).

% ERH	Volume of Glycerol (ml)	Volume of Water (ml)
55	75	25
65	68	32
75	58	42
85	45	55
95	22	78

2.4 Statistical analysis

Analysis of variance (ANOVA) was done using StatGraphics Plus (3.0) Statistical Software Program. The level of significance used was $p < 0.05$. The least significant difference test (LSD-test) was applied for mean separation.

3 Results and Discussion

3.1 Effects of irradiation on *Callosobruchus maculatus* and on cowpea storage

Table 2 shows the percent mortality of *Callosobruchus maculatus*, 144 hours (six days) after irradiation.

Table 2 Percent (%) mortality of *C. maculatus* 144 hours (six days) after irradiation

DOSE (kGy)	Percent Mortality (%) of <i>C. maculatus</i>			
	“ASONTEM”	“NHYIRA”	“NIGERIA”	“TOGO”
0.0	0.00 ^b	0.00 ^c	0.00 ^d	0.00 ^b
0.25	55.08±2.82 ^a	55.08±1.3 ^b	28.77±1.45 ^c	25.53±2.2 ^a
0.5	94.19±1.12 ^a	74.64±1.4 ^a	32.96±1.15 ^c	33.50±1.58 ^a
0.75	100 ^a	85.51±1.32 ^a	52.24±1.07 ^b	53.09±2.34 ^a
1.0	100 ^a	90.78±1.17 ^a	70.96±1.07 ^a	57.28±1.82 ^a
1.5	100 ^a	95.72±1.07 ^a	95.06±1.10 ^a	60.81±1.86 ^a

Means ± standard deviation with different superscripts in the same column are significantly different ($p < 0.05$).

The percent mortalities in the irradiated weevils were significantly higher ($p < 0.05$) than in the non-irradiated (Table 2). However, there were varietal differences in percent mortality of the weevils. The percent mortality among the cowpea cultivar weevils decreased in the order “Asontem” > “Nhyira” > “Nigeria” > “Togo”.

In “Asontem”, a hundred percent mortality was experienced by the weevils irradiated with 0.75 kGy, 1.0 kGy and 1.5 kGy within 48 hours (2 days) and within 96 hours (4 days) for the weevils irradiated with 0.25 kGy and 0.5 kGy. The weevils in the control experienced a hundred percent mortality at 192 hours (8 days). There were no significant differences ($p > 0.05$) in the percent mortality between all the irradiated weevils

within 144 hours (6 days) after the irradiation. On the other hand, there were significant differences ($p < 0.05$) in the percent mortality of the weevils in all the irradiated and the non-irradiated.

In “Nhyira”, a hundred percent mortality was attained within 96 hours (4 days) by the weevils irradiated with 0.75 to 1.5 kGy whilst those irradiated with 0.5 kGy had 100 % mortality within 144 hours (6 days); the non-irradiated and 0.25 kGy had 100 % mortality within 192 hours (8 days).

In “Nigeria”, weevils irradiated with 1.5 kGy had 100 % mortality within 96 hours (4 days). A hundred percent mortality in weevils was experienced within 192 hours (8 days) for radiation doses from 0.5 to 1 kGy while the control and 0.25 kGy had 100% mortality within 288 hours (12 days).

In “Togo”, weevils irradiated with the 0.75 to 1.5 kGy doses had 100 % mortality within 144 hours (6 days) and those irradiated with doses of 0.25 and 0.5 kGy had 100 % mortality within 240 hours (10 days). In the control, the highest mortality experienced was 86 % which occurred within 240 hours (10 days) of storage. In the control, the highest mortality experienced was 86 % which occurred within 240 hours (10 days) of storage.

In all the four cultivars, the insect mortality increased with the radiation dose (Table 2). The significant differences ($p < 0.05$) in the percent mortality of the irradiated and non-irradiated weevils suggest that the radiation doses facilitated the death of the weevils. The mortality rate of the adult forms after irradiation depended on their imaginal dimorphism (Gill et al., 1971). The flight forms can evade difficult living conditions and which live longer than the flightless forms (Gill et al., 1971).

The weevils stored on “Asontem” attained higher percent mortalities (55-100 %) compared to the weevils on the other three cultivars. This could be attributed to factors such as hard and compact seed coat (Table 2). As such, inaccessibility to the seed content by the weevils could have resulted in starvation, hence higher mortality. The radioresistance of *C. maculatus* (cowpea weevil) as measured by percent mortality increased gradually with its development in the seed (Diop et al., 1997). “Togo” had the lowest percent mortality (26-61 %) among the four cultivars (Table 2). In the control, 86 % mortality was achieved but was higher in the other three cultivars.

The use of radiation dose of 0.25 kGy was effective in killing the *C. maculatus* at any stage of development of the adults in less than 4 days to a maximum of 12 days. These results were similar to those obtained by (Musasa and Onyembe, 1993) at a lower radiation dose (0.2 kGy) and (El Kady and Hekal, 1991) at 0.4 kGy. It was observed that no matter which form was studied, the radiation dose necessary to bring about a rapid mortality of these weevils appeared to be relatively high, greater than 2 kGy (Diop et al., 1997). The ionizing radiation at low doses has the disadvantage of leaving the cowpea weevils alive in the packaging film. However, their life expectancy is greatly reduced (the mortality rate after irradiation at 0.05 kGy has been estimated at 97% after 20 days), but their presence may be unacceptable to the consumer (Diop et al., 1997).

There was no subsequent development of weevils in the irradiated seeds (all four cowpea cultivars) stored for the period of study (one month). Since a radiation dose of 0.25 kGy disinfested cowpea as effectively as the 1.5 kGy within a few days, the 0.25 kGy had economic benefit over the other radiation doses.

3.2 Moisture sorption isotherm (MSI) of irradiated cowpea seed cultivars

Figs 1-20 show the moisture sorption isotherms of both the irradiated and control cowpea seed cultivars (*V. unguiculata*) at the various equilibrium relative humidities (ERH).

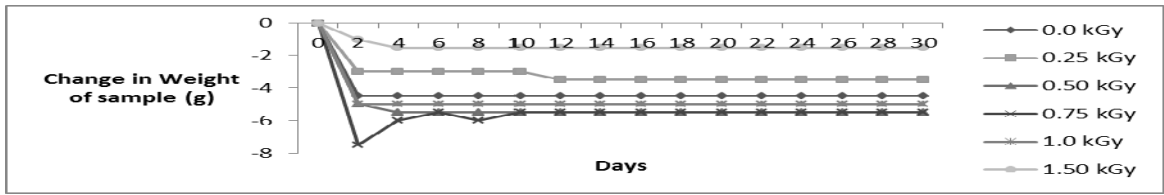


Fig. 1 Moisture Sorption Plot for "Asontem" at 55 % ERH

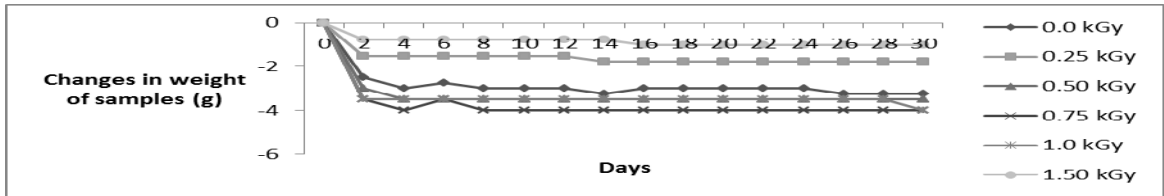


Fig. 2 Moisture Sorption Plot for "Asontem" at 65 % ERH

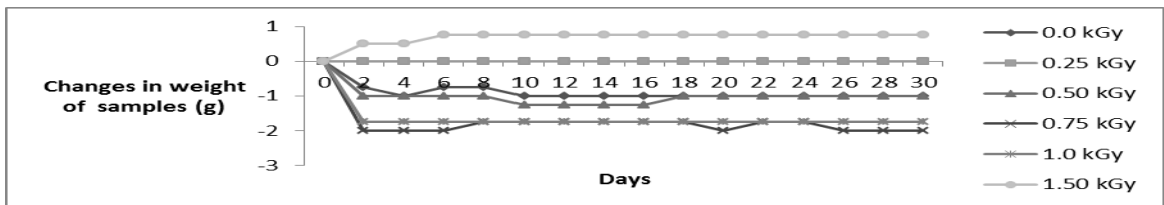


Fig. 3 Moisture Sorption Plot for "Asontem" at 75 % ERH

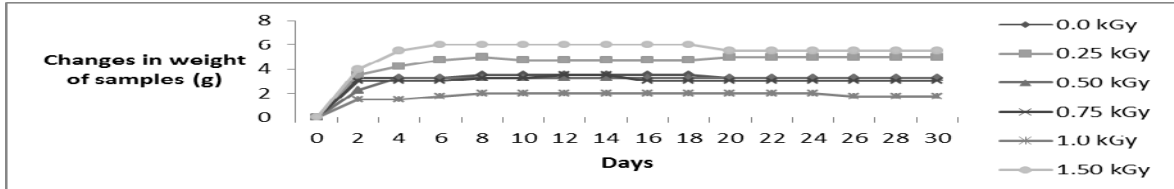


Fig. 4 Moisture Sorption Plot for "Asontem" at 85 % ERH

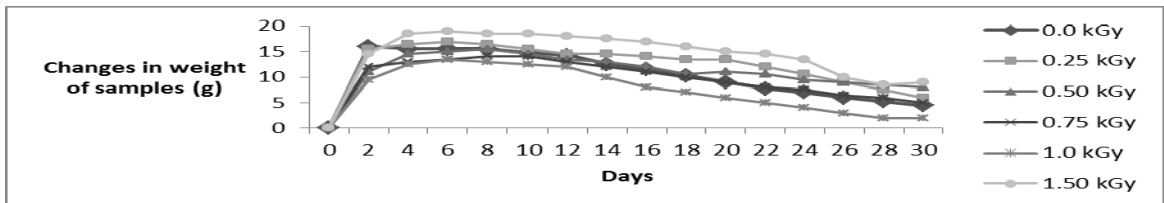


Fig. 5 Moisture Sorption Plot for "Asontem" at 95 % ERH

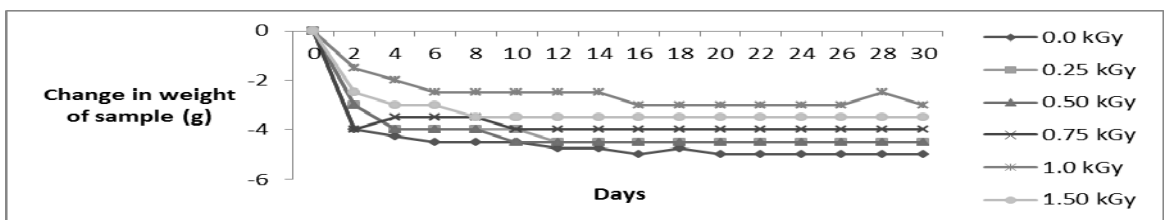


Fig. 6 Moisture Sorption Plot for "Nhyira" at 55 % ERH

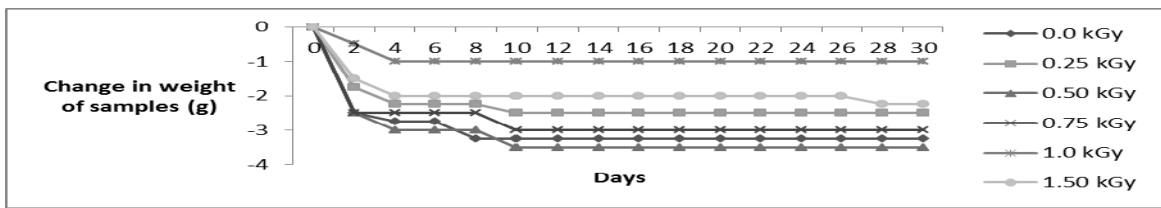


Fig. 7 Moisture Sorption Plot for “Nhyira” at 65 % ERH

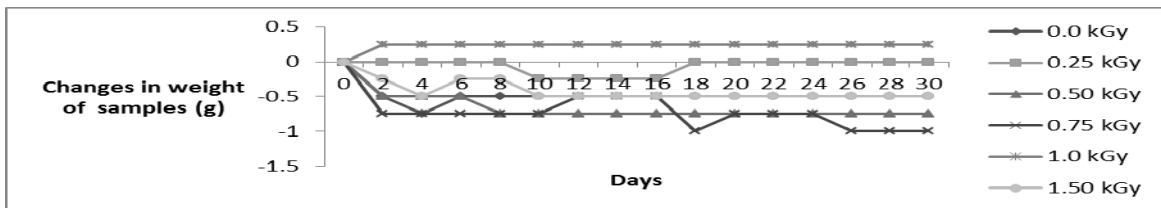


Fig. 8 Moisture Sorption Plot for “Nhyira” at 75 % ERH

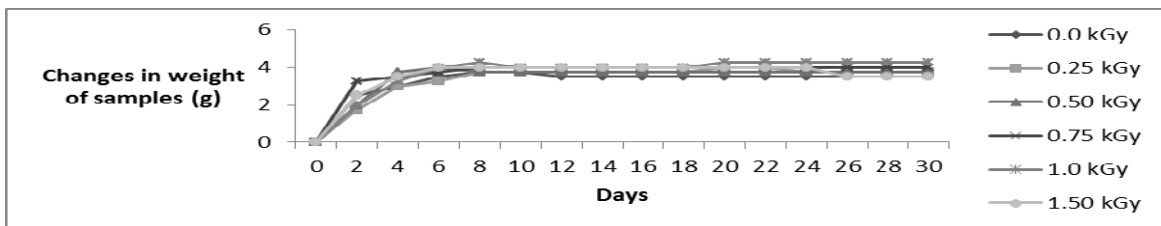


Fig. 9 Moisture Sorption Plot for “Nhyira” at 85 % ERH

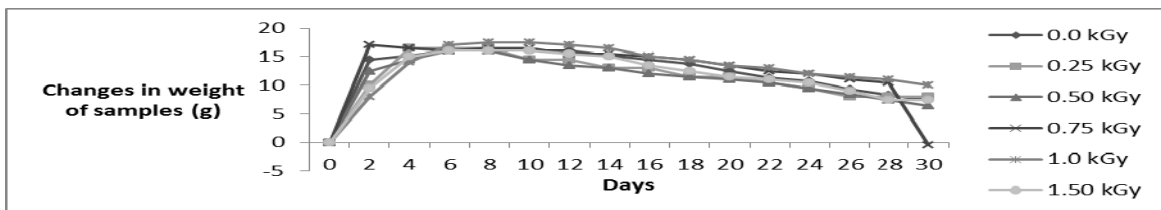


Fig. 10 Moisture Sorption Plot for “Nhyira” at 95 % ERH

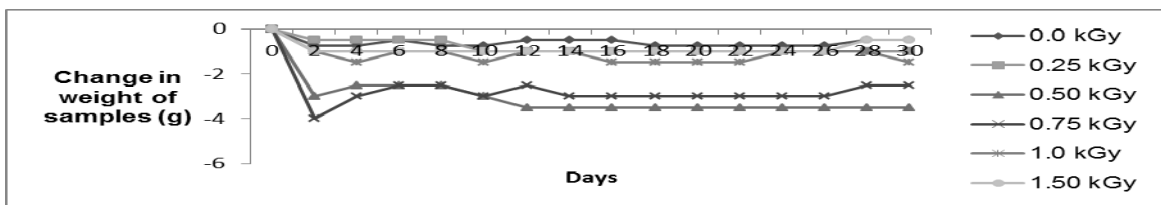


Fig. 11 Moisture Sorption Plot for “Nigeria” at 55 % ERH

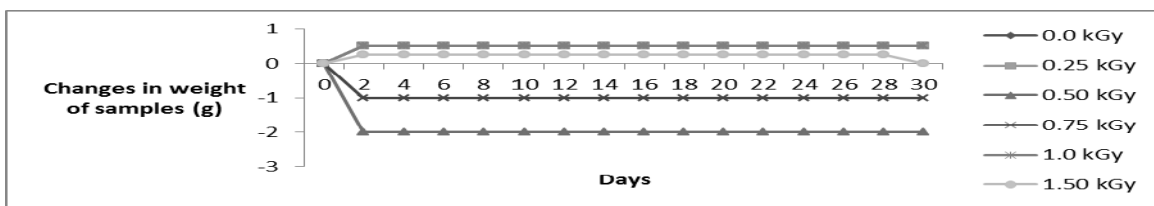


Fig. 12 Moisture Sorption Plot for "Nigeria" at 65 % ERH

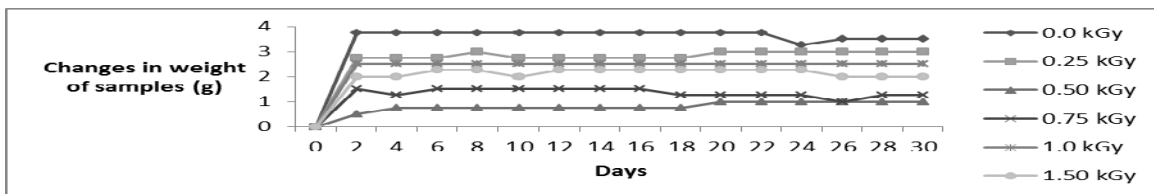


Fig. 13 Moisture Sorption Plot for "Nigeria" at 75 % ERH

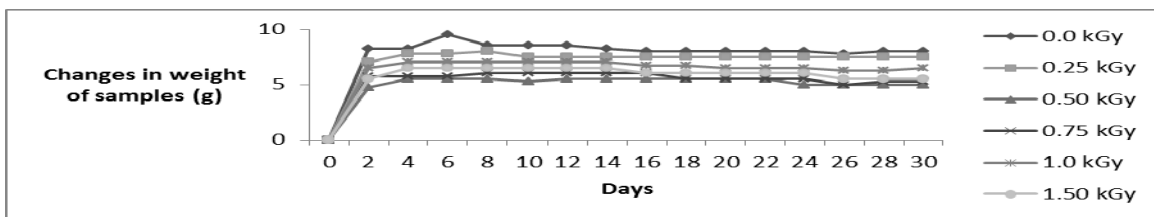


Fig. 14 Moisture Sorption Plot for "Nigeria" at 85 % ERH

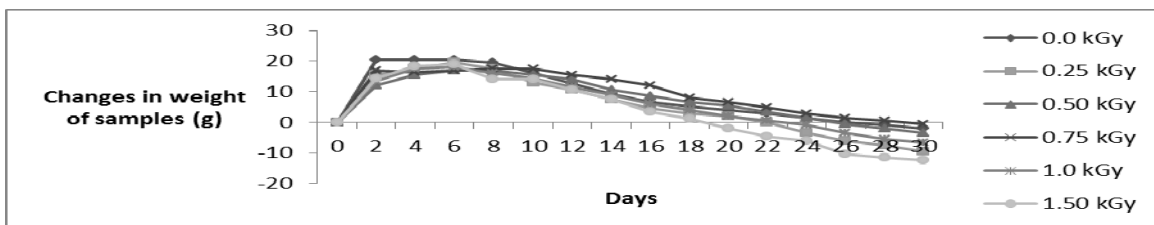


Fig. 15 Moisture Sorption Plot for "Nigeria" at 95 % ERH

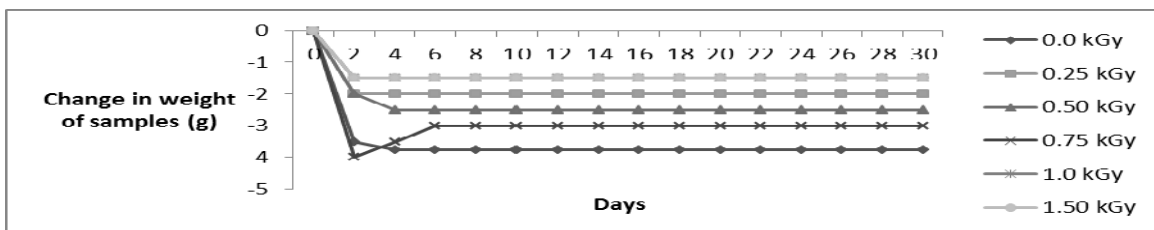


Fig. 16 Moisture Sorption Plot for "Togo" at 55 % ERH

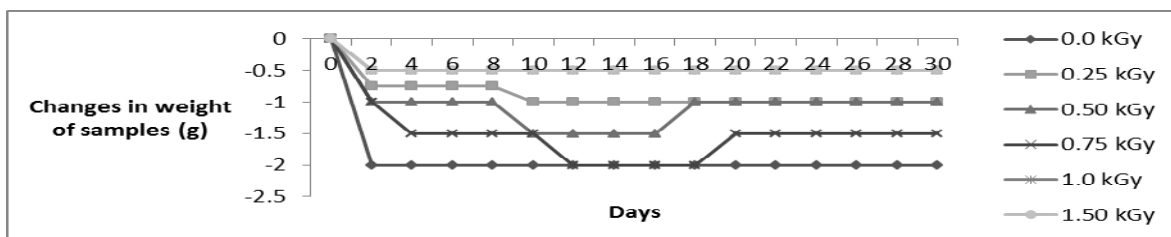


Fig. 17 Moisture Sorption Plot for “Togo” at 65 % ERH

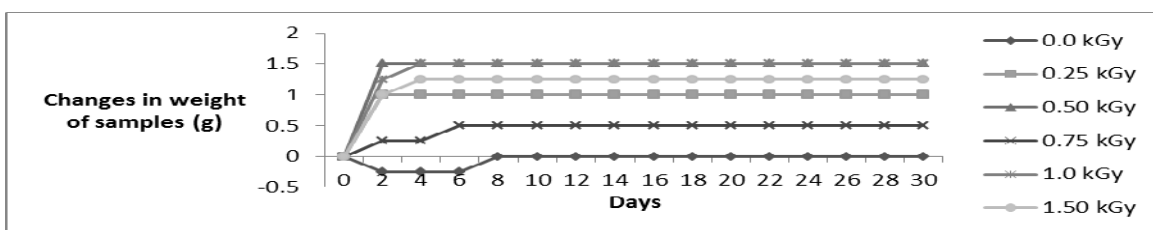


Fig. 18 Moisture Sorption Plot for “Togo” at 75 % ERH

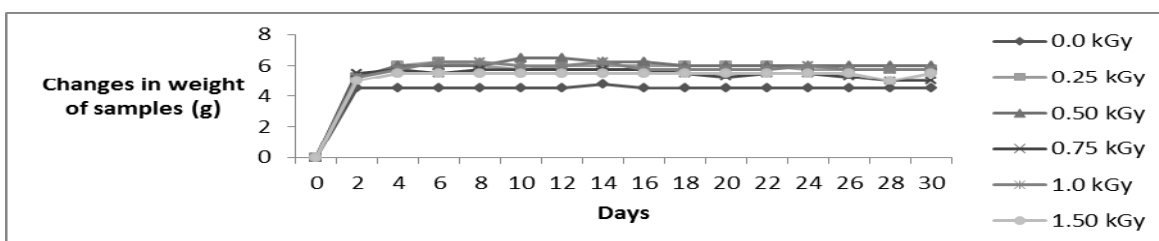


Fig. 19 Moisture Sorption Plot for “Togo” at 85 % ERH

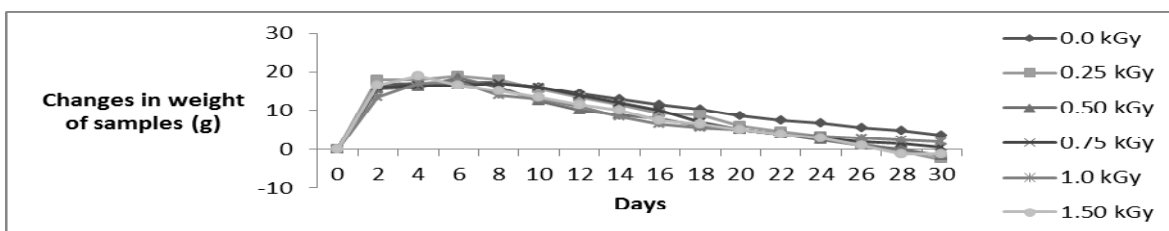


Fig. 20 Moisture Sorption Plot for “Togo” at 95 % ERH

For all the four cultivars, at the ERH of 55, 65, 75 and 85 % equilibrium sorption was attained between 2-6 days. All samples stored at 55 and 65 % ERH lost moisture content with the exception of the 0.5 and 0.75 kGy samples of “Nigeria” which gained moisture content. At 75 % ERH moisture content of all the samples (except “Togo”) began to rise and at 85 % ERH, high moisture was gained by all samples before attaining equilibrium. But for the “Togo”, at 75 % ERH, the control initially lost moisture within the first 8 days after which the moisture content was maintained. However, all the irradiated samples (“Togo” at 75 % ERH) gained some amount of moisture while high moisture was gained by both the irradiated and non-irradiated samples before attaining equilibrium at 85 % ERH. At 95 % ERH, there was an exponential gain in moisture content from 6-8

days after which there was a steep reduction in moisture content due to the visible growth of moulds on all the samples after day 4.

There has been an establishment that equilibrium relative humidity (ERH) is an important parameter, as it determines the amount of water available to microorganisms and hence an indication of the biological activity of the product (Ayerst, 1965; Jelle, 2003). Therefore the moisture content of the product itself, as well as the moisture content of surrounding air, is important for safe storage (Jelle, 2003). Each product has its own characteristic balance (or equilibrium) between the moisture it contains and the water vapour in the air surrounding it. This equilibrium is known as the moisture content or the relative pattern (Jelle, 2003).

The moisture contents of all the irradiated and non-irradiated samples of the four cowpea cultivars were 13-14 % (averagely 14 %) (Figs 1-20). This was in agreement with (Jelle, 2003), which stated that beans and pulses have safe moisture content of 13-15 % (on average, 14 %). It had been showed also that safe storage is reached in flour at 13 % moisture content (Wilson and Payne, 1994). Most of the moisture sorption isotherms for the four cowpea cultivars (irradiated and non-irradiated) showed a near sigmoid curve (Figs 1-20). This was in agreement with the publications of (Banu et al., 2008) for cassava accessions and (Ashaye, 2006) for irradiated and non-irradiated cowpea seeds.

For the ERH of between 55-85 %, equilibrium sorption was attained between 2-6 days (Figs 1-20) which is not far from the 4-6 days attained by (Banu et al., 2008) and (Odamtten et al., 1980) for cassava and maize respectively. The minimal loss of moisture at 65 % and 75 % ERH is an indication that cowpea (both irradiated and non-irradiated) cultivars can be stored at ERH of between 60-75 % (Figs 1-20). This was in agreement with (Alakali et al., 2007; Ashaye, 2006; Odamtten et al., 1980). It was found that maize and maize flour were better stored at 55-75 % ERH (Odamtten *et al.*, 1980). There had been reports that ERH for safe storage of beans lies about 60 % ERH for temperature of 20- 40 °C (Alakali et al., 2007), and also 60 % ERH to be safe for irradiated cowpea samples (Ashaye, 2006). The physical growth of moulds on the stored grains from the 6-8 day at 95 % ERH (Figs 1-20) was due to the high humidity which was favourable for the growth of moulds. Similarly, Banu et al. (2008) observed growth of moulds on the cassava flour samples within 6-12 days at 95 % ERH.

In general, the samples irradiated at 0.00 kGy, 1.0 kGy, 0.25 kGy and 1.50 kGy (in increasing order of absorption) had maximum moisture absorption at 85 % ERH and 95 % ERH (Figs 1-20). It had also been indicated that irradiated cowpea samples adsorb more moisture than non-irradiated cowpea up to 15 Gy (Ashaye, 2006). This is because irradiation weakens the inter-molecular bonds between starch and water thereby enhancing water uptake (Rao and Vakil, 1985).

Irradiated and non-irradiated cowpea samples of moisture content of around 14 % could be stored safely at ERH of between 60 and 75 % under room temperature (Fig. 1-20). It had been stated that, food water activity values of less than 0.70_{a_w} (ERH 70 %) are unlikely to support spoilage by microorganisms (Wilson and Payne, 1994). There is also an established fact that the maximum allowable moisture content for safe storage of a product, known as the safer moisture content is taken to be the equilibrium moisture content which corresponds to a relative humidity of 65-70 % (Jelle, 2003). When a product's moisture content is equal to or below the safe moisture content, the danger of attack by bacteria and fungi is negligible.

4 Conclusion

The studies have shown that gamma irradiation dose of 0.25 kGy was effective in disinfesting the cowpea seeds. There were also significant increases in the percent mortality of the irradiated cowpea weevil, *C. maculatus* compared to the non-irradiated *C. maculatus*. The moisture sorption isotherm favourable for storage of cowpea samples at room temperature was not significantly affected by the radiation at the doses studied.

Irradiated and non-irradiated cowpea samples of moisture content of around 14 % could be stored safely at ERH of between 60 and 75 % under room temperature.

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