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The effects of processing on cyanide and mineral contents of *Garri* from different sources

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Abstract

In this study, garri from different sources were analysed in order to investigate the effects of processing on cyanide and mineral contents in the test samples using AOAC (2006). Cyanide and mineral analysis carried out on the test sample revealed the presence of cyanide and minerals in both yellow and white garri but in varying amounts. The level of cyanide in the yellow garri was 1.19 ± 0.01^{a} (Obubra), 1.16 ± 0.02^{a} (Yakurr), 1.08 ± 0.02^{b} (Bekwarra) compared to the white variety which was 1.24 ± 0.02^a (Obubra), 1.21 ± 0.01^b (Yakurr), 107.12 ± 0.02^c (Bekwarra) compared to the white garri 128.15 ± 0.05^a (Obubra), 124.2 ± 0.1^b (Yakurr), 114.5 ± 0.2^c (Bekwarra). Sodium in yellow *garri* was 3.27 ± 0.25^a (Obubra), $3.7 \pm 0.10a$ (Yakurr), 2.47 ± 0.25^{b} (Bekwarra), for white *garri* was 4.05 ± 0.05^{a} (Obubra), 4.6 ± 0.10^{b} (Yakurr), $3.08 \pm 0.02^{\circ}$ (Bekwarra). Calcium in yellow garri was $9.6 \pm 0.10^{\circ}$ (Obubra), $7.53 \pm 0.03^{\circ}$ (Yakurr), $8.43 \pm 0.02^{\circ}$ while in white *garri* was $12.117 + 0.08^{a}$ (Obubra), $11.597 + 0.03^{b}$ (Yakurr), $10.24 + 0.02^{c}$ (Bekwarra). Magnesium in yellow garri was 12.3 + 0.25^a (Obubra), 13.02 + 0.02^b (Yakurr), 12.72 + 0.03^a while in white garri was 14.6 + 0.10^a (Obubra), 15.397 ± 0.03^b (Yakurr), 16.01 ± 0.01^c (Bekwarra). Iron in yellow *garri* was 0.02 ± 0.01^a (Obubra), 0.03 ± 0.01^b (Yakurr), 0.02 ± 0.01^{b} while in white garri was $0.05 \pm 0.01^{\text{a}}$ (Obubra), $0.06 \pm 0.01^{\text{b}}$ (Yakurr), $0.033 \pm 0.02^{\text{c}}$ (Bekwarra). Zinc in yellow *garri* was 0.13 ± 0.01^a (Obubra), 0.15 ± 0.02^a (Yakurr), 0.14 ± 0.02^a (Bekwarra) while in white *garri* was 0.16 ± 0.02^a (Obubra), 0.22 ± 0.02^{b} (Yakurr), 0.18 ± 0.02^{c} (Bekwarra). Copper in yellow garri was 0.02 ± 0.01^{a} (Obubra), 0.013 ± 0.01^{a} 0.01ª (Yakurr), 0.027 <u>+</u> 0.01ª (Bekwarra) while in white *garri* was 0.03 <u>+</u> 0.01ª (Obubra), 0.02 <u>+</u> 0.01ª (Yakurr), 0.033 <u>+</u> 0.02^a (Bekwarra). Phosphorus in yellow *garri* was 23.04 <u>+</u> 0.04^a (Obubra), 21.5 <u>+</u> 0.10^b (Yakurr), 22.27 <u>+</u> 0.02^c (Bekwarra) while in white garri was 26.2 + 0.10^a (Obubra), 23.827 + 0.03^b (Yakurr), 24.68 + 0.02^c (Bekwarra). From the result of the study, yellow colour *garri* contains less amount of cyanide compared to white colour *garri*, it could be recommended that yellow colour garri is good for consumption. Also, considering minerals, white colour garri contained the highest amount of minerals which helps in proper functioning of the body and help in body build-up, it could be inferred that moderate consumption of white colour *garri* could be a source of mineral supplement.

Keywords: Cyanide; White Garri; Yellow Garri; Cassava; Mineral; Yakurr; Obubra; Bekwarra

1. Introduction

This research is limited to the assessment of levels of cyanide and minerals of different sources of *garri* in three communities (Yakurr, Obubra and Bekwarra) in one of the South-Southern States (Cross River State) of Nigeria.

Garri gotten from cassava is a commonly consumed food in Nigeria, mostly southern and eastern parts of Nigeria, is found to contain minerals and cyanide. Consumption of cyanide and its accumulation in the human body normally leads to neurological disorders and goiter (Ojo and Akande, 2013). Cyanide has been found to be greatly reduced during the processing of cassava to *garri*. Unit operation such as peeling, washing, grating, fermentation, dewatering and roasting

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have been found to effectively reduce the residual cyanide contents of the product (Ojo and Akande, 2013). Chijioke *et al.*, (2010) reported that the traditional method of *garri* production requires the cassava slurry to be fermented for 72hours during which the cyanides (linamarin and lotaustralin) are hydrolyzed by linamarase enzyme to yield hydrocyanic acid which has a low boiling point and easily escape during roasting render the *garri* safe for consumption. Cutting corners by so many processors for the sake of profit has led to the production of *garri* with excess cyanide content (Ojo and Akande, 2013).

After consumption, cyanide quickly enters the bloodstream. The body handles small amounts of cyanide differently than large amounts. In small doses, cyanide in the body can be changed into thiocyanate, which is less harmful and is excreted in urine. In the body, cyanide in small amounts can also combine with another chemical to form vitamin B12, which helps maintain healthy nerve and red blood cells. In large doses, the body's ability to change cyanide into thiocyanate is overwhelmed. Large doses of cyanide prevent cells from using oxygen and eventually, these cells die. The heart, respiratory system and central nervous system are most susceptible to cyanide poisoning, hence the need for this research.

Garri is a lactic acid-fermented product of cassava root that can be processed with palm oil rich in carotenoid ("yellow *garri*") or without palm oil. In Nigeria, *garri* is widely acceptable and consumed by both the poor, the middle men or average Nigerian, and also the rich because it serves as a major source of carbohydrate. *Garri* can be taken in various forms; some people use it to make eba or soak in water along with groundnut, mashed beans, or bean cake (*akara*). The major problem of consuming *garri* is the toxicity that may arise from the poor processing of cassava which is rich in cyanogenic glucosides. Cassava Manihot esculenta cranta is a dicotyledonous plant and widely grown root crop in tropical regions of Africa, Latin America and Asia (Ihenkoronye & Ngoddy, 1985). Two varieties of cassava are known; the sweet cassava is known for its low cyanide content and the bitter cassava with its characteristic high content of cyanogenic glycosides (CGs) that is highly toxic when consumed (Ihenkoronye & Nnenna, 1998). Total cyanide in cassava products exists in the form of content of cyanogenic glycosides (Linamarin and Lotaustralin), cyanohydrin and free hydrocyanic acid (HCN) (Tewe, 1983). The CGs notwithstanding, cassava meal provides dietary energy to over 500 million people of the world (Otto, 1998).

According to FAO, (FAO, 2001) 172 million tons of cassava were produced worldwide in 2000 with Africa accounting for 45 %, Asia 28 % and Latin America and the Caribbean 19%. The five top-producing countries are Nigeria, Brazil, Thailand, Congo (DRC) and Indonesia. The crop plays a prominent role in the daily subsistence of many indigenous communities in southern Nigeria. Some commonly proceeded cassava meals include chips, "Abacha", "fufu", "liolio", tapioca, cassava flour and grit also known as "*Garri*" (Iwuoha & Eke, 1996). Also, it is known as "*Garri*" in Yoruba, Hausa and Efik/Ibibio.

Mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life (Zoroddu *et al.*, 2019; Berdanier *et al.*, 2013). However, the four major structural elements in the human body by weight (oxygen, hydrogen, carbon and nitrogen), are usually not included in lists of major nutrient minerals (nitrogen is considered a "mineral" for plants, as it often is included in fertilizers). These four elements compose about 96 % of the weight of the human body and major minerals (macrominerals) and minor minerals (also called trace elements compose the remainder.

Evidently, no work has been published on the processing methods, cyanide and elemental levels in *garri* of any colour in the South-Southern part of Nigeria. So, the findings of this study will generate literature for future researchers and will have relevance in nutritional toxicology, food biochemistry, nutrition & dietetics, and public health nutrition.

2. Materials and methods

2.1. Preparation of the samples

Two cups of yellow and white *garri* samples from different local markets were purchased in the three communities (Yakurr, Obubra, Bekwarra), Nigeria and taken for analysis in the laboratory.

2.2. Materials

Several materials were used during the analysis.

2.3. Determination of Hydrocyanic acid (A.O.A.C., 2006)

2.3.1. Apparatus

- Steam distillation set-up
- Beakers, 200 ml
- Erlenmeyer flasks (25 ml capacity)
- Round-bottom flasks (25 ml capacity)
- Volumetric flasks (1000 ml, 500 ml capacity)
- Micro-burette (910 ml capacity)
- Reagents
- 0.02 N silver nitrate: 1.699 g of AgNOs were dissolved in 500ml of distilled water
- 2.5% (w/v) sodium hydroxide solution
- 5% (w/V) potassium iodide
- 6N ammonium hydroxide 99.3 ml of cone. NH₄OH were made up to 250 ml with distilled water in a volumetric flask.

2.3.2. Procedure

- 10.0 g of the sample was weighed into a round-bottom flask and left to soak for about hours.
- It was then steam distilled into 20 ml of 2.5 ml (w/v) NaOH contained in an Erleineyer flask. The distillation continued until 25 ml of the distillate was collected.
- 8.0 ml of 6N NH₄O and 2 ml of 5% 9w/v) ki was added to the distillate
- The distillate was a faith but permanent turbidity

2.4. Determination of Zinc (A.O.A.C., 2006)

- Reagents
- Components concentration
- Reagent A:
- Zinc (buffer) 200 mmol/L
- Liquid (complexants) <0.1%
- Volume = 50/ 100 ml (5-Br-PAPS) < 0.1%
- Standard-zinc 200 µg/L
- Volume = 10 ml (zinc) 30.6 μmol/L

2.4.1. Test procedure

Three sets of test tubes were labeled B (blank), T (test) and S (standard) respectively. 1000 μ L reagent (A) was added to the three tubes, 50 μ L of distilled water to tube B, 50 μ L of sample samples to tube T and 50 μ L of zinc standard solution to tube S the content of the tubes were mixed, incubated for l0minutes at room temperature (15-25 °C) and absorbance and standard measured at 560 nm against reagent blank.

2.4.2. Calculation

Zinc (μ g/dL) = $\frac{\text{Abs sample x conc. of standard}}{\text{Abs standard}}$

Concentration of standard = $2000 \ \mu g/dL$

2.5. Determination of Iron (A.O.A.C., 2006)

2.5.1. CAB reagent composition

- CAB
- CTMA (cetyltrimethyl-ammonium bromide) 2.2 mmol
- Guanidinium chloride
- Sodium acetate buffer (pH 4.7)
- Iron standard

0.18 mmol/L 2.2 mmol/L 2.6 mmol/L 45 mmol/L 100 µg/dL or 17.9 µmol/L

2.5.2. Procedure

Three sets of test tubes were labeled B (blank), S (sample), and STD (standard). 50 μ L of the sample and the blank were pipette into tubes S and STD respectively. 50 μ L of distilled water was added into tube B and 1000 μ L each of the working reagent (CAB) into the three tubes. The contents of the tubes were mixed incubated for I5 minutes at room temperature (20-25 °C) and the absorbance of the sample and the standard was measured at 623 nm against the reagent blank within 60 minutes.

2.5.3. Calculation

Iron concentration $(\mu g/dL) \frac{\text{Change in sample A x conc. of standard}}{\text{Change in standard A}}$

Concentration of standard = $100 \mu g/dL$

2.6. Determination of Potassium (A.O.A.C., 2006)

2.6.1. Reagents composition

•	Precipitant	
•	Trichloroacetic acid (TCA)	0.3 mol/L
•	TPB-Na-Reagent	
•	Sodium tetraphenylboron	0.2 mol/L
•	NaOH Reagents	
•	Sodium hydroxide	2.0 mol/L
•	Standard	
•	Potassium ion	5.0 mmol/L

2.6.2. Reagent preparation

Working reagents

The content of bottle (TPB) was mixed with the content of bottle (sodium hydroxide) and allowed to stand for 15-30 minutes.

Test procedure

To 50 μ L of the samples in their respective tube was added 50 μ L of trichloroacetic acid (TCA). This was mixed carefully. To 100 μ L of this mixture, was added 100 μ L of working reagent and this tube labeled sample, while 1000 μ L of the working reagent was mixed with 100 μ L of the potassium standard and labeled STD. These were mixed carefully and allowed to stand for 5 minutes. The absorbance of the STD and the samples were read against working reagent between 5 and 30 minutes at 578 nm.

Calculation

Potassium cone (mEq/L) = $\frac{\text{Change in sample A x conc. of standard}}{\text{Change standard}}$

Concentration of standard = 5 mEq/L

2.7. Determination of Sodium (A.O.A.C., 2006)

2.7.1. Reagent composition

- Filtrate reagent: Uranyl acetate 2.1 mM and magnesium acetate 20 mM in ethyl alcohol
- Acid reagent: a diluted acetic acid
- Sodium colour reagent: Potassium ferrocyanide, non-reactive stabilizers and filters
- Sodium standard: sodium chloride solution: 15 mmol/L of sodium

2.7.2. Materials

- Spectrophotometers
- Centrifuge
- Test tubes/racks

2.7.3. Test procedure

50 μ L of the digest was put into all the test tubes except the blank which had 50 μ L of distilled water. 50 μ L each of standard and distilled water was mixed in the tube labeled STD (standard), the tubes were shaken vigorously and mixed thoroughly for 3 minutes. 50 μ L of the mixture and 50 μ L of colour reagent was put into each clean test tube containing 1.0 ml of acid reagent and mixed. The absorbance of each tube was read at 550 nm against distilled water.

2.7.4. Calculation

 $\frac{(Abs of blank - Abs of S)x cone, of STD (mmol/L)}{(Abs of blank - Abs of STD)} = \text{ concentration of S (mmol/L)}$

Where Abs = Absorbance S = Sample STD = Standard

2.8. Determination of Calcium (A.O.A.C., 2006)

2.8.1. Atomic absorption spectrometry was used for the determination of calcium and magnesium

Apparatus

- Perkin-Elmer atomic spectrophotometer model 306 with digital readout (USA)
- 100 ml volumetric flask
- Muffle furnace
- Porcelain crucible

Procedure

2.7639 g of oven dried calcium chloride was dissolved in 100 ml of deionized water and made up to 1 liter. This solution contained 1000 ppm calcium ions. From this stock solution, calcium standard solutions of concentration 2 part per million 9 ppm), 4 ppm, 8 ppm were prepared. Strontium chloride solution was added such that 1500 mg/ml strontium ions were in the final solution.

2.8.2. Sample materials and reagents

- Conc. nitric acid
- Perchloric acid
- Digestion flask (conical flask)
- Stuart hot plate
- Strontium chloride solution

2.8.3. Procedure

- An acid mixture of 65 ml conc. HNOs, 8 ml percholic acid, 2 ml conc. was prepared
- 20ml acid mixture was put into a digestion flask containing l.0 g of the sample.
- The digestion flask was heated gently (50 °C 70 °C) on a Stuart hot plate until clear digest was obtained.
- The digest was made up to 100 ml with distilled water
- Appropriate dilutions were made for each element. 10,000 mg/ml strontium chloride solution containing 1500 mg/ml Sr2+ was added to the final solution.
- Calibration curves were prepared for each element using the standard solution. Appropriate lamps and correct wavelength for each element was specified in the instrument manual. Calcium wavelength was 422.7 nm.
- The concentration of calcium was determined using calibrated curve.

2.9. Determination of Phosphorus (A.O.A.C., 2006)

2.9.1. Apparatus

- Colorimeter
- Test tubes
- Centrifuge (centaur 2 MSE) (UK)
- Volumetric flask, 100 ml
- Flask shaker (Gallenkamp) (UK)

2.9.2. Reagents

- 10% (w/v) trichloroacetic acid. L0g of tricholoroacetic acid (CC13COOH) crystals were dissolved in 100 ml of deionized water.
- Molybdate reagent: 200 ml of distilled water was added to 83.0ml of conc. sulphuric acid solution, 25.0 g of ammonium molybdate tetrahydrate was then added to the sulphuric acid solution. Obtained solution was then made up to 1 litre with deionized water.
- Sulphuric acid reagent: 0.125 g of 1,2,4-amino naphthosulphuric acid, 728 g of sodium bisulphate and 0.25 g of sodium sulphite were dissolved in 50 ml distilled water, filtered and stored in a well stopped dark bottle.

2.9.3. Procedure

- 0.5 ml of mineral digest and 9.5 ml of 10% trichloroacetic acid were added to a 16 x 25 mm test tube.
- The mixture was agitated, centrifuged for 5 minutes and then filtered through Whatman filter paper.
- 5 ml of filtrate and 5 ml of working standard were measured into two 19mm cuvette.
- 0.5 ml of molybdate reagent was added to each cuvette and shaken.
- 0.2 ml of sulphuric acid reagent was added to both cuvettes.
- The cuvette were stopped, shaken and allowed to stand for 10 minutes.
- The absorbance of the test and standard were read at 660 nm with the blank set at zero, in a spectrophotometer.

2.9.4. Calculation

$$P(Mg\%)or(mg/dl) = \frac{Abs of test x conc. of standard (5 mg\%)}{Abs of standard}$$

Phosphorus Concentration can also be obtained from the calibration curve of standard.

2.9.5. Standardization

Phosphorus standard (l000 ppm^{-p}) was prepared by dissolving 4.394 g of potassium dihydrogen orthophosphate dried at 105 °C in 250 ml of deionized water. This was then diluted to 1 litre from which concentration of 2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and l0.0 ppm was prepared.

3. Results

Table 1 Mean variation in levels of cyanide in yellow garri and white garri from different stations (mg/100 g dry matter)

		Obubra	Yakurr	Bekwarra
Yellow garri	Cyanide	1.19 <u>+</u> 0.01 ^a	1.16 <u>+</u> 0.02 ^a	1.08 <u>+</u> 0.02 ^b
White garri	Cyanide	1.24 <u>+</u> 0.02 ^a	1.21 <u>+</u> 0.01 ^b	1.15 <u>+</u> 0.01 ^c

Values are mean + SD of triplicates. Means with different superscripts in the same row are statistically different at P<0.05.

	Elementals	Obubra	Yakurr	Bekwarra
	К	113.26 + 0.12 ^a	120.23 + 0.15 ^b	107.12 + 0.02 ^c
	Na	$3.27 + 0.25^{a}$	$3.7 + 0.10^{a}$	2.47 + 0.25 ^b
	Са	9.6 + 0.10 ^a	7.53 + 0.03 ^b	8.43 + 0.02 ^c
Yellow garri	Mg	$12.3 + 0.25^{a}$	13.02 + 0.02 ^b	$12.72 + 0.03^{a}$
	Fe	0.02 + 0.01 ^a	0.03 + 0.01 ^b	0.02 + 0.01 ^b
	Zn	0.13 + 0.01 ^a	0.15 + 0.02 ^a	$0.14 + 0.02^{a}$
	Cu	0.02 + 0.01 ^a	0.013 + 0.01 ^a	0.027 + 0.01 ^a
	Р	$23.04 + 0.04^{a}$	21.5 + 0.10 ^b	22.27 + 0.02 ^c
	К	128.15 + 0.05 ^a	124.2 + 0.1 ^b	114.5 + 0.2°
	Na	4.05 + 0.05 ^a	4.6 + 0.10 ^b	3.08 + 0.02 ^c
	Са	$12.117 + 0.08^{a}$	11.597 + 0.03 ^b	10.24 + 0.02 ^c
White garri	Mg	14.6 + 0.10 ^a	15.397 + 0.03 ^b	16.01 + 0.01 ^c
	Fe	0.05 + 0.01 ^a	0.06 + 0.01 ^b	0.033 + 0.02 ^c
	Zn	0.16 + 0.02 ^a	0.22 + 0.02 ^b	0.18 + 0.02 ^c
	Cu	0.03 + 0.01 ^a	0.02 + 0.01 ^a	0.033 + 0.02 ^a
	Р	26.2 + 0.10 ^a	23.827 + 0.03 ^b	24.68 + 0.02 ^c

Table 2 Mean variation in levels of elementals in yellow and white garri from different sources (mg/100 g dry matter)

Values are mean <u>+</u> SD of triplicates. Means with different superscripts in the same row are statistically different at P<0.05.

Table 3 Mean variation in levels of cyanide and elementals in yellow and white *garri* (mg/100 g dry matter)

	Yellow garri	White garri		
Cyanide	$1.14 + 0.05^{a}$	$1.20 + 0.04^{b}$		
К	113.57 + 5.73 ^a	122.28 + 6.08 ^a		
Na	$3.15 + 0.57^{a}$	3.91 + 0.67 ^b		
Са	8.52 + 0.90 ^a	11.32 + 0.84 ^b		
Mg	$12.69 + 0.33^{a}$	15.34 + 0.61 ^b		
Fe	0.02 + 0.01 ^a	0.05 + 0.02 ^b		
Zn	$0.14 + 0.02^{a}$	0.19 + 0.03 ^b		
Cu	0.02 + 0.01 ^a	0.03 + 0.01 ^a		
Р	$22.27 + 0.67^{a}$	24.90 + 1.04 ^b		

Values are mean + SD of triplicates. Means with different superscripts in the same row are statistically different at P<0.05.

4. Discussion

This research work was carried out to investigate the effects of processing on cyanide and mineral contents of *garri* from different sources. Yellow and white *garri* were gotten from three sources (Yakurr, Obubra and Bekwarra) in Cross River State after processing.

The result in table 1, the mean variation of cyanide in yellow *garri* and white showed that cyanide levels at (P<0.05) was not significantly different in two sources (Obubra and Yakurr) but there was significant difference in Bekwarra. Meanwhile in white *garri* cyanide levels at (P<0.05) was significant difference in all the sources.

The mean variation of the elementals in yellow *garri* and white *garri* in table 2 revealed that potassium level in yellow garri at (p<0.05) was significantly different in the three sources. Meanwhile, potassium level in white *garri* at (P<0.05) was significantly different in the three sources.

Sodium level in yellow *garri* at (P<0.05) was not significantly different in two sources (Obubra and Yakurr) but there was significant difference in Bekwarra. Also, sodium level in white *garri* at (P<0.05) was significantly different in all the sources.

Calcium (Ca) and Phosphorus (P) levels at (P<0.05) in yellow *garri* were significantly different in the three stations. Meanwhile, calcium (Ca) and Phosphorus (P) levels at (P<0.05) in white garri were significantly different in the three sources.

Magnesium level at (P<0.05) in yellow *garri* in two sources (Obubra and Yakurr) was significantly different but there was no significant difference in Bekwarra. Also, magnesium level at (P<0.05) in white *garri* was significantly different in all the sources.

Iron (Fe) level at (p<0.05) in yellow *garri* was observed with a significant difference in Obubra but there was no significant difference between Yakurr and Bekwarra. Meanwhile, iron level at (p<0.05) was observed a significant difference in white *garri* in all sources.

Also, Zinc (Zn) and copper (Cu) levels at (P<0.05) in yellow *garri* was analyzed with no significant difference in the three sources. Zinc (Zn) at (P<0.05) in white *garri* was significantly different in all the sources and copper (Cu) levels at (P<0.05) in white *garri* was no significant difference in the three stations.

This result agrees with the work carried out by Akinsola *et al.*, (2015) on the assessment of cyanide content in white, Light Yellow and Deep Yellow Cassava Grit (*Garri*) sold in Damaturu Metropolis. It was observed that deep yellow *garri* showed the lowest cyanide concentration compared to the other products. This is because of the presence of palm oil which may be the factor leading to reduction of cyanide content as a result of complex ion formation. World Health Organization recommendation on cyanide level in food and water is 10mgkg⁻¹. A person could die instantly if the HCN concentrations are respectively 180 and 130ppm (National Academic Press., 2002). Evidently, no work has been published on the elementals level in garri of any colour or location, making this work a novel study.

The mean variation of cyanide and elementals in both yellow and white *garri* in table 3 showed that cyanide level and mineral levels at (P<0.05) in white *garri* were significantly increased compared to yellow garri.

5. Conclusion

In summary, since yellow colour *garri* contains less amount of cyanide compared to white colour *garri*, it could be recommended that yellow colour *garri* is good for consumption. Also, considering minerals, white colour *garri* contained the highest amount of minerals which helps in proper functioning of the body and help in body build-up, it could be inferred that moderate consumption of white colour *garri* could be a source of mineral supplement.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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