Chromosomal Aberrations Induced in Root Tips of *Allium cepa* by Squeezed *Garri* Extracts

Daniel I. Olorunfemi and Emmanuel O. Ehwre

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria
udanfem@yahoo.com,

Abstract: The potential genotoxic effects of squeezed extracts from toasted cassava granules popularly known as *garri*, a popular Nigerian cassava meal, obtained from different fermentation days were investigated using the *Allium cepa* assay. The squeezed extracts were prepared with potable water as practiced conventionally by soaking 1-day fermented, 2-day fermented, 3-day fermented and 4-day fermented *garri* and squeezing out the liquid. A series of 5 onion bulbs were exposed to 1, 2.5, 5 and 10% (effluents, v/v) concentrations of each of the extracts for macroscopic and microscopic analyses. There was fermentation and concentration-dependent and statistically significant ($P < 0.05$) inhibition of root growth by the extracts when compared with the control. The EC_{50} obtained for 1-day, 2-day, 3-day and 4-day fermented toasted cassava granules were 2.5, 2.8, 3.1 and 4.0 respectively. All the tested extracts were observed to have mitodepressive effects on cell division in the increasing order 1-day > 2-day > 3-day > 4-day fermented *garri* extracts. The results further go to confirm findings from other studies that proper fermentation aids in the reduction of toxic cyanogenic components present in poorly processed cassava products including *garri*.
[Daniel I. Olorunfemi and Emmanuel O. Ehwre. Chromosomal Aberrations Induced in Root Tips of *Allium cepa* by Squeezed *Garri* Extracts. Report and Opinion 2010;2(12):166-171]. (ISSN: 1553-9873). http://www.sciencepub.net.

Key words: *garri*, genotoxicity; mitotic index; cyanide; *Allium cepa*

1. Introduction

Among the numerous processed forms of cassava roots (*Manihot esculenta*, Crantz), roasted granular product popularly known as *garri* is the most widely consumed in the West Africa sub region (Ofuya and Akpoti, 1988). In Nigeria, it is a staple for many of the 374 ethnic groups (Akoroda, 1992). It is popularly referred to as the common man's bread (Meludu *et al*., 2001). Its relative low cost, ease of handling and preparation before serving as a meal and its improved shelf life makes it amenable to the increasing trend in commercialization and urbanization (Irtwange and Achimba, 2009). The most widespread method of *garri* consumption in Nigeria is by pouring it into measured quantity of boiling water to produce a thick paste popularly called *eba* which is eaten by dipping small balls of it into soup or stew and swallowed. *Garri* is also consumed as snack when dispersed in cold water (soaked *garri*) and eaten directly with or without sweeteners (like sugar and salt), coconut, banana, peanut and smoked fish (Ogiehor and Ikenebomeh, 2006). The quality of *garri* available in the local market varies from batch to batch among the traders. Variations are observed in the colour, fibre content, moisture, particle size, starch content and toxic residual which are due to the cyanogenic glucosides, linamarin and lotaustralin present in all parts of the plant with the possible exception of the seeds (Coursey, 1973). The variations in commercially prepared *garri* are caused by cassava variety, age at the time of harvest, processing method and equipment and duration of fermentation (Irtwange and Achimba, 2009).

Fermentation is a method by which cyanogenic glucosides in *garri* can be reduced. The time allowed for fermentation is critical, if too short, the detoxification process will be incomplete, resulting in a potentially toxic product, if too long the product will have a strong sour taste and the texture will be poor (Azam-Ali *et al*., 2003). Collard and Levi (1959) reported that the acceptability of *garri* is influenced by its sourness and is directly related to the degree of fermentation. Poorly processed cassava products are undoubtedly associated with increased cyanogenic potential or ability to generate hydrogen cyanide, a well known poison with potential acute and chronic metabolic effect in humans.

The cyanogenic glucoside content is an important quality parameter of *garri*, due to its toxicity, if consumed in amount greater than 30ppm (Olarewaju and Boszoromanyi, 1975). Studies have shown that increased total cyanogens content of *garri* diet deplete as fermentation period increased (Ihedioha and Chineme, 1999).

There is likelihood that cyanide has been linked to pancreatic islet damage and diabetes (Akanji, 1994). Various test systems, using human and animal models (especially with animals e.g. rats) has been used to provide data, as a scientific basis for improving the processing of cassava product; however literature is scanty on the use of plant models. *Allium cepa* assay is undoubtedly a rapid and sensitive first tier assay for the cytological screening of toxic

effluents and chemicals (Fiskesjö, 1993). This study was undertaken to access the toxicity or otherwise of squeezed *garri* extracts processed with varied fermentation periods.

2. Materials and Methods

Collection of *Garri* Samples

Four types of *garri* samples based on the number of days of fermentation they were subjected to, were purchased from four local markets in Edo State of Nigeria and used for this study. The markets are well known for stocking specific types of *garri*, a reflection of the processing methods employed by the respective farmers/traders in these local communities.

One-day fermented *garri* was collected from Ugbejobo market located in Ovia-South-East Local government Area while two-day fermented *garri* was collected from Abudu market in Orhionwon Local Government Area. 3-day fermented *garri* was purchased from Auchi in Estako West Local Government Area while and four-day fermented *garri* was got from Ibillo in Akoko-Edo Local Government Area.

Ugbejobo market is located on the ever-busy Benin-Sagamu expressway while Abudu market is on the Benin-Asaba dual carriage way. Auchi market is located on the Benin-Abuja highway while Ibillo market is on the Ibadan-Ife-Abuja highway. Traffic on these roads is extremely high.

2.2 Preparation of Extracts

400g of *garri* was measured and dispersed into a plastic bowl containing 120cl of tap water at room temperature to soak the *garri*. This was left for 5 minutes to soak properly, after which it was stirred and the extract squeezed out and sieved with the aid of a cheese cloth. The residual *garri* particles were discarded.

2.3 *Allium cepa* Assay

Onion bulbs *(Allium cepa* L., 2n=16*)* bulbs of the purple variety of average size (15-22 mm diameter) were purchased locally in Benin City, Edo State in Nigeria. They were sun-dried for six weeks and the dried roots present at the base of the onion bulbs were carefully shaved off, with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primodial cells from drying up. To account for a number of bulbs in the population that would be naturally slow or poor growing, seven replicate bulbs were used for each test sample and control (tap water) and the best five bulbs were chosen at the approximate time for examination (Rank and Nielsen, 1993). The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water.

For root growth inhibition evaluation, freshly prepared stock extracts were diluted into five concentrations of 20, 10, 5, 2.5, and 1%. Seven onion bulbs were utilized for each concentration of each extract and the control (tap water). The base of each of the bulbs was suspended on the extracts inside 100mL beakers in the dark for 72 h. Test extracts were changed daily. At the end of the exposure period, the roots of five onion bulbs with the best growth at each concentration were removed with a forceps and their lengths measured (in cm) with a metre rule. From the Weighted averages for each concentration and the control, the percentage root growth inhibition in relation to the negative control and the EC_{50} (the effective concentration where root growth amounts to 50% of the controls) for each extract was determined (Fiskesjo, 1985). The effect of each sample on the morphology of growing roots was also examined.

For the evaluation of induction of chromosomal aberration, 5 onion bulbs were suspended on 10, 5, 2.5, and 1% concentrations (v/v) of each of the extracts and the control for 48 h. At the end of 48 h, root tips from these bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v). These were hydrolyzed in 1N HCl at 60^{o} C for five minutes after which they were washed in distilled water. Two root tips were then squashed on each slide, stained with acetocarmine for 10 min and cover slips carefully lowered on to exclude air bubble. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). This is to prevent drying out of the preparation by the heat of the microscope (Sharma, 1983).

Six slides were prepared for each concentration and the control out of which five (at 1000 cells per slide) were analyzed at ×1000 magnification for induction of chromosomal aberrations. The mitotic index was calculated as the number of dividing cells per 1000 observed cells (Fiskesjo, 1985, 1997). The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of each extract (Bakare *et al*., 2000).

2.4 Statistical Analysis

The means, with 95% confidence limits and the standard errors for results of the root inhibition and chromosome aberrations of each concentration of the extracts were calculated. Data were expressed as Mean ± Standard Error of Mean (SEM). Differences between the control and the different concentrations of the extract were analyzed by means of the Student's unpaired *t*-test. P values of < 0.05 were

considered to be statistically significant. All statistical analyses were carried out using SPSS®14.0 statistical package.

3. Results

Table 1 shows the results of the effects of the squeezed extracts of *garri* on root growth of *Allium cepa*. Maximum root growth was achieved in the control and without any morphological deformities. The roots were whitish in colour, straight and unbroken. At tested concentrations, root growth was highest at the 1% concentration of the 4-day fermented extract. On the other hand, mean root length was least at 10% 1-day fermented *garri* extract. Inhibition of root growth was concentration-dependent and statistically significant ($P < 0.05$) at tested concentrations. Morphological deformities observed in tested concentrations were short, bent, spiral and crochet-like roots. There was no root growth at a concentration of 20% of all extracts. The EC_{50} for the 1-day fermented, 2-day fermented, 3-day fermented and 4-day fermented extracts were 2.5, 2.8, 3.1 and 4.0 respectively.

Results of the microscopic analysis are presented in Table 2. Compared to the mitotic index (MI) value of 92.8% in the control, there was concentration-dependent decrease in the mitotic indexes in all concentrations and fermentation periods. In particular, the lowest MI value of 83.6 was recorded for 10% 1-day fermented *garri* extract. There were no dividing cells at the 20% concentration of the extracts. All the tested extracts induced chromosomal aberrations in all the concentrations of the extracts (Fig. 1) and they were statistically significant ($p < 0.05$).

Table 1 - Root length of *A. cepa* grown in squeezed *garri* extracts

Conc (%)	1-day fermented garri			2-day fermented garri			3-day fermented garri			4-day fermented garri		
	Mean Root length±S.E.	RG(%) of control	95% CL	Mean Root length±S.E.	RG(%) of control	95% CL	Mean Root length±S.E.	RG(%) of control	95% CL	Mean Root length±S.E	RG(%) of control	95% CL
0	3.75±0.21	-	0.34	3.75±0.21	-	0.34	3.75±0.21	-	0.34	3.75±0.21	-	0.34
1	2.13±0.17	73.09	0.98	2.31±0.16	88.54	0.50	3.23±0.17	89.75	0.70	3.31±0.25	91.35	0.31
2.5	2.04±0.14	64.65	1.10	2.08±0.18	65.24	0.50	2.83±0.11	67.94	0.56	3.08±0.22	68.79	0.52
5	1.56±0.08	36.62	0.44	1.28±0.05	37.58	0.68	2.10±0.18	40.43	0.60	2.65±0.04	42.17	0.70
10	0.33±0.08	20.51	0.46	0.44±0.07	28.73	0.40	1.17±0.07	31.85	0.40	1.53±0.09	35.67	0.40
20	No root growth			No root growth			No root growth			No root growth		
EC_{50}	2.5%			2.8%			3.1%			4.0%		

RG (%) of control expressed as % root growth of the control.
95%CL: 95% Confidence limit.
* $P < 0.05$, level of significance of root growth inhibition compared with the control. Values are Mean ± SEM

Table 2 - Cytological effects of squeezed *garri* extracts on cells of *Allium cepa*

Conc (%)	1-day fermented garri			2-day fermented garri			3-day fermented garri			4-day fermented garri		
	No. of dividing cells	Mitotic index	% of aberrant cells	No. of dividing cells	Mitotic index	% of aberrant cells	No. of dividing cells.	Mitotic index	% of aberrant cells	No. of dividing cells.	Mitotic index	% of aberrant cells
0	464	92.8	-	464	92.8	-	464	92.8	-	464	92.8	-
1	450	90	18	454	90.8	14	455	90.8	10	458	91.6	03
2.5	430	87	19	449	89	16	450	90	11	452	90	05
5	425	85	21	440	88	18	445	89	15	446	89.2	08
10	418	83.6	28	430	86	25	434	86.8	18	436	87.2	11
20		-			-			-			-	

*5000 cells (5 slides) per concentration of each effluent and the control

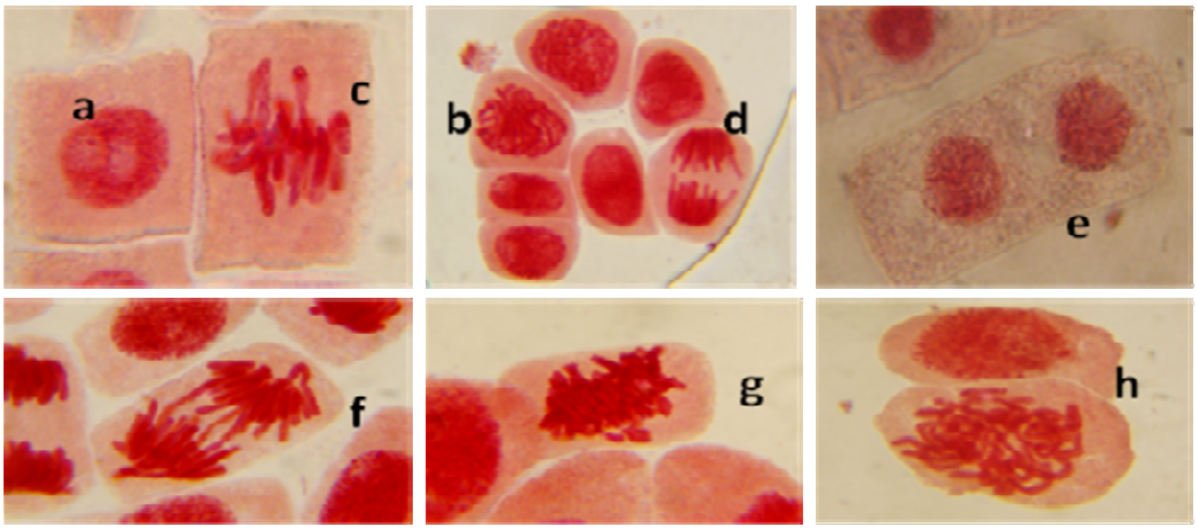

Figure 1. Stages of mitotic division in cells of *Allium cepa* treated with water extracts of *garri.* (a–e) Normal stages of mitotic division. (a) interphase (b) prophase (c) metaphase (d) anaphase (e) telophase (f) chromosome bridges (g) sticky chromosomes (h) spindle disturbance at prophase. Magnification 1000×.

Discussion

Garri is one of the most popular forms in which cassava is consumed in West Africa (Ofuya and Akpoti, 1988). Production methods vary from one locality to another resulting in products of non-uniform quality and the quality of *garri* available in the local market varies from batch to batch among the traders (Ogiehor and Ikenebomeh, 2006). There is likelihood that cyanide has been linked to pancreatic islet damage and diabetes (Akanji, 1994) and fermentation has been reported to be a veritable tool for reducing the total cyanide content of *garri* to appreciable safe levels (Ihedioha and Chineme, 1999; Asegbeloyin and Onyimonyi, 2005). Soaked *garri* is a *ready-to-eat* meal as it does not involve preparation with boiling water and accompaniment of soup or stew. This mode of *garri* consumption in West Africa and Nigeria in particular, is as popular as boiling water to produce a paste popularly called *eba* which is eaten by dipping small balls of it into soup or stew and swallowed in morsels. *Garri* soaking is an easier and more convenient mode of *garri* consumption as a snack than *eba* (Ogiehor, 2002).

In this study, the potential cytotoxic and genotoxic effects of squeezed extracts of *garri* on *Allium cepa* were evaluated. There was a linear relationship between macroscopic and microscopic parameters for all the extracts. There was concentration-dependent decrease in root growth and the order of induction of root growth inhibition based on EC_{50} values was 1-day >2-day > 3-day > 4-day fermented *garri* extracts. These values indicate that the samples were toxic; comparatively, the 1-day fermented squeezed *garri* extract has more inhibitory and mitodepressive effects than all the other extracts. This might be due partly to the cynogenic component in the *garri* as in all poorly fermented cassava products (Coursey, 1973).

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Our results showed among other aberrations, induction of sticky chromosomes, bridges and disturbance of spindle fibres at different stages of mitotic division in the onion root cells. In *A. cepa*, whenever chromosome aberrations occurred, there were almost always certain growth restrictions (Fiskesjo, 1997). Most of these aberrations are lethal which can cause genetic effects, either somatic or inherited (Swierenga *et al.*, 1991). The total root-growth inhibition in the onion bulbs and complete arrest of cell division by the squeezed extract of *garri* at the 20% concentration may suggest turbagenic activity of these extracts.

In spite of repeated warnings from research findings (Sanni, 1994; Asegbeloyin and Onyimonyi, 2007) on the danger of consumption of inadequately fermented *garri*, there is presently no legislation on sale of cassava processed into *garri* the same day in Nigeria by appropriate regulatory bodies. Furthermore, post process handling practices such as spreading *garri* on the floor, display in open bowls in the market and sales points and the use of various

packaging materials to haul finished products from rural to urban areas where large buyers live have been identified as possible additional ways exacerbating contamination (Ogiehor and Ikenebomeh, 2006). Results from this study further go to confirm suggestions fromother studies that proper fermentation is imperative if the level of toxic components of substances present in poorly processed cassava products must be reduced, especially in *garri*.

Acknowledgements
The authors are grateful to Dr. M.S.O. Aisien of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria; for helping with digital microscopy.

Correspondence to
Dr. Daniel I. Olorunfemi,
Department of Plant Biology and Biotechnology, Faculty of Life Sciences,
University of Benin, Edo State, Nigeria
E-mail: udanfem@yahoo.com
Phone Number: +2348023372455

References

Akanji A.O. (1994). Cassava intake and risk of diabetes in humans. *Acta Horticulture* **375:**349-359.

Akoroda M.O. (1992). State of the Cassava Production in Adamawa Cameroon and Implication for Improvement. Proceedings of the 4th Triennial Symposium of the International Society for Tropical Root Crop. Kinshasha, Zaria. 5-8 December 1989, pp 179-201.

Asegbeloyin J.N. and A.E. Onyimonyi (2005). The effect of fermentation time and variety on the cyanide content of cassava (Manihot esculenta Crantz). Proceedings of the 30th Annual Conference of the Nigeria Society for Animal Production, pp 199-200.

Asegbeloyin J.N. and A.E. Onyimonyi (2007). The effect of different processing methods on the residual cyanide of 'gari'. *Pakistan Journal of Nutrition* **6:** 163-166.

Azam-Ali S, E. Judge, P. Fellows, M. Battcock (2003). Small-Scale Food Processing: A Directory of Equipment and Method, ITDG Publishing, London 256 pp.

Bakare A.A, A.A. Mosuro, O. Osibanjo (2000). Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* (L). *Journal of Environmental Biology* **21:** 263–271.

Collard P. and S. Levi (1959). A two stage fermentation of cassava. *Nature* **183:** 620-621.

Coursey, D.G. (1973). Cassava as Food: Toxicity and Technology. In: Chronic cassava toxicity. Nestle B and MacIntyre R (eds) International Development Research Centre, IDRC-010e Ottawa, Canada.

Fiskesjö, G. (1985). Allium test on river water from bran and sexan before and after closure of a chemical factory. *Ambiologia* **14:** 99-103.

Fiskesjö, G. (1993). The *Allium* test in wastewater monitoring. *Environmental Toxicology and Water Quality* **8:** 291-298.

Fiskesjö, G. (1997). Allium Test for Screening Chemicals; Evaluation of Cytologic Parameters In: Plant for Environmental Studies. Wang W, Gorsuch JW, Hughes JS (eds) CRC Lewis Publishers, BOG Raton, New York, pp 308-333.

Grant, W.F. (1982). Chromosome aberration assay in allium. A report of the United States Environmental Protection Agency. Gene toxicity program. *Mutation Research* **99:** 273-291.

Ihedioha, J.I. and C.N. Chineme (1999). Effect of varied fermentation periods on the diabetogenic potential of toasted cassava granules. *Plant Foods and Human Nutrition* **53:** 159-168.

Irtwange, S.V. and O. Achimba (2009). Effect of the duration of fermentation on the quality of garri. *Journal of Biological Sciences* **1:** 150 – 154.

Meludu, N.T, O.O. Ajani, M.O. Akoroda (2001). Garri: Food for the Rich or Poor in Nigeria. Proceedings of the 8th International Society for Tropical Root Crop Symposium, Ibadan, pp 160-169.

Ofuya, C.O. and P. Akpoti (1988). Post processing Microflora and shelf stability of garri. *Journal of Applied Bacteriology* **64:** 389-394.

Ogiehor, I.S. (2002). Extension of Shelf Life of Garri by Combinations of Preservative Factors. PhD Thesis, University of Benin, Nigeria.

Ogiehor, I.S. and M.J. Ikenebomeh (2006). The effects of different packaging materials on the shelf stability of garri. *African Journal of Biotechnology* **5:** 2412-2416.

Olarewaju, O.C. and Z. Boszoromanyi (1975). The process of detoxification and residual cyanide content of commercial garri samples. *West African Journal of Biology and Applied Chemistry* **18:** 7 – 14.

Rank, J. and M.H. Nielsen (1993). A modified *Allium* test as a tool in the screening of the

genotoxicity of complex mixtures. *Hereditas* **118:** 49–53.

Sanni, O.N. (1994). Garri Processing in Ibadan Metropolis: Factors Controlling Quality. Proceedings of the 5th International Society for Tropical Root Crops, pp 256-261.

Sharma, C.B. (1983). Plant meristem as monitors of genetic toxicity of environmental chemicals. *Current Science* **52:** 1000-1002.

Submission Date: 6th October, 2010

Swierenga, S.H., J.A. Heddle, E.A. Sigal, J.P. Gilman, R.L. Brillinger, G.R. Douglas, E.R. Nestmann (1991). Chromosome aberrations and sister-chromatid exchange in Chinese hamster ovary. *Mutation Research* **24:** 301-322.