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EFFECTS OF STORAGE TIME AND PACKAGING MATERIALS ON NUTRITIONAL AND MICROBIOLOGICAL CONTENT OF GARRI

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Abstract
Garri, a product of granulated cassava tuber, is a major staple food in Nigeria with huge potential for export. Garri has a short shelf life, which further deteriorates with time under poor packaging conditions. This study examined how packaging material and storage time affect the proximate composition, carotenoid content, cyanide content, energy content, and microbial content of the samples. Garri was stored in four packaging materials, namely, paper, nylon, plastic and jute bags. The responses were measured and recorded every 2 weeks for 24 weeks. The findings showed that the amounts of crude protein, crude fat, and ash in garri stored in the four packaging materials did not differ significantly ($p > 0.05$). There was a significant difference ($p<0.05$) in carbohydrate, moisture content, crude fibre, cyanide, carotenoid, energy, and total viable microbial count between samples stored in paper and jute bags compared to those stored in plastic and polythene materials. Storage time had a significant linear effect ($p<0.01$) on all the parameters measured and had a significant quadratic effect ($p<0.05$) on all the parameters except crude protein and energy content. The optimized results showed that samples stored in plastics gave the best results overall.

1.0 INTRODUCTION
Garri is a popular derivative of cassava (*manihot esculenta Crantz*) in West Africa. Cassava is a tropical root tuber cultivated in Africa and Asia that is eaten in different processed forms such as *fufu*, *abacha*, *garri* etc [1] for both human and animal consumption [2], [3]. Nigeria is presently the world's top producer of cassava, yielding over 40 million MT annually [4]. While cassava alone accounts for roughly 5% of Nigeria's agricultural GDP, most of it is processed and used domestically for food purposes, with very little of it being used for industrial uses [4], [5].

Cassava, previously known as food for subsistence level, is now gaining traction as an important raw material in the food, beverage and pharmaceutical industry [6], [7], [8] and even the energy industry as the wastes are being considered as important feedstocks for bioenergy [9], [10], [11]. The food value of cassava is significantly reduced by the presence of cyanogenic glucosides, specifically linamarin and lotaustralin. These compounds release acetone cyanohydrin and hydrogen cyanide when

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they are broken down by the natural enzyme linamaras [11], [12]. This is why it is very essential that cassava is processed to minimize the quantity of the cyanide compound and also stabilize the product to extend the shelf life [13]. The processes employed include fermenting, drying, boiling, roasting, steaming, and frying [12], [14].

Processing cassava into garri is the most widely acceptable form of food for most people in West Africa, constituting over 70% of total cassava consumed in Nigeria [15] . This is because the shelf life is increased in this form. Garri processing includes harvesting, peeling, washing, grating, bagging, fermentation and dewatering [16]. The dewatered cassava is then sieved and fried into a more stable form known as garri. Garri can be consumed in its granular form with or without cereals, sugar and milk. By using hot water (at 100^{o}C), garri can also be converted into a starchy food that can be swallowed with rich soups or stew spiced with beef, stockfish or mutton

Storage food packaging has an effect on the storability of foods [17]. The growing world population emphasizes the necessity of ensuring food capacity, quality, and security [18]. Packaging helps extend the shelf life of processed products after harvesting. By protecting the products from exposure to air, moisture, and contaminants, packaging preserves their freshness and quality for longer periods. This reduces spoilage and waste, ensuring that consumers receive safe and nutritious food items. Food packaging has multiple functions, including protecting products from physical vibrations and shocks during transportation, as well as from chemical and biological changes. It also helps prevent product loss and pollution and provides important information, such as ingredient and allergen labelling [19]. The food packaging operations has been producing new functional packaging to meet the primary difficulties of food packaging such as managing food drainage, food shelf life, waste from food, and packaging recyclability [20]. Food packaging is essential for storing, handling, transporting, and preserving food, which helps prevent food waste [21]. Packaging is important for maintaining the quality and safety of food and beverages throughout their distribution, sale, and consumption. It extends the shelf-life of food and decreases food waste. Additionally, packaging provides consumers with valuable information about the product, such as ingredients, nutritional facts, storage instructions, and the product's environmental impact [22]. Therefore, the objective of this study is to evaluate the impact of storage duration and packaging materials on the physicochemical properties, microbiological quality, and energy composition of garri.

2.0 MATERIALS AND METHODS

2.1 Sample Preparation and Experimental Design

Processed garri was purchased from producers at the Okwo Ngbo market in Ohaukwu Local Government, Ebonyi State, Nigeria. Figure 1 shows the unit operations involved in the processing of garri from raw cassava to the finished product. 500 g of garri was portioned and packaged into different packaging materials, which are paper, plastic, nylon and jute bags (Figure 2). Each packaging material was used, had 12 portions and was stored at room temperature (29^{o}C) and relative humidity ranged from 65% to 75%. Each portion from the four different packaging materials was collected and tested in triplicate every 2 weeks for 24 weeks.

Figure 1: Flow diagram for garri processing [23]

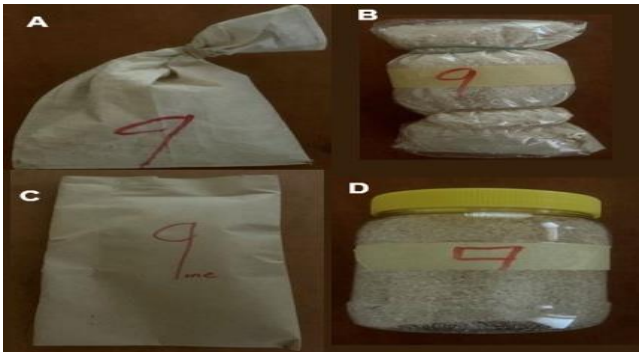

Figure 2: Garri samples stored in (A) jute bag, (B) nylon, (C) paper and (D) plastic packages

2.2 Proximate composition determination

The Association of Official Analytical Chemists' (2023) guidelines were followed in determining the

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proximate parameters, which included moisture content, ash, protein, fat, and carbohydrate [24]. The thermogravimetric method, which involved drying the sample in an oven set at 105°C for six hours until a constant weight was reached, was used to determine the moisture content. The sample was then burned for four hours at 600°C in a furnace to assess the amount of ash present. The Kjeldahl method, which involves the phases of destruction, distillation, and titration, was used to determine the protein concentration. Total nitrogen was converted to protein using a conversion factor of 6.25. Using a hexane solvent and the Soxhlet technique, the lipid content was determined. Lastly, the moisture, ash, protein, and fat content were subtracted from 100% to determine the carbohydrate level.

2.3 Cyanide Determination

The cyanide content of the samples was measured using the method detailed by Kalu [25]. Twenty grams of the sample were placed in an extraction flask, followed by the addition of 100 cm³ of distilled water. The mixture was left to stand for 2 hours to release all bound hydrocyanic acid. After this period, another 100 cm³ of distilled water was added to the slurry, which was then steam distilled. The distillate was collected in 20 cm³ of 0.01N $AgNO_3$ (silver nitrate) that had been acidified with 1 cm³ of HNO_3 (nitric acid). The distillation process continued for 40 minutes. Once 150 cm³ of distillate was obtained, it was filtered with a small amount of water, and the excess $AgNO_3$ was titrated with 0.02N KSCN (potassium thiocyanate) using ferric alum as an indicator. The endpoint was indicated by the appearance of a faint reddish colour upon the addition of 0.02N KSCN.

2.4 Energy Content Determination

The methodology outlined by A.O.A.C [26] was employed to determine the energy content in the samples using a bomb calorimeter (model XRY-1A, manufactured by Shanghai Changji, China). This process involves igniting the waste sample within an oxygen bomb calorimeter under high pressure of oxygen gas. The released heat energy is absorbed by the surrounding water inside the calorimeter, causing an increase in the water's temperature. This temperature rise is then used to estimate the energy value of the sample. Specifically, 1 gram of the sample was pelleted and placed in the oxygen bomb calorimeter. The heat of combustion was calculated as the gross energy. The energy content of the sample was computed using Equation 1 [9].

$$Energy\ content = \frac{E^{*}\Delta T\text{-}2.3^{*}L\text{-}1}{g} \quad (1)$$

Where,
E is energy equivalent of the calorimeter (kj)
ΔT Is change in temperature$(^{\circ}C)$

L is length of burnt wire (m)
V is titration volume (cm3) and g is weight of sample (g)

2.5 Carotenoid Determination

A UV visible spectrophotometer (Shimadzu UV 1800, Japan) was used. A 1g sample was weighed and placed in a test tube with 5 cm³ of hexane. It was pulverized with a glass stick until discoloured, then transferred into a 50 cm³ flask. The test tube was rinsed with hexane until clear, and the flask was filled to 35–40 cm³ with more hexane. After mixing, the sample stood at room temperature in the dark for 30-40 minutes until colourless. The flask was topped up to 50 cm³ with hexane and mixed again. After particle settlement, the clear carotenoid-hexane solution was extracted for spectrophotometric analysis at 450 nm compared to a blank. Total carotenoids were calculated using the standard, which was produced and read at a wavelength of 450 nm [27].

2.6 Total Viable Count (TVC)

Total viable count (TVC) was determined by the dilution plate technique as described by [28]. Using a sterile pipette, sterile distilled water was added to the suspension that was produced during the bacterial isolation process. A dilution of roughly 30 cells per 0.015 cm³, or 0.015 volumes per drop, was the desired result. An indelible marker was used to split the agar plates into eight segments. Each section received an inoculation drop of the suspension. After that, these plates were incubated at 37°C for 24 hours. Equation 2 was used to count developed colonies, while Equation 3 was used to determine the TVC [29].

$$\text{Mean count} = \frac{N}{8} \quad 2$$

$$\text{TVC} = \frac{mean\ count \times dilution\ factor}{volume\ per\ drop} \quad 3$$

Where, N = number of colonies in each segment,
TVC = total vriable count, dilution factor = 10^4

and volume per drop = $0.015\,cm^3$

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2.7 Statistical Analysis

Design-Expert was used to perform regression analysis and ANOVA on the acquired data. The parameters were also optimized to determine the most effective method of storing garri. To identify significant differences at a 5% probability level, the Duncan Multiple Range Test was used to compare means.

3.0 RESULTS AND DISCUSSION

As seen in Figure 3, crude protein, crude fat, and ash content reduced as storage time increased. This trend occurred for the four packaging materials tested. However, the rate of reduction for paper and jute bags was more than that of plastic and nylon. This can be attributed to the increased porosity of the paper and jute bag [30]. The amount of crude protein, crude fat, and ash was negatively impacted by storage duration. As storage time increases, the crude fat reduces.

Table 1 demonstrated that the crude protein of the garri was not significantly impacted by the packaging material. Crude protein ranged from 1.11 to 1.92% which was higher than what was obtained by [31] who studied the proximate composition of garri stored at ambient temperature for 8 weeks and lower than what was obtained by [32]. The slight differences in values could be attributed to variations in the species of cassava used for making the garri. The crude fat content in the samples decreased from an initial value of 1.27% to 0.5, 0.87, 0.82 and 0.45% for paper, plastic, nylon and jute bags, respectively. This was also higher than what was obtained by [31]. Ash content values reduced from 1.19% to 0.49, 0.93, 0.85 and 0.56 for paper, plastic, nylon and jute bags, respectively, which was lower than values of 1.25% and 1.78% obtained by [31] and [32] respectively. The samples that were wrapped in papers had higher moisture content values (9.48 to 11.76% and jute bags (9.48 to 10.67%), whereas plastic samples in plastic (9.48 to 7.85%) and nylon (9.48 to 7.33%) recorded a decrease. The initial carbohydrate content of 86.44% in the samples at Day 0 was similar to what was obtained by [31]. Storage time had a reverse effect on the carbohydrate content in the samples. As storage time increased, the carbohydrate content of the garri increased as well. The carbohydrate content of the samples wrapped in paper and jute bags, as well as nylon and plastic, varied significantly ($p < 0.05$) with time.

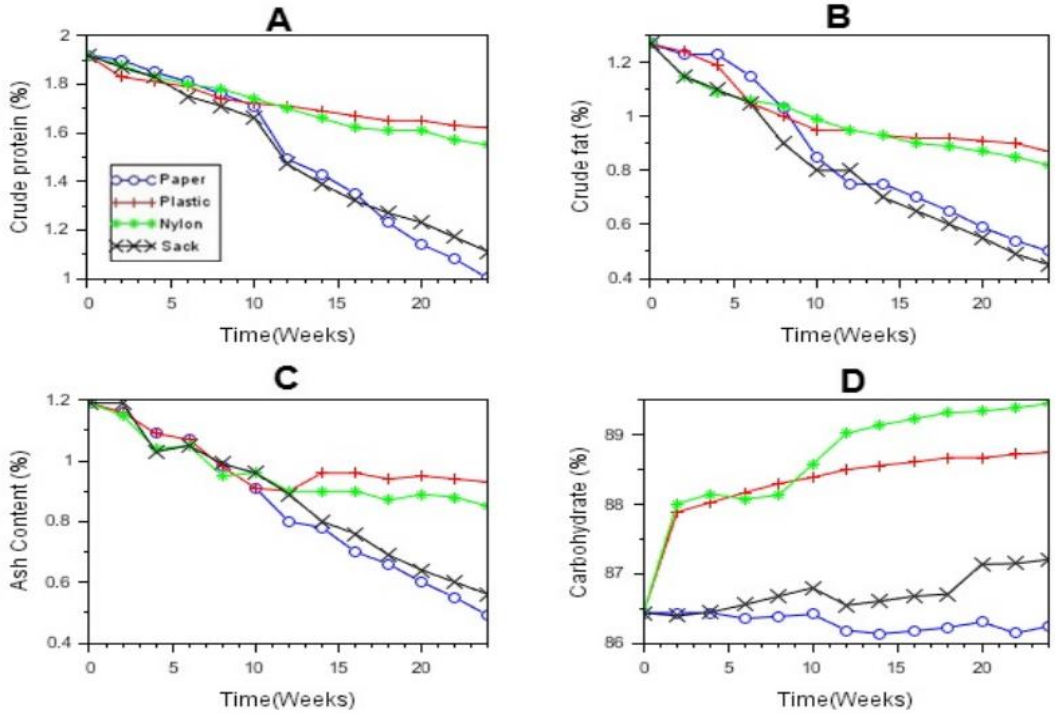

Figure 3: Variation in crude protein (A), crude fat (B), ash content (C) and carbohydrate (D) against storage time

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Figure 4 shows that the crude fiber of garri in paper and jute bags decreased with increasing storage time, but it grew with storage time for garri in packing materials made of plastic and nylon. Similar to carbohydrates, the crude fiber content of garri packaged in paper and jute bags and plastic and nylon bags varied significantly ($p < 0.05$). This trend was similar to what was observed by Haruna et.al [33] for garri stored in a warehouse using propylene bags. The energy value of the samples in plastic, nylon and jute bag increased slightly and then took a downward turn after 8 weeks. Plastic and nylon maintained a stable energy value up to 24 weeks while the energy content of the samples packaged with paper and jute bag reduced as the storage time increased.

The energy content of garri packaged in paper and jute bags, as well as plastic and nylon, did not change significantly ($p \geq 0.05$). The moisture content of the samples stored in nylon and plastic reduced with increased storage time, while that of paper and jute bags increased with increased storage time. The moisture level of the samples packaged in plastic, nylon, paper, and jute bags differed significantly ($p < 0.05$). There was no significant difference between the paper and the jute bag, and between the plastic and the nylon. The carotenoid content reduced with increased storage time. The average carotenoids of the samples kept in plastic and nylon bags and those kept in paper and jute bags differed significantly ($p < 0.05$).

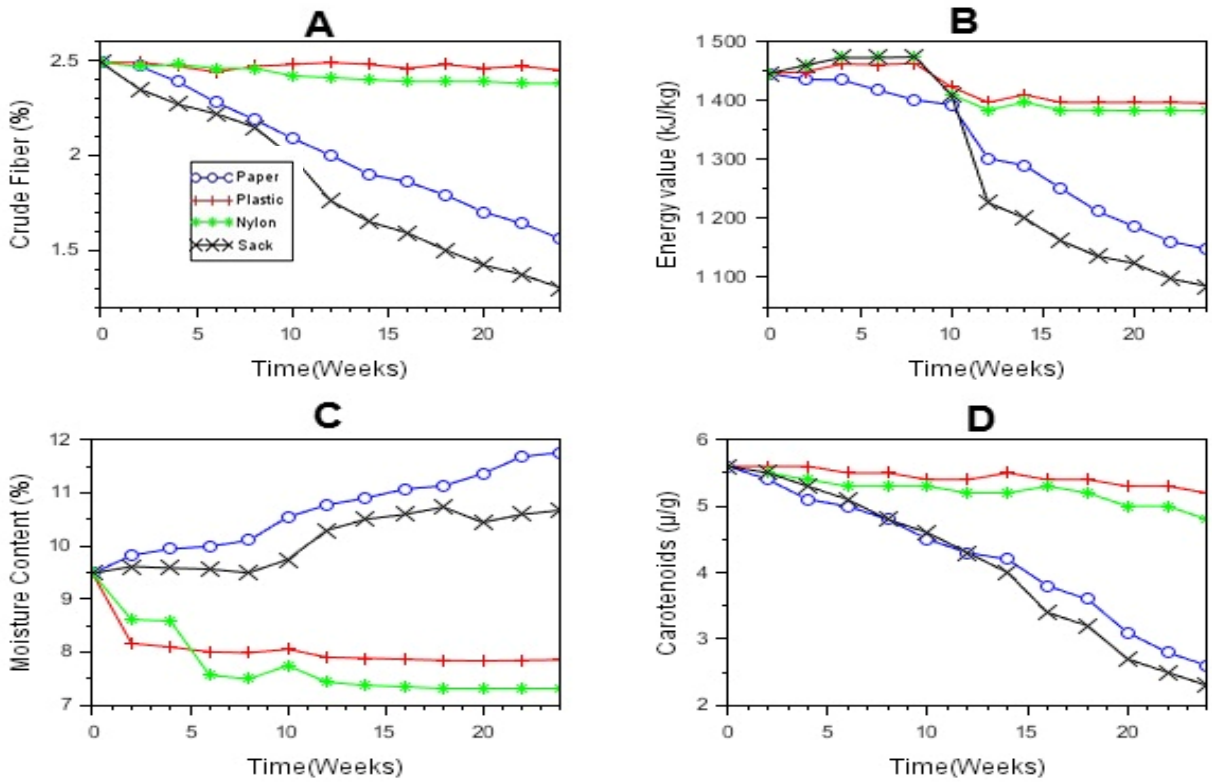

Figure 4: Variation of crude fiber (A), energy value (B), moisture content (C) and carotenoids (D) against time

The cyanide content of the samples packaged in paper and jute bags increased with increased storage time, while those packaged in plastic and polythene decreased slightly as storage time increased (Figure 5). The average cyanide concentration of samples preserved in plastic and nylon, and those packaged in paper and jute bags, differed significantly ($p<0.05$). The microorganisms present in the samples packaged in paper and jute bag increased with increased storage time. The samples packaged with plastic and nylon maintained a relatively constant microbial load. The microbial load of garri samples stored in paper and jute bags differed significantly ($p < 0.05$) from that of samples housed in plastic and nylon bags. This observation also agreed with that of Adebola et al. [35] on the microbial content of garri during storage.

The type of packaging material significantly influences both the chemical safety and microbial stability of *garri* during storage. *Garri* stored in paper and jute bags tends to deteriorate faster, as

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indicated by increasing cyanide content and microbial load with prolonged storage.

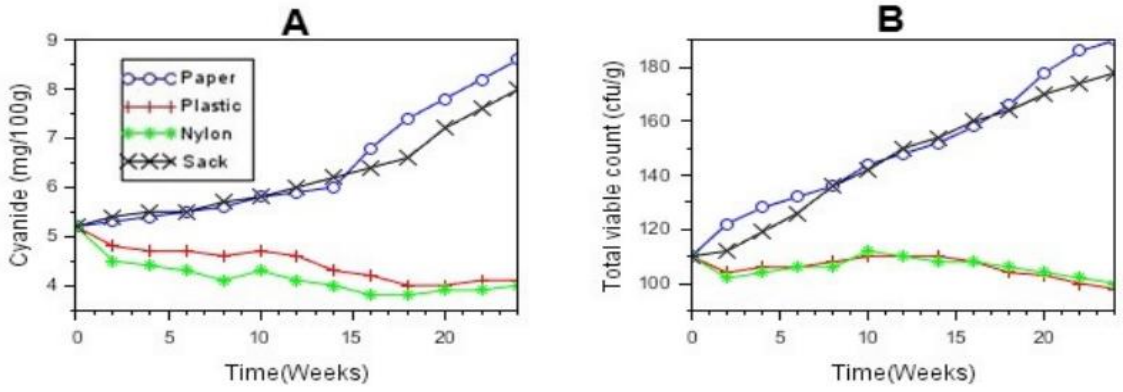

Figure 5: Effect of cyanide content (A) and total viable count (B) with time

In contrast, plastic and nylon packaging provide better protection, maintaining lower cyanide levels and relatively constant microbial populations over time. This suggests that airtight, moisture-resistant packaging materials such as plastic and nylon are more effective in preserving the quality and safety of *garri* during long-term storage.

3.1 Effect of Packaging Material on The Properties of Garri Samples

Table 1 displays the results of the Duncan Multiple Range Test (DMRT) and analysis of variance (ANOVA). The crude protein, crude fat, and crude ash content of the samples in the four packaging materials did not differ significantly ($p \geq 0.05$). This indicates that the crude protein was not significantly affected by the packaging material ($p \geq 0.05$).

The carbohydrate content of the samples kept in paper and jute bags did not change significantly ($p \geq 0.05$). The amount of carbohydrates in plastic and nylon packaging did not differ significantly ($p \geq 0.05$). However, the difference between the paper and jute bags was substantial ($p < 0.05$). The same trend was observed for crude fiber, energy value, moisture content, total carotenoid, total cyanide and total viable microbial count.

Table 1: Effect of packaging material on the properties of the garri samples.

Properties	Paper	Plastic	Nylon	Jute bag
Crude protein	1.51 ± 0.32^{a}	1.73 ± 0.09^{a}	1.72 ± 0.13^{a}	1.52 ± 0.28^{a}
Crude fat	0.86 ± 0.27^{a}	1.01 ± 0.13^{a}	0.99 ± 0.13^{a}	0.81 ± 0.26^{a}
Ash content	0.84 ± 0.23^{a}	1.00 ± 0.09^{a}	0.96 ± 0.11^{a}	0.87 ± 0.21^{a}
Carbohydrate	86.30 ± 0.11^{a}	88.28 ± 0.59^{b}	88.64 ± 0.83^{b}	86.72 ± 0.27^{a}
Crude fiber	2.03 ± 0.31^{a}	2.47 ± 0.02^{b}	2.42 ± 0.04^{b}	1.85 ± 0.40^{a}
Energy value	1314 ± 11^{a}	1423 ± 28^{b}	1418 ± 39^{b}	1290 ± 16^{a}
Moisture content	10.66 ± 0.71^{a}	8.06 ± 0.42^{b}	7.76 ± 0.66^{b}	10.10 ± 0.50^{a}
Total carotenoids	4.22 ± 0.94^{a}	5.44 ± 0.12^{b}	5.24 ± 0.21^{b}	4.10 ± 1.13^{a}
Total cyanide	6.42 ± 1.15^{a}	4.46 ± 0.36^{b}	4.18 ± 0.37^{b}	6.24 ± 0.85^{a}
Total viable microbial count	150 ± 23.91^{a}	105.92 ± 3.81^{b}	106.00 ± 3.42^{b}	145.77 ± 22.67^{a}

The values are shown as mean±SD. Within a single row, values with distinct superscripts indicate statistically significant differences ($p<0.05$).

3.2 Effect of Storage Time on The Proximate, Energy and Cyanide Content of Garri

Tables 2 and 3 illustrate the impact of storage time on the proximate, energy, and microbiological contents of samples. Storage time significantly reduced crude protein ($p < 0.001$), more so in paper and jute bags than plastic and nylon ones. Ash content decreased linearly over time across all packaging types ($p < 0.001$). Negative quadratic effects were noted in garri samples in paper, nylon, and jute bags ($p < 0.001$), while plastic packaging showed a positive quadratic effect ($p < 0.001$).

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Crude fat content in all four packaging materials decreased linearly over time, more so in paper and jute bags than in plastic and nylon. Storage time had a significant quadratic effect on crude fat ($p<0.001$). Moisture content increased in paper and jute bags ($p<0.01$) but decreased in plastic and nylon bags ($p<0.01$). This suggests that paper and jute are more permeable to water. The moisture content change across all materials showed a significant quadratic effect ($p<0.001$), indicating a complex relationship influenced by factors like packaging permeability, ambient humidity, and sample nature. Understanding these factors is key for optimizing storage conditions and predicting shelf life [34].

Storage time linearly increased carbohydrate content ($p<0.001$) in all samples across the four packaging materials. As storage duration extends, carbohydrate levels rise at a constant rate. A significant negative quadratic effect ($p<0.01$) was also found, indicating the increase rate slows over time. These findings emphasize the importance of considering both linear and non-linear effects in food storage studies to understand the impact on food quality comprehensively.

Storage time reduced crude fibre content in all packaging materials ($p<0.001$). For paper, the crude fibre declined faster before potentially slowing down again (negative quadratic effect, $p<0.05$). In plastic, nylon, and jute bags, the rate of reduction increased over time (positive quadratic effect, $p<0.05$). These results show that different storage materials affect crude fibre levels differently, important for industries focused on nutritional quality.

Storage time negatively affected the energy content of garri samples ($p<0.001$) but had no significant quadratic effect ($p<0.05$). Longer storage increased cyanide levels in samples stored in paper and jute bags ($p<0.001$), while it decreased cyanide in plastic and jute bags ($p<0.001$). Storage time also positively influenced total cyanide content across all packaging types ($p<0.001$).

Storage time significantly reduced total carotenoids in all packaging materials ($p<0.001$), showing both linear and quadratic effects. Carotenoid concentration declined with longer storage across plastic, glass, metal, and paper. Storage time also increased the microbial load ($p<0.001$), with a more pronounced effect in paper and jute bags than in plastic and nylon. It showed a significant quadratic effect ($p<0.001$) on microbial counts.

Table 2: Effect of time on proximate content of garri

Packaging material	Regression coefficient	Crude protein (%)	Ash content (%)	Crude fat (%)	Moisture content (%)	Carbohydrate (%)	Crude fibre (%)
Paper	C_o(Intercept)	1.915	1.190	1.254	9.701	86.362	2.493
	C_1	-0.039***	-0.041***	-0.052***	0.214**	0.057***	-0.005***
	C_{11}	-0.003	-0.004***	-0.015***	0.012***	-0.008**	-0.005*
Plastic	C_o(Intercept)	1.872	1.211	1.281	9.051	86.935	2.498
	C_1	-0.008***	-0.021***	-0.032***	-0.071**	0.271***	-0.026***
	C_{11}	-0.001	0.001***	-0.006***	0.017***	-0.014**	0.008*
Nylon	C_o(Intercept)	1.907	1.194	1.256	9.385	86.860	2.489
	C_1	-0.013***	-0.024***	-0.033***	-0.347**	0.305***	-0.018***
	C_{11}	-0.001	-0.001***	-0.002***	0.019***	-0.014**	0.008*
Sack	C_o(Intercept)	1.936	1.183	1.258	9.514	86.332	2.486
	C_1	-0.034***	-0.038***	-0.050***	0.276**	0.081***	-0.107***
	C_{11}	-0.002	-0.003***	-0.003***	0.012***	-0.008**	0.027*
	R^2	0.973	0.986	0.991	0.986	0.975	0.998
	Adj R^2	0.964	0.98	0.986	0.982	0.968	0.997
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Subscripts: 1= linear effect, 11= quadratic effect, * Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

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Table 3: Effect of time on energy, biochemical and microbiological content

Packaging material	Regression coefficient	Energy value (kJ/kg)	Total cyanide (mg/100g)	Total carotenoids (ug/g)	Total viable count (cfu/g)
Paper	C_o(Intercept)	1430.58	5.193	5.555	110.800
	C_1	31.953***	0.057***	-0.084***	3.725***
	C_{11}	-4.022	0.003***	-0.001***	-0.163***
Plastic	C_o(Intercept)	1439.50	5.198	5.611	110.016
	C_1	16.239***	-0.138***	-0.016***	0.252***
	C_{11}	-3.155	0.066***	0.000***	1.173***
Nylon	C_o(Intercept)	1444.08	5.168	5.523	109.446
	C_1	6.235***	-0.134***	-0.022***	0.390***
	C_{11}	-3.618	0.074***	0.000***	1.256***
Sack	C_o(Intercept)	1429.71	5.249	5.703	109.968
	C_1	29.368 ***	00.015***	-0.101***	3.565***
	C_{11}	-7.276	0.025***	-0.002***	1.006***
	R^2	0.974	0.997	0.994	0.999
	Adj R^2	0.962	0.995	0.992	0.998
	P-value	<0.001	<0.001	<0.001	<0.001

Subscripts: $_1$= linear effect, $_{11}$= quadratic effect, * Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

3.3 Optimization

The results obtained were optimized to determine the best packaging material for the longest time. The goal was to get the best material to store for the longest time and maximize the crude protein, ash content, crude fat, carbohydrate, crude fibre, energy value, total carotenoids and to minimize the moisture content, total cyanide and total viable microbial count.

It is observed that plastic had the highest optimized results and could be stored for up to 21 weeks, followed by nylon which could stored for up to 15 weeks. Garri samples packaged in Jute bag and paper could only be stored for three weeks. It is important to note that the initial moisture content of garri is very important and should be below the critical moisture content (8%).

4.0 CONCLUSION

In conclusion, the choice of packaging material plays a crucial role in maintaining the quality of garri during storage. Plastic containers offer the best long-term protection by preventing moisture absorption and pest infestation, making them ideal for humid environments. For medium-term storage, polythene bags provide a practical and cost-effective option when properly sealed, while paper and jute bags serve well for short-term storage due to their breathability and eco-friendliness. Ultimately, selecting appropriate packaging based on storage duration and environmental conditions is essential for preserving the freshness and quality of garri.

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