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COMPARATIVE MOISTURE SORPTION, INSECT INFESTATION AND AFLATOXIN PRODUCTION BY RESIDENT *ASPERGILLUS FLAVUS* LINK SPORES IN SOLAR AND SUN DRIED CASSAVA ACCESSIONS BEFORE AND AFTER GAMMA IRRADIATION

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ABSTRACT

Ten accessions of Solar and Sun dried cassava (Manihot esculenta Crantz) were studied for their comparative ability to absorb moisture and harbour insects under varying Environmental Relative Humidities, (ERH's) representative of the Ghanaian tropic conditions. The colour change during storage was also assessed by the Hunter's L* a* b* colour system. The production of aflatoxin (B_1 B_2, G_1, and G_2) before and after gamma irradiation (0, 20KGy) by resident Aspergillus flavus spores was also investigated. The moisture sorption isotherms of both solar and sun dried cassava flours followed a near sigmoid curve and equilibration at ERH's (55, 75, 95 %) was attained after 4-6 days at 32^0C. The drying method did not significantly ($P>0.01$) influence the sorption isotherms. A gamma irradiation dose of at least 5KGy eliminated the infesting insects predominated by Araecerus fasciculatus and Lasioderma serricorne. Analysis of variance to determine the influence of incubation humidity (A) accession number (B) and radiation treatment (C) as well as the interaction of these factors showed that A, B, C significantly ($P<0.01$) influenced colour change of flours. Interaction between AB, AC as well as BC and ABC were significant. The natural uninoculated and unirradiated cassava flour (122II, 032II, DMA030) did not contain aflatoxin G_1. There was an apparent enhanced formation by A. flavus spores in the artificially inoculated cassava flour samples after irradiation with 20 KGy but this was not attributable to the irradiation treatment per se but rather to the tendency of reduced inoculum of A.flavus to produce more aflatoxins.

INTRODUCTION

The challenge of post harvest deterioration of cassava because of the high moisture content of the tubers poses a serious post-harvest conservation problem. Drying roots into various storable forms is the acceptable practice. Dried cassava chips, if well preserved, is one form in which cassava can be preserved for subsequent sale or further processing (Fish and Trim, 1993). Cassava chip, could serve as a major income earner particularly through export. Among the local products of cassava chips is kokonte flour. The proc-

essing and marketing of kokonte flour constitutes an important source of income, especially among women in rural farming communities (Anon, 2004). This has the potential of being developed into a cottage industry, since kokonte, a Ghanaian local dish, is widely patronized by many people, especially in chop bars.

Most consumers especially the Ghanaian consumer prefer a white or less often pure yellow appearance of cassava products; deviation from these colours to brown or black may ward of some consumers. Brown to black coloured flour may be an indication of moldiness or chemical reaction during drying or processing. The visual quality of cassava chips depends on the drying rate; chips dried rapidly under good conditions either on concrete at 5kg/m^2 or in trays at 10kg/m^2 are brilliant white; so are chips dried in a solar dryer (CIAT, 1976).

Insect infestation, leading to degradation and weight loss of chips could be a major setback in cassava chips storage. During traditional sun-drying, chips are exposed to insect- eggs deposit which hatched when favourable conditions are created during storage. Losses in Togo from pest have been estimated at up to 30% after 6 months of storage (Wright, 1993). Inefficient drying and storage can also result in the significant reduction in cassava flour quality as a result of rapid mould spoilage and mycotoxin production. Aflatoxin (and other mycotoxins) contamination of cassava has been documented in Mozambique and Zaire (Mota and Lourenco, 1974; Brudzynski *et al.,* 1977), Burundi (Sajise and Illang, 1987), Ghana (Lokko, 1978; Wareing *et al.,* 2001).

This paper reports the findings of a research carried out to provide information on the susceptibility of ten new cassava accessions in Ghana to insect-mould contamination before and after irradiation treatment. Aflatoxin formation by resident *A.flavus* in naturally and artificially inoculated samples was also investigated. Finally the moisture sorption isotherms of the flours of the cassava were also determined at Environmental Relative Humidities ERH's representative of the Ghanaian ambient tropic conditions.

MATERIALS AND METHODS

Sample Preparation of Cassava Chips and Flour

Root tubers of ten accessions of Cassava (*Manihot esculenta* Crantz) tubers namely; 006II, 007II, 009II, 032II, 042II, 053II, 122II, 167II, 175II, DMA030II, were obtained from the University of Ghana Botanical Gardens. These were among accessions selected from germplasm collection maintained at the various Germplasm Conservation Centers and farming fields throughout Ghana and used in the Presidential Special Initiative programme on cassava. Ten shrubs of each accession uprooted were peeled and cut manually into thin sizes as practiced locally. The average chip size was 3.0 X 1.6 X 0.3 cm. Cassava chips were dried using solar-drying and sun drying methods. The solar dryer was made of a 120cm X 59 cm wooden rectangular tray roofed by a dense transparent polyethylene sheet erected on stands 150cm high. Three circular holes of circumference 19.6cm were made at the two extreme ends of the wooden tray, which were sealed with nylon mesh to facilitate effective percolation of air current over the samples. This encouraged drying by convection currents. Compartments were made in the rectangular tray for each accession to avoid spillovers. Samples to be sun dried were spread on 10 flat trays and dried daily in the open sun for up to 14 days. Both samples were turned regularly for free movement of air. Dried cassava chips were later milled into flour using a model 4–E Quaker City Mill, USA and a hammer mill of sieve size 20μm. Grinding was done repeatedly to obtain very fine powders.

Moisture Sorption Isotherms Studies Of Flour Samples

Transparent plastic bags of dimensions 90cm X 40 cm were made into hoods by the insertion of a metal frame into these plastics. Glycerol: water mixtures were prepared to obtain Equilibrium

Relative Humidities ERH of 55%, 75% and 95%. The glycerol: water mixture of appropriate humidities were poured into 12.5cm diameter Petri dishes and each placed at the centre of hood to create the specific ERH condition required. The ambient ERH's were checked by using Diplex Electronic '6 in1' maximum and minimum thermometer/hygrometer (Diplex Limited Wafford, England). 5g of the cassava flour was poured into plastic Petri dishes (diameter 9.0 cm). These were also arranged in the hoods and sealed. The samples were weighed at pre-determined intervals of 2, 4, 6, 8, 10, 12, 22, 24 days. The increase in weight was noted and plotted on a graph. The ambient ERH's were checked daily using an electronic Thermometer/ Hygrometer (Diplex Limited, Watford, U.K).

Culturing Pure Culture of *A. flavus*

Pure cultures of *A. flavus* were raised on Oxytetracycline Glucose Yeast Extract Agar (Oxoid, CM 545). 30g of maize grits placed in large Petri dishes of diameter 12.5cm was moistened with sterilized distilled water. These were autoclaved for 15min at 121^{0}C steam pressure. The plates were allowed to stand for 5days to ensure that the maize grains were not contaminated. Under aseptic conditions pure cultures of *A. flavus* were scraped with a flame-sterilized forcep and mixed with the moistened maize grains. The samples were incubated at 32^{0}C. The population of *A. flavus* after incubation for 7 days was determined using the decimal serial dilution technique up to 1:10^4 dilutions. This gave a population of 1.26 X 10^{6}CFU/g sample.

Irradiation of Cassava Flour Inoculated with Spores of *A. flavus*

250g of cassava flour was poured into each Beatson Clark bottle. The sealed bottles with contents were treated with gamma irradiation from a ^{60}Co source (Gamma Irradiator, Budapest, Hungary) at dose levels of 5KGy, 10KGy and 20KGy. Selected cassava flour of the controls and 20KGy were either untreated or inoculated with spores of *Aspergillus flavus* (1.26 X 10^6 spores/ml) and then covered for 4 days before irradiation. The spores were harvested dry from sterilized (121^{0}C for 20min) cracked maize. The seven day old spores were separated from the maize medium by sieving with a plastic mesh and 0.1g of spores transferred into a McCartney tube. NB. The unirradiated and uninoculated samples were code named as 0KGy

Determination of Aflatoxin Levels in Cassava Flour Samples

This was determined using the High Performance Liquid Chromatography (HPLC) Equipment at the Food Research Institute, CSIR- Ghana, following the method outlined by Pons (1979). Only the named samples in Table 3, of the controls (unirradiated and uninoculated) and the 20KGy samples (the highest dose applied) were subjected to aflatoxin determination.

Moisture Content Determination

Moisture content of flour samples was determined using an Electronic Moisture Meter (Shimadzu, Japan Model EB - 330 MOC). 4g of the dried cassava flour was weighed on the silverpan on the balance section on the meter. As this sample was heated up by the heater unit of the meter, the decrease in weight and the ERH values were registered automatically.

Colour Measurement of Samples

The Minolta Chromameter 310 (Osaka, Japan) was used for this measurement. It is a compact tristimulus colour analyser for measuring reflective colour of surfaces. This instrument consists of a measuring head and data processor, which measures the chromaticity (colour quality of light). The internationally accepted ICI system of colour measurement recorded the L*a*b* values automatically.

L values are, L=100(white), L=0(black); a values are, +a (red), –a (green); b values are, +b (yellow); -b (blue).

Preparation of cassava chips and flour for storage

Samples of the chips produced from the 10 cassava accessions were either sun-dried or solar-dried for 14 days. The chips were then stored in Beatson Clark Bottles and tightly covered. The bottles were stored at room temperature (30-32°C) on rubber bungs in aluminum trays containing dirty engine oil. This was to avoid insects crawling from the exterior into the bottles. Portion of the chips were blended into powder and kept in a similar manner as the chips. After a six-month period of incubation, the powder produced as a result of insect degradative activity was sifted and weighed. The insects present (dead and alive) were recorded and identified. The weight of the remaining portion of the cassava chips and flour were also recorded.

Statistical Analysis

Data collected were subjected to statistical analysis with Genstats software using the randomized complete block design.

RESULTS

Dry Weight and Moisture Content of Cassava Chips

The total water content of the fresh cassava accessions varied from 69.6 % (175II) to 55.5 % (167II). Each accession was unique in its ability to hold and retain water (Data not shown). The sun-dried flour had a moisture content varying from 9.35% (009II) to 12.17% (122II) while the solar-dried samples varied in moisture content from 8.8% (042II) to 12.1% (167II). No generalisation could therefore be made about the moisture content of the sun-dried and solar dried chips although in some instances the moisture content was higher in the sun-dried samples (006II, 032II, 042II, 053II) than in the solar dried samples.

Moisture sorption isotherms of ten accessions of cassava flour stored at 55%, 75% and 95% ERH for 24 days at 28-30^{0}c

The cassava flour prepared from the ten different accessions behaved differently in their ability to absorb moisture at the same temperature. There was however no significant difference (P>0.01) in moisture sorption pattern between the sun-dried samples and the solar dried samples (Figs. 1 and 2).

Generally, moisture sorption by all the samples (solar-dried and sun-dried) followed a sigmoid curve reaching approximate equilibrium after 4-6days at either 55% or 75% ERH. The only exceptions were accessions 030IIDMA at 55% ERH that showed an increase in weight from day 12 to day 24 and accessions 007II and 009II at 75% ERH that also showed a decrease from day 12 to day 24. At 95% ERH, the sorption curve of the sun-dried and solar dried samples kept rising. At 55% and 75% ERH, the highest value was obtained in accession 167II (Figs. 1&2) and the least in 175II.

Comparative moisture content of ten accessions of sun-dried and solar dried cassava flour incubated at ERH'S of 55%, 75% and 95% before and after irradiation

The higher the storage ERH, the greater the final moisture content after 50 days. Samples stored at 95% ERH had a final moisture content ranging from 16.30% to 19.58% as compared to 11.75-15.68% at 75% ERH and 12.1-14.02% at 55% ERH (Fig 3a&b). The differences in moisture content between accessions, radiation treatment, drying method, and ERH were statistically significant ($p < 0.01$).Analysis of variance of the interaction between drying method and ERH, the final moisture contents were not statistically significant ($p>0.01$)

Influence of storage humidity on colour of cassava flours before and after treatment with varying doses of gamma irradiation

Application of analysis of variance in order to examine the influence of incubation humidity (A) accession type (B) and radiation treatment (C) as well as the interaction of these factors showed

that A, B, and C significantly ($P < 0.01$) influenced the L*a*b* values of the different accessions. Closer examination of the interaction of A and C showed that 'L' values became darker with increasing radiation dose and storage humidity; 'a' values became reddish with increasing radiation dose and storage humidity. Finally, 'b' values increased and became more yellow but the extent depended on the accessions (Figs.4a-c). There were also marginal differences in colour change between the solar-dried and sun-dried samples. Though both samples were very white in colour with very high L values these values decreased, skewing towards black with increasing ERH.

Hidden infestation of cassava chips and flour stored for varying periods at 60-70% ERH and 28-32°C

The species of insects in the cassava chips are shown in Table 1 and that of the flour is shown in Table 2. There were large numbers of insects (dead and alive) in the flour of sun-dried accessions 006II, 042II, 053II, 167II and 175II (ranging from 92 to 1151). The insects encountered were predominantly *Araecerus fasciculatus* (De Geer) Anthribidae and *Lasioderma serricorne*(Fab) Anobiidae. From Table 1 solar dried cassava flour were not infested at all after 6months storage. Gamma irradiation of fresh dry flours with 5-20KGy drastically reduced or eliminated all insects (Table 2). Solar-dried samples were not susceptible to insect-attack.

Aflatoxins production by artificially inoculated cassava flour before and after gamma irradiation

The uninoculated samples of cassava flour accessions 122II, 032II did not contain any aflatoxins (B_1, B_2, G_2) after storage at 28-30°C for 6 months. Only DMA030II contained 19.0µg/kg aflatoxin G_1 (Table 3). Aflatoxin G_2 production was attenuated in samples of irradiated accession 122II and DMA 030II. There were some instances of enhanced total aflatoxins formation in the rest of the artificially inoculated samples after irradiation

Table 1: Hidden infestation in cassava chips of the indicated accessions stored in Beatson's bottles for 6 months at 28± 3°C

Cassava accession number	Type of Drying	Weight of cassava chips (g) Initial	Final	% Weight loss	Total no. of insects (Dead and Alive)	Type of Insect
006II	Sun	123.4± 0.2	114.3±4.1	7.4	578±0.0	*Araecerus fasciculatus*
	Solar	126.7± 0.5	118.3±1.0	6.6	0.0	-
032II	Sun	102.9+0.4	80.2± 0.6	22.1	0.0±0.0	*Araecerus fasciculatus*
	Solar	118.9±0.7	112.6±0.5	5.3	0.0	-
042II	Sun	80.4±1.1	75.2±0.8	6.5	1151±0.0	*Araecerus fasciculatus* & *Lasioderma serricorne*
	Solar	127.0±0.4	122.4±2.0	3.6	0.0	-
053II	Sun	83.1±0.3	70.8±0.9	14.8	660±0.0	*Lasioderma serricorne*
	Solar	86.9±1.0	83.1± 2.0	4.4	0.0	-
122II	Sun	120.0±1.0	101.1±1.1	15.8	92±0.0	*Lasioderma serricorne*
	Solar	127.0±2.0	122.4±0.4	3.6	0.0	-
167II	Sun	105.1±1.1	100.0±1.2	4.9	630±0.0	*Araecerus fasciculatus* & *Lasioderma serricorne*
	Solar	110.1±2.0	106.0±1.8	3.7	0.0	-
175II	Sun	79.7±1.4	69.6±3.0	12.7	578±0.0	*Lasioderma serricorne*
	Solar	133.2±2.0	126.0+0.4	5.4	0.0	-

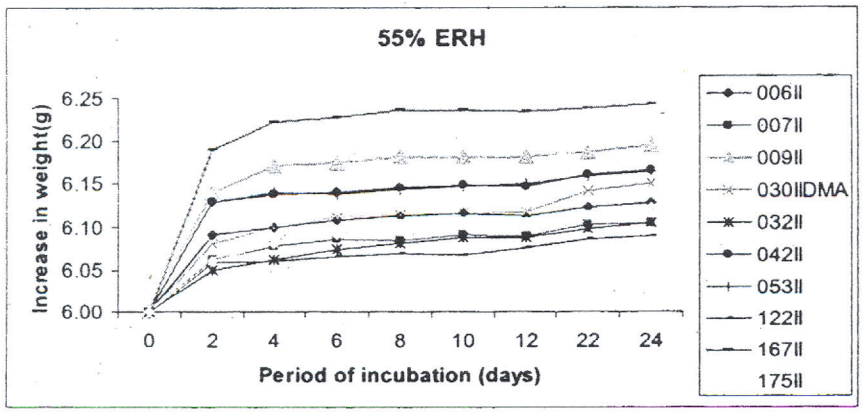
Table 2: Record of number of live insects recorded in the indicated dry cassava flour treated with varying doses of gamma irradiation and then stored for 6months in Beatson's Bottle at 28± 3°C

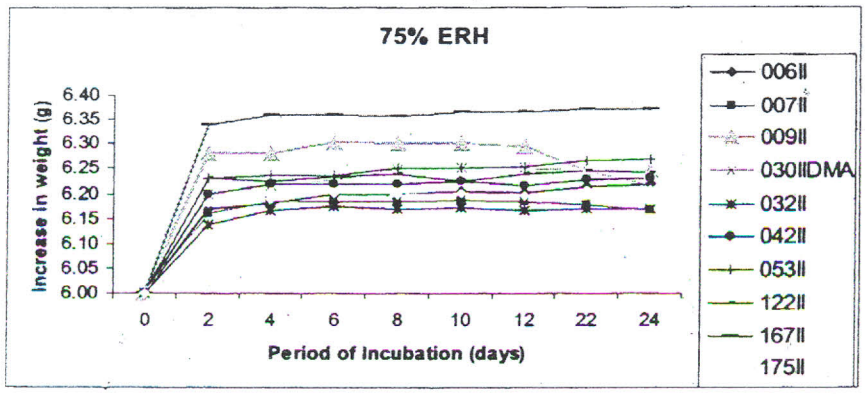
Cassava accession number	Type of Flour	Dose applied (KGy) 0(control)	5	10	20
		Number of insects	Number of insects	Number of insects	Number of insects
006II	Sun	1	0	0	0
	solar	2	0	0	0
007II	Sun	56	3	0	0
	solar	10	0	0	0
009II	Sun	42	0	0	0
	solar	31	0	0	0
DMA030II	Sun	44	3	0	0
	solar	4	0	0	0
032II	Sun	8	0	0	0
	solar	3	1	0	0
042II	Sun	6	1	1	0
	solar	0	0	0	0
053II	Sun	8	0	0	0
	solar	0	1	0	0
167II	Sun	62	1	0	0
	solar	21	2	0	0
175II	Sun	4	1	0	0
	solar	1	2	0	0
122II	Sun	22	5	5	3
	solar	20	3	5	2

Table 3: Aflatoxin production by A. *flavus* in Cassava flour of the indicated accessions before and after gamma irradiation and storage at 28-30°C for 6 months

Accession Number (Code)	Aflatoxin (mg/kg)	Uninoculated samples 0KGy		20KGy		Inoculated samples 0KGy		20KGy	
		Solar	Sun	Solar	Sun	Solar	Sun	Solar	Sun
122II	B_1	ND	ND	ND	ND	92.0	278.0	308.4	308.6
	B_2	ND	ND	ND	ND	3.5	92.0	8.8	9.2
	G_1	ND	ND	ND	ND	ND	48.6	ND	ND
	G_2	ND	ND	ND	ND	ND	ND	ND	ND
	Total	-	-	-	-	**95.5**	**340.0**	**317.2**	**312.8**
032II	B_1	ND	ND	ND	ND	203.6	830.2	718.9	2458.9
	B_2	ND	ND	ND	ND	12.9	12.3	50.6	67.3
	G_1	ND	ND	ND	ND	288.0	31.0	299.3	ND
	G_2	ND	ND	ND	ND	435.0	21.7	477.9	ND
	Total	-	-	-	-	**939.8**	**895.2**	**1546.7**	**2526.2**
DMA030II	B_1	ND	ND	ND	ND	72.8	60.8	732.0	393.7
	B_2	ND	ND	ND	ND	2.1	5.1	18.0	13.8
	G_1	19	ND	ND	ND	10.7	ND	ND	6.9
	G_2	ND	ND	ND	ND	7.2	209	ND	ND
	Total	-	-	-	-	**92.8**	**274.9**	**750.0**	**414.4**

ND = NOT DETECTED
\- = Nil





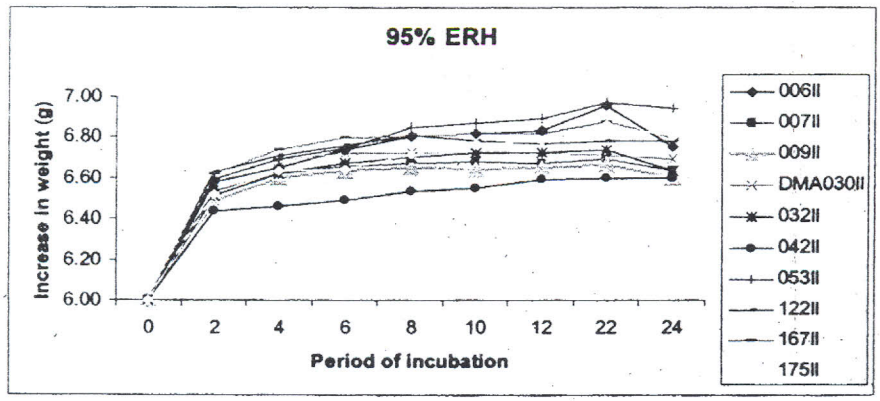


Fig.1: Moisture sorption by sun-dried cassava flour samples incubated at Environmental Relative Humidities of (55, 75 and 95) % at 28 ^{0}C up to 24 days.

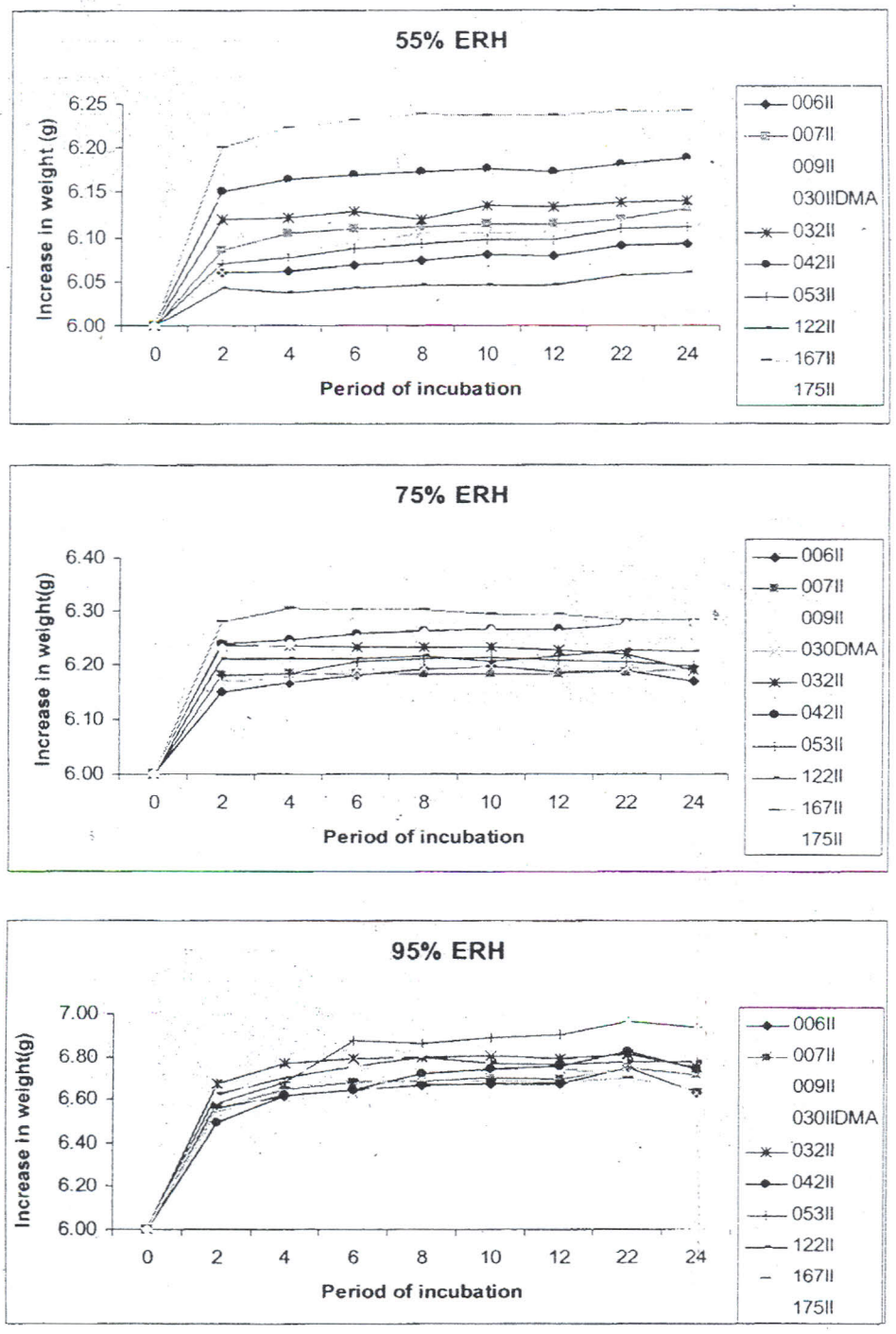


Fig.2: Moisture sorption by sun-dried cassava flour samples incubated at Environmental Relative Humidities of (55, 75 and 95) % at 28 ^{0}C up to 24 days.

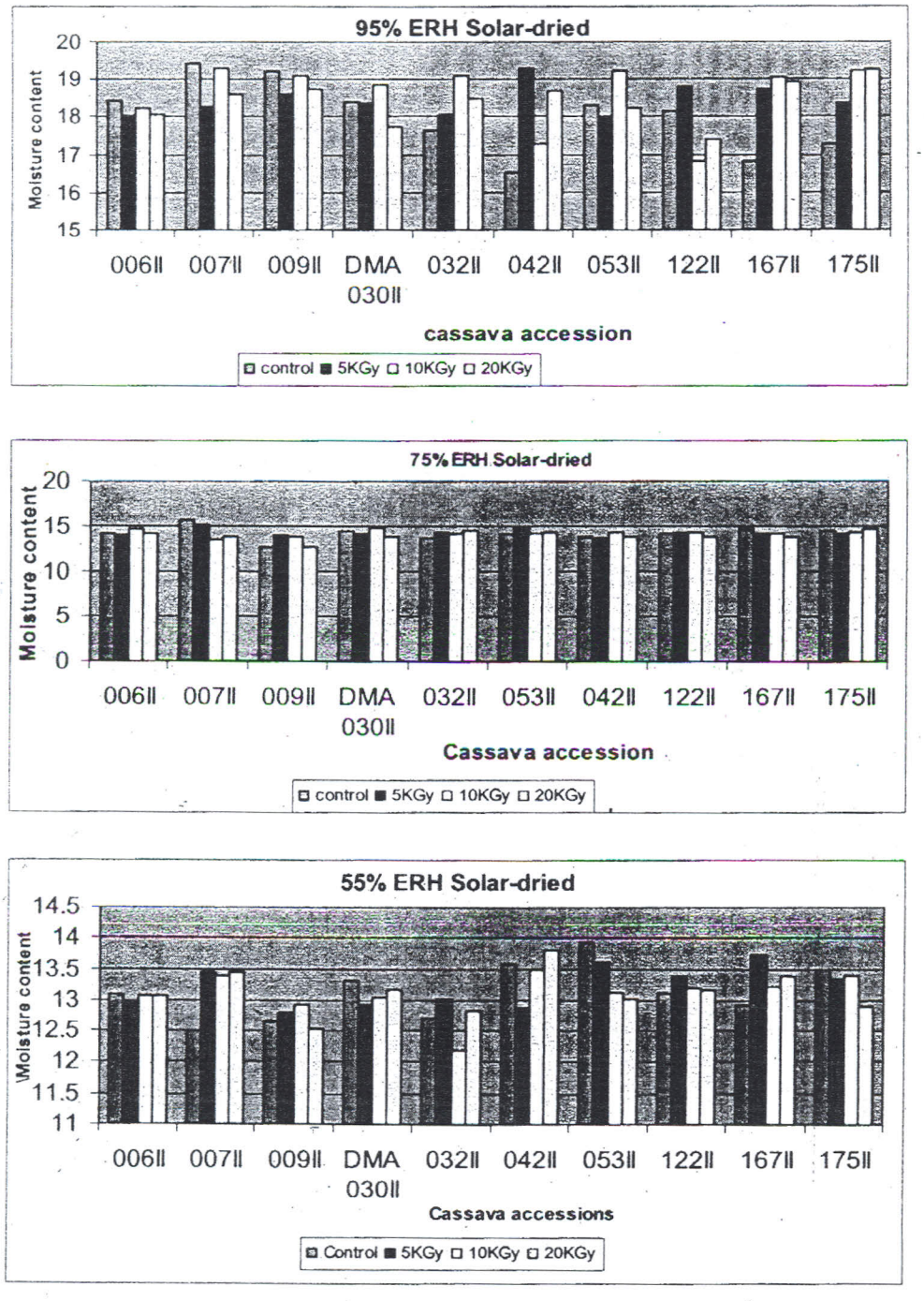


Fig3a Moisture contents of different solar-dried accessions of cassava flours stored at varying ERH's (95, 75, 55) % for 50 days.

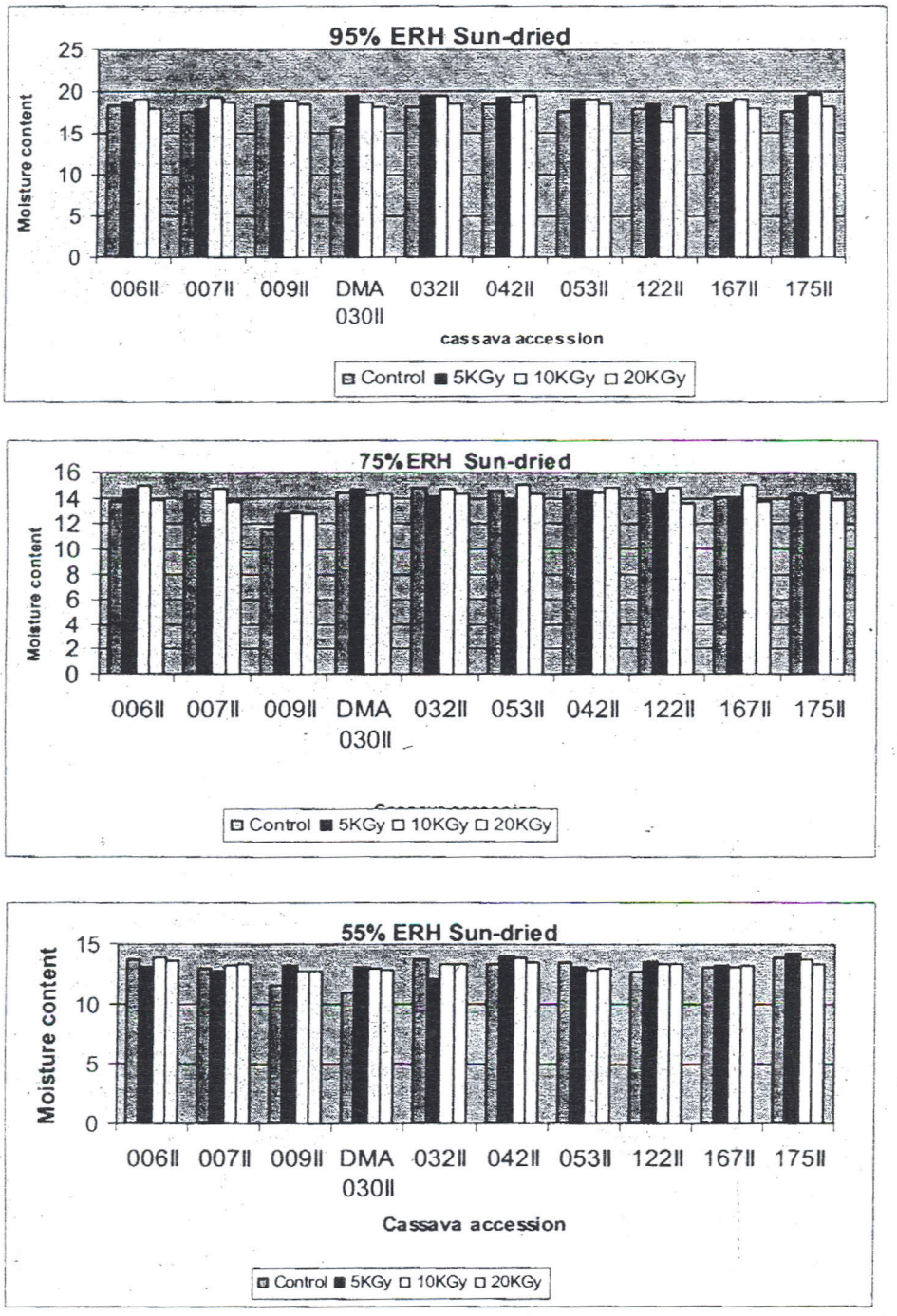


Fig3b Moisture contents of different sun-dried accessions of cassava flours stored at varying ERH's (95, 75, 55) % for 50 days

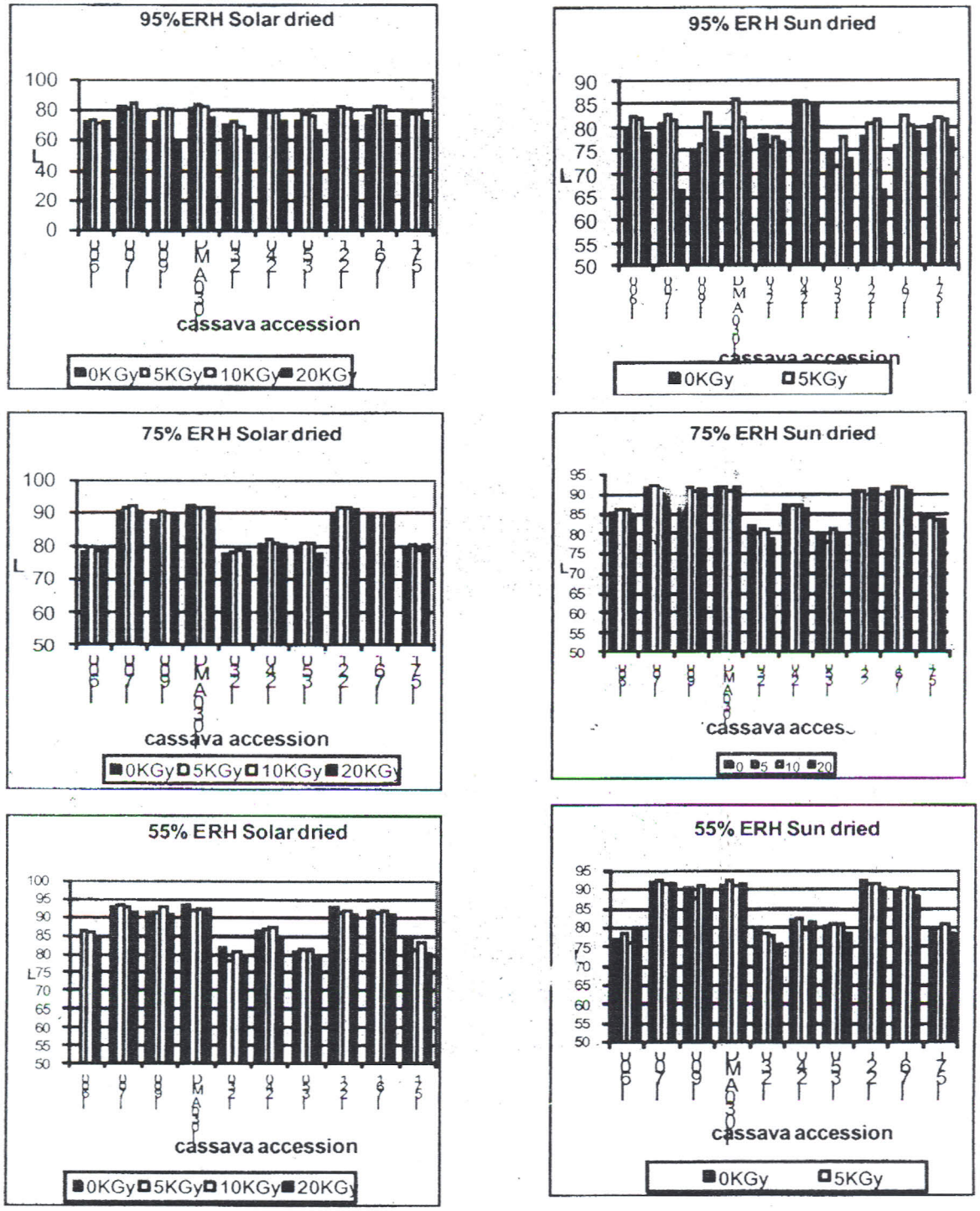


Fig.4a **Changes in L (L=100, white; L=0, black) value of radiation treated cassava flours of different indicated accessions stored at either (95, 75, 55) % ERH for 50days.**

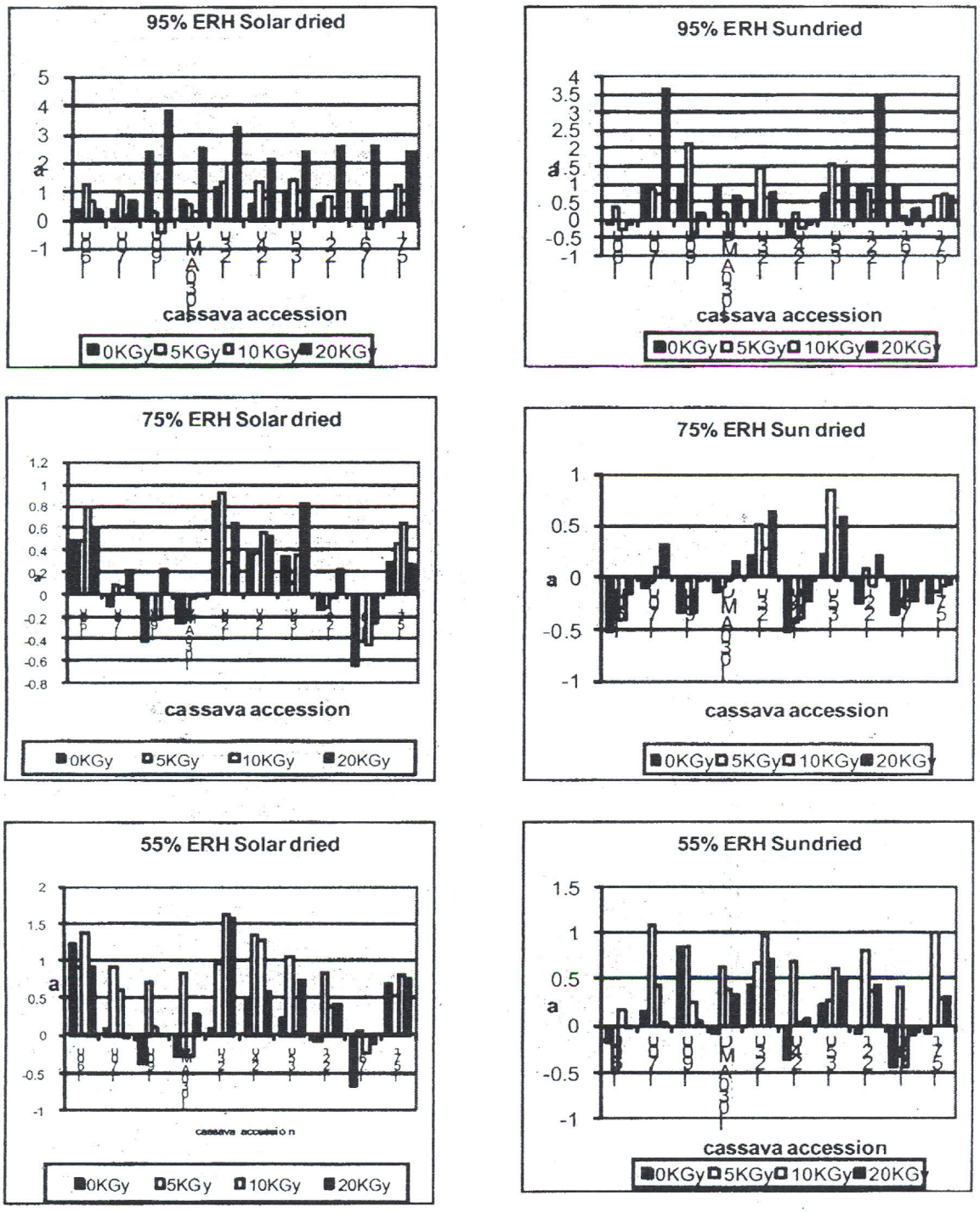
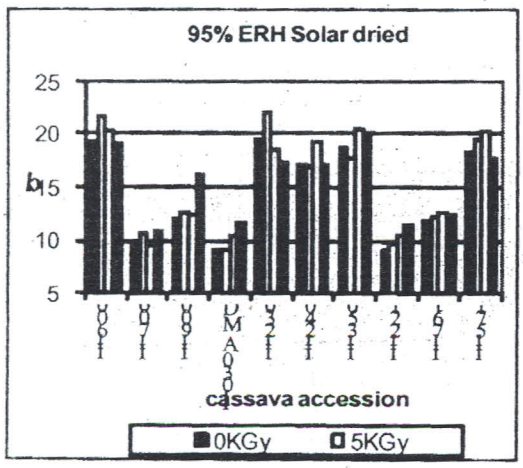
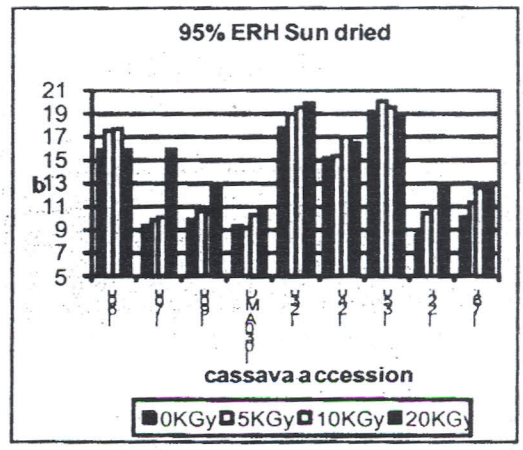
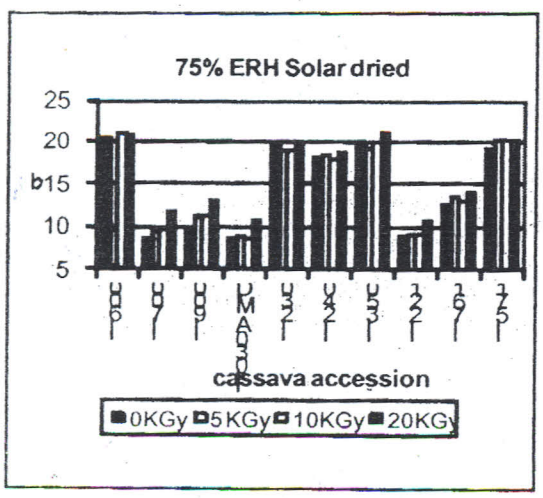
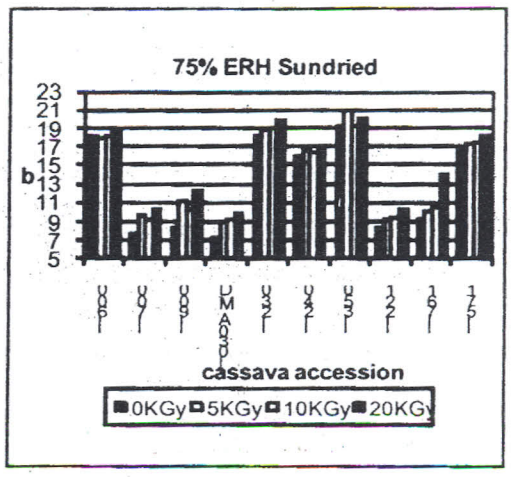


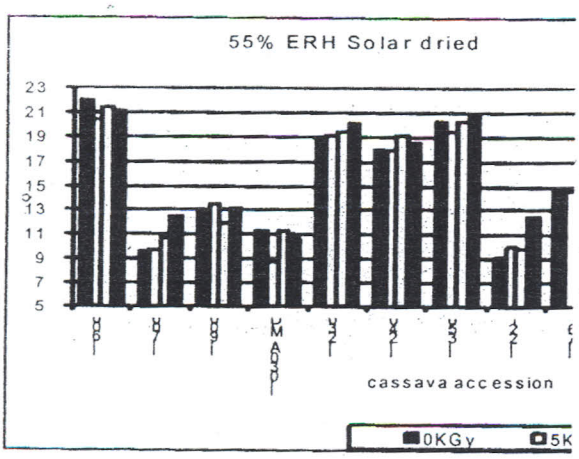
Fig.4b: Changes in a (+a=red; -a=green) value of radiation treated cassava flours of different indicated accessions stored at either (95, 75, 55) % ERH for 50days

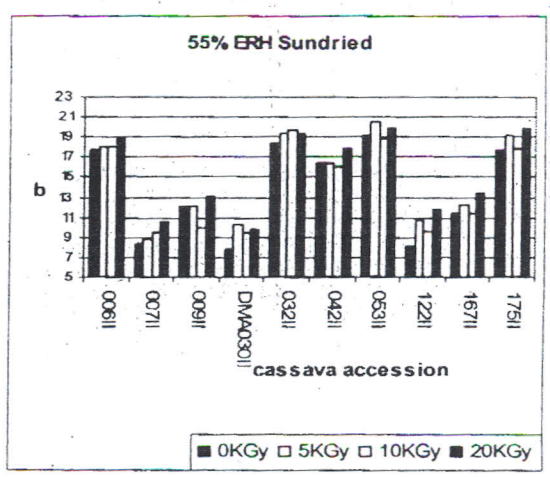

95% ERH Solar dried
b
cassava accession
0KGy
5KGy


95% ERH Sun dried
b
cassava accession
0KGy
5KGy
10KGy
20KGy


75% ERH Solar dried
b
cassava accession
0KGy
5KGy
10KGy
20KGy


75% ERH Sundried
b
cassava accession
0KGy
5KGy
10KGy
20KGy


55% ERH Solar dried
cassava accession
0KGy
5K


55% ERH Sundried
b
cassava accession
0KGy
5KGy
10KGy
20KGy

(Table 3). The total aflatoxin formed in accession 032II was largest (2526.2µg/kg) and least in accession 122II (312.8µg/kg). There were varietal differences in the susceptibility of the accession to aflatoxin contamination.

Cassava chips and flours are very hygroscopic and absorb moisture commensurate with the ambient conditions. The water activity, a_w of foods determines their susceptibility to fungal infection and subsequent deterioration. Wilson and Payne (1994), stated that food water activity values of less than $0.70a_w$ (i.e. ERH 70%) are unlikely to support spoilage by microorganisms. In considering storage potential, the ambient 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). The moisture sorption isotherms of the ten cassava accessions followed a near sigmoid curve and Equilibrium Relative Humidity was attained after 4-6 days (Figs. 1 and 2). The only exceptions were accessions 030IIDMA at 55% ERH that showed an increase in weight from day 12 to day 24 and accessions 007II and 009II at 75% ERH that also showed a decrease from day 12 to day 24. Samples stored at 95% ERH had very high moisture content ranging from 16.3-19.6% followed by 75% ERH (11.8-15.7%) and 55% ERH (12.1-14.0%) (Figs. 3a and b). Wilson and Payne (1994) showed that safe storage of dry vegetables is reached when moisture content is 10-20% and in flour at 13% moisture content. The cassava flour accessions stored at 95% ERH had very high moisture content and were visibly mouldy within 6-12 days. It is therefore safer to store samples at ERH 55% and below to extend the shelf-life. Recently, Obadina *et al.* (2007) showed that fufu flour (also dehydrated cassava) would keep best when stored under condition of 33% ERH at room temperature. Odamtten *et al.* (1980) found that maize and maize flour also equilibrated within 4 -6 days at ERH 55%-75% and attained safe moisture content of 12-14%. Cassava flours (kokonte) for sale on the market exposed to high ERH values could promote growth of resident mycoflora and lead to mycotoxin contamination. Barimah and Mantey (2002) showed that different drying methods (sun, oven, tray and solar) have no significant effect on the moisture content, ash, amylose and solubility of cassava starches. In this present study, moisture content of sundried samples ware not significantly different from the samples that were solar-dried. However, the differences in moisture content within the flour accessions stored at the varying ERHs were significant ($p<0.01$) (Figs. 3a and b).

Work by Rombo *et al.* (2001) on irradiated maize and beans flours showed that the flours became darker as well as more red and yellow with increased irradiation doses, probably owing to Maillard's browning reaction. In a similar pattern, flour in this work became darker, reddish and yellowish (Figs 4a – c) with increasing irradiation dose and with increasing ERHs for both sun and solar-dried accessions. The darker colour of samples at 95% ERH could be attributed to the effect of prolific growth of the resident mycoflora with the fungi appearing on the substrates in different colour hues. The fungal growth exudates could also impact shades of colours to the substrates.

Post harvest deterioration in fresh cassava (Wenham, 1995; Beeching *et al.*, 1998, Rodriguez *et al.*, 2000) occurs in two stages: Primary Physiological Deterioration (PPD) characterized by the discolouration of the vascular system initiated from 24hr to 48hr after harvesting depending on the genotype; and secondary (microbial deterioration), which is decay due to microbial invasion through wounds within 5-7days (Plumbley and Rickard, 1991).These processes may influence the development of the dark colouring effect of end product of cassava flour since chips samples are usually dried for 2 weeks.

The insect population in the stored flour accessions were killed with increasing radiation dose (5-20KGy) (Table 2). Bhuiya *et al.* (1985) disinfested pulses, oil seeds and tobacco leaves of in-

sects such as *Callosobrochus anailis* (Fab). *Sitotroga cereallela* (OL) *Oryzaephilus surinamensis* (L) and *Lasioderma serricorne* (F) using a dose of up to 0.5KGy. A dose of 0.1 to 0.25KGy were effective in eliminating adult survivors from irradiated older larvae and pupae of *Araecerus fasciculatus* in copra, desiccated coconut and coffee bean (Manoto *et al.*, 1985). The dose used in the present study is therefore adequate to eliminate the predominant insects (*A. fasciculatus* and *L. serricorrne*) in the stored cassava accessions.

The insects encountered in the cassava accession flours showed food preference. There was large numbers of *A. fasciculatus* and *L. serricorne* in accession 009II, 007II, 167II and DMA030II (Table 1). In Malaysia, *Rhizopertha dominica* was the most destructive insect in the well-dried cassava chip, while *L. serricorne* and *A.fasciculatus* were noted as serious pests of dry cassava chips (Parker and Booth, 1979). A male variety of insects feed directly on dried foodstuffs and some mould feeding insects have been reported causing weight loss in stored cassava chips. It is known that infestation of cassava chips occur during the sun-drying process (Parker and Booth, 1979). Data from this study also confirms this finding because more insects were associated with the sun-dried cassava flour samples which may be attributed to the presence of deposited insect's eggs in the chips during the exposed sun-drying as compared to the covered solar-drying. High quality, insect-free cassava chips may be produced by rapid drying of chips in a confined solar-dryer and milling immediately to keep product away from major insect infestation. Flours are the generally preferred way to preserve cassava chips since the bulky chips predispose them to attack by borers which do not thrive in flours (Balagopalan *et al.* 1988).

The aflatoxins formed in the uninoculated and irraditated accessions were variable. There was also varietal differences in the susceptibility of the cassava accessions to aflatoxin contamination (Table 3) before and after irradiation. Several environmental, nutritional and genetic factors considerably influence the type and amount of aflatoxins produced by moulds of the genus *Aspergillus* (Maggon *et al.*, 1977; Zuber and Lillehoj, 1979). For *A. flavus* and *A. parasiticus* the optimal nutritional availability of certain trace metals is a crucial factor (Maggon *et al.*, 1977).

Cassava leaves and peels are known to contain variable concentrations of calcium, phosphorous, magnesium, copper, iron, manganese, zinc, vitamin A, riboflavin, thiamine, niacin, vitamin C etc. (Kudjoe and Amoa-Awua, 2001). Although the mineral concentration profile was not determined in the cassava accessions, the stimulatory effect of Zinc on aflatoxin production is well documented (Lee *et al.*, 1966; Maggon *et al.*, 1977; Lillehoj *et al.* 1974; Gupta and Venkitasubramanian, 1975; Obidoa and Ndubuisi, 1981).

Surprisingly, gamma irradiation enhanced aflatoxin B_1B_2 and G_1 production by *A.flavus* in accessions (122II, 032II and DMA 030II). Studies by Schindler *et al.* (1980) indicated an increase in aflatoxin production after gamma irradiation of maize. However, subsequent studies by Odamtten *et al.* (1986; 1987) showed that the irradiation per se does not bring about enhanced aflatoxin production and that reducing the inoculum size of *A. flavus* spores by 3.0-4. log cycles after irradiation with 5.0KGy was followed by abundant sporulation and enhanced aflatoxin formation. Aflatoxin production was therefore inversely proportional to inoculum size of *A. flavus* spores (Odamtten *et al.*, 1987). Heavy inoculum used in the control resulted in vegetative growth predominating over sporulation. Detroy *et al.* (1971) showed that aflatoxin synthesis occurs during the period of intense sporulation of the fungus and that good aflatoxin production correlated with yellowing of mycelium and medium. There is another explanation for enhanced aflatoxin formation after irradiation. The growth of fungi is restricted to the apical region of the mycelium for cell multiplication and branching (Burnett, 1968). Sharma *et al.* (1980) suggested that aflatoxin production in a medium might be associated with mycelium

branching and differentiation. With smaller spore populations of *A. flavus*, more lateral branching and secondary mycelial growth is afforded, which may in turn result in higher yield of aflatoxin at least in culture. A novel approach to control fungal contamination of stored fresh and dehydrated produce is by the combination treatment of heat and gamma irradiation. Radiation treatment combined with heat has been found to be more effective than radiation treatment alone (Roy *et al.* 1972; Odamtten *et al.* 1985; Langerak and Cañet-Prades 1979). Aflatoxin B_1, B_2, G_1 and G_2 formation by *A. flavus* NRRL 5906 was completely inhibited in maize by a combination treatment of moist heat (60°C for 30min) applied under high ambient conditions (> 85%R.H.) and 4KGy of gamma irradiation (Odamtten *et al.* 1985). It was shown that in addition to the temperature and duration of treatment, humidity markedly affected the lethality of heat applied.

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