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Quality Characteristics and HCN in Gari as Affected by Fermentation Variables

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Abstract

The effect of some fermentation variables (temperature, relative humidity and duration of fermentation) on the fermentation of grated cassava mash for gari production was studied. The proximate composition, functional, chemical and pasting properties were determined using standard analytical methods. The sample fermented at 40°C, 85% relative humidity and 96h (sample 12) had the highest values for protein (2.11%), ash (2.17%) and swelling index (3.62 ml/ml). These values were significantly different ($p<0.05$) from the values obtained from the other samples. The HCN content of sample 12 was also very low (4.77 mg/kg) and was not significant different ($p>0.05$) from the least value obtained in the study (4.31 mg/kg). It also had the lowest pH value at the end of fermentation of the mash. The length of fermentation was observed to have the most significant effect on most of the parameters monitored while temperature had the least effect. Fermentation of grated cassava mash at 40°C, 85% relative humidity and for 96h will therefore give gari with higher protein content, low HCN and good eating quality.

Key words: *Gari; Fermentation variables; HCN; Functional properties; Chemical properties.*

Introduction

Gari is a granular pre-gelatinized cassava starch with a slightly fermented flavor and a slightly sour taste made from grated, fermented, gelatinized fresh cassava roots (Sokari and Karibo, 1992). This partially gelatinized dried cassava product is commonly consumed directly or soaked in cold water with sugar, coconut, roasted peanut or boiled cowpea as compliments, or as a stiff gel made with hot water and eaten with soup or stew. The acceptance and popularity of gari in urban and rural areas of west and Central Africa is attributed to its ability to store well, its convenience and ready-to-eat form (Flach, 1990).

A safety concern in the consumption of cassava based products like gari arises from the presence of cyanogenic glucoside, which upon hydrolysis produces cyanohydrins that further breaks down to release hydrogen cyanide (Bokanga, 1994; Ernesto *et al*., 2002).Although all the processing steps are important to determine the quality of the end product, grating and fermentation remain the critical process steps in gari processing in that hydrolysis is initiated by intimate contact between the compartmentalized linamarin and the degrading enzyme linamarase (Vasconcelos *et al*., 1990). Fermentation is also crucial to the development of the characteristic aroma and sour flavor, and detoxification of the mash by liberation of free HCN (Achinewhu and Owuamanam, 2001). Gari production till date is carried out at local levels mostly by rural women who do so to enhance household food security (Fapojuwe, 2008), and according to Nweke *et al*. (2002) production of gari remains labour intensive.

Large scale production of gari failed probably due to the limited information on processing variables that promote detoxification and fermentation of cassava to produce unique flavor characteristics associated with gari (Achinewhu and Owuamanam, 2001; Nweke *et al*., 2002). Traditional processing of cassava by fermentation is centered on reduction of cyanide in the resultant product through extended period of fermentation for up to seven days as important strategy for safety of product (Sanni, 2005). However, the traditionally processed gari contain varied amounts of residual cyanide because of the tendency by local processors to shorten duration of fermentation in order to meet growing market demand (Nweke *et al*., 2002). Therefore, there is need to optimize the traditional gari processing methods with the view to reducing fermentation time and achieving the elimination of cyanide while maintaining gari quality. In this direction Owuamanam *et al*. (2011) studied the impact of seeding fermenting cassava mash with preferment liquor, and fermenting at different temperature and time regimes. This resulted in a residual cyanide level of 8.36 mg/kg in gari when preferment liquor concentration of 20% was used, however, the resultant flavor was intense and rated low by panelists.

The present study considered the variability in the fermentation conditions (temperature, relative humidity and duration of fermentation) as they affect quality characteristics of gari and cyanide reduction.

Materials and Methods

The cassava roots used in the study were obtained from National Root Crop Research Institute, Umudike, Umuahia, Nigeria.

Extraction of linamarase:

200g of fresh cassava peels were homogenized in 1600mL of 0.1M acetate in a blender for 3min. The homogenate was centrifuged at 10000xg for 30 min. The supernatant liquid was brought to 60% saturation of $(NH_4)_2SO_4$ by adding 724g of $(NH_4)_2SO_4$ in 1600mL supernatant. The salt was added slowly with continuous gentle agitation. The precipitate was collected by centrifugation at 10000xg for 1h. The supernatant was discarded and the precipitate dissolved in 150mL of phosphate buffer (pH 6.0) before freezing.

Preparation of KCN standard: A stock solution was prepared by dissolving 50 mg of KCN in 0.2 M NaOH. The stock solution was diluted1:50 with 0.2 M NaOH. The automatic pipette was used to pipette into marked tubes: 0.025, 0.050, 0.075 and 0.100 mL of the diluted KCN stock, the volume was made up to 0.100 mL corresponding to 5, 10, 15 and 20µg mL^{-1} with 0.2 M NaOH. The 0.5 mL of 0.1 M phosphate buffer pH 6.0 was added followed by addition of 0.6 mL, chloramines-T and 0.8 mL of the colour reagent. The absorbance reading was obtained using (visible) spectrophotometer against blank at 605nm wavelength.

Sample preparation and fermentation

The cassava tubers were washed, drained and peeled. Working in a sterile hood, the tubers were rapidly grated into a sterile container with a sterile hand grater. 500g of the sample was weighed rapidly into small jute bags and tied with fishing line thread. They were then put in incubators set at the appropriate temperatures (30^OC, 35^OC and 40^OC) and allowed to fermentation for the desired length of time (48h, 72h and 96h).

Generation of relative humidity (RH)

The various relative humidities were generated as follows:

i) 65% RH was generated by dissolving 50g of anhydrous $NaNO_3$ in 33.3mL of hot water.
ii) 75% RH was generated by dissolving 20g of NaCl in 50mL of hot water.
iii) 85% RH was generated by dissolving 25g of KCl in 50mL of hot water.

The salt solutions were put in the incubators to create the required relative humidities in the environment of fermentation.

Experimental design: A Box Behnken rotable response (Lawson and Madrigal, 1994) for k = 3 was employed to study the effect of the independent variables on the fermentation process. The variables were of three levels (Tables 1 and 2).

Table 1: The independent variables and their levels

Independent variables	Variable levels		
	-1	0	+ 1
Temperature ^OC (x_1)	30	35	40
Relative humidity % (x_2)	65	75	85
Time h (x_3)	48	72	96

Table 2: Combination of the independent variables

Run	X_1	X_2	X_3
1	-1	0	0
2	+1	0	0
3	0	-1	0
4	0	+1	0
5	-1	0	-1
6	+1	-1	-1
7	-1	-1	+1
8	+1	-1	+1
9	+1	0	-1
10	0	+1	-1
11	-1	+1	+1
12	+1	+1	+1
13	0	0	0

Samples were collected on 24 h basis for analysis. The parameters analyzed for were: pH, total titrable acidity and cyanide content of fermenting mash.

pH: The pH of the fermenting mashes was determined using the methods described in AOAC (1990). Ten grammes of the samples were put into 200 mL beaker and 100 mL of distilled water added to it. The pH was then measured using a standardized pH meter (Prazisions pH meta ES10 model).

Total titrable acidity (TTA): 10g of sample was homogenized in 200mL of distilled water and filtered using Whatman filter paper. 80mL of filtrate was titrated with 0.1M NaOH using 1% phenolphthalein as indicator (Obilie *et al*., 2004).

Determination of cyanide in fermenting mash: The free cyanide was determined as described by O'Brien *et al*. (1991). 50g of sample was homogenized in 200ml of extraction medium, which was previously prepared by adding 6.75mL of H_3PO_4 to 200ml of distilled water. The homogenizer operated at 15 sec. at low speed, then 2 min. at high speed with 1 min. rest in between periods. The sample was subsequently centrifuged for 10min. at 4000xg. The supernatant was collected for analysis for free cyanide (HCN).

Determination of free cyanide: 0.1mL of sample extract was mixed with 3.9mL of phosphate buffer (pH 4.0). Then 0.1mL of linamarase enzyme was added and incubated for 15 min. at 30^{O}C. Thereafter 0.6mL of 0.2M NaOH was added and left for 5 min. after which, 2.5ml of phosphate buffer (pH 6.0) was added. Subsequently, 0.2mL of chloroamine T was added to the test tube already containing 4.0ml of buffered extract to stabilize the colour formation. The content was mixed and the test tubes placed in iced water bath for 5 min and transferred to the fume cupboard. Then 0.8mL of pyridine/pyrazolone reagent was added and left for 90 min. followed by measurement of the absorbance at 620nm wavelength.

At the end of fermentation the various samples were toasted over fire to obtain 'gari'. The proximate composition, functional, chemical and pasting properties of the gari samples were determined as follows;

Determination of Proximate Composition of the gari samples:
The proximate composition of the final product (gari) was determined and this was done according to the methods described in AOAC (1990). The parameters determined included the following; crude protein, crude fibre, moisture content, ash and carbohydrate by difference.

Determination of functional properties of the gari samples:
The following functional properties of the gari samples were determined:

i) Swelling index

The method of Ukpabi and Ndimele (1990) was followed with slight modification. Ten (10 g) grammes of the sample was transferred into a clean, dried, calibrated measuring cylinder. The gari was gently leveled by tapping the cylinder and the initial volume recorded. 50 ml of distilled water was poured into the cylinder and allowed to stand for 4h. The value for swelling index (SI) was taken as the multiples of the original volume.

ii) Water absorption capacity (WAC)

The method of Soluski (1962) as described by Abbey and Ibeh (1988) was followed. One (1.0 g) gramme of gari was weighed into an already weighed clean dried centrifuge tube. 20ml of distilled water was poured into the centrifuge tube and stirred thoroughly; centrifuge at a speed of 3500 rpm for 45min. The supernatant was discarded and the tube and its content reweighed. The gain in mass was taken as the water absorbed.

iii) Bulk density

The method of Akpapunam and Markakis (1981) was followed. Ten (10 g) grammes of the gari were transferred into 50 ml measuring cylinder. The cylinder was tapped repeatedly for 5 min. The bulk density of gari was calculated as the mass of gari over the volume at the end of tapping. The mean value was recorded from triplicate determinations.

Determination of residual cyanide of the gari samples:
Thirty grammes of gari was milled and homogenized with 250 mL of 0.1 M otthophosphoric acid. The homogenate was centrifuged and the supernatant taken as the extract; 0.1 mL of the enzyme was added into 0.6 mL of the extract. 3.4 mL of acetate buffer (pH 4.5) was added and stirred to mix, after which 0.2 mL of 0.5% chloramin-T and 0.6 mL of colour reagent were added and allowed to stand for 15 min. for colour development. The absorbance value was obtained at 605 nm wavelength against a blank similarly prepared containing all reagents and 0.1 mL phosphate buffer instead of KCN.
The data from the standard were used to obtain a standard curve and its slope (b) by plotting absorbance values (Y-axis) against standard concentration (X-axis). The unknown mean absorbance (A) and the weight of the sample (W) were used to calculate the residual cyanide, using the formula;

Residual cyanide = A x 250 x 0.4151 b x W
Unit = mg HCN equivalent kg^{-1}

Determination of pasting properties of the gari samples:
The pasting properties will be determined using the Brabender Visco-amylograph as described by Almanza (1988).

Statistical analysis
The data from the study was analyzed using Analysis of Variance and the means separated using Fisher's Least Significant Difference (LSD).

Results and Discussion

Table 3 shows the proximate composition of gari samples obtained from the study. The highest value for protein (2.11%) was obtained in sample 12 while the least value (1.76%) was obtained in sample 13. For carbohydrate sample 12 had the least value (81.88%). Sample 9 had the highest value for crude fibre (1.12%), however, the least vaue (0.94%) was obtained in sample 3. Sample 12 had the highest value for ash (2.17%) while the least value (1.71%) was obtained in sample 5.

Table 3: Proximate composition of the gari samples after production (%)

Sample/Run	Moisture content	Ash	Crude fibre	Crude protein	Carbohydrate
1	$11.09^{a,c}$	$1.75^{a,d,e}$	$0.95^{a,b}$	$1.90^{a,c}$	$84.31^{a,b}$
2	11.22^{a}	$1.86^{b,c,h}$	$0.96^{a,b}$	1.94^{b}	84.02^{a}
3	11.29^{a}	$1.85^{b,c,e,h}$	0.94^{a}	$1.90^{a,c}$	84.02^{a}
4	12.53^{b}	$1.82^{b,d,e}$	$0.95^{a,b}$	1.84^{d}	82.86^{c}
5	$10.88^{c,d}$	1.71^{a}	$1.08^{d,e}$	$1.88^{c,d}$	84.45^{b}
6	10.74^{d}	$1.83^{b,d}$	$1.03^{c,e,f}$	1.85^{d}	84.55^{b}
7	10.77^{d}	$1.90^{c,f,h}$	$1.05^{e,f}$	1.95^{b}	$84.33^{a,b}$
8	11.92^{e}	$1.85^{b,c,e}$	0.98^{b}	1.93^{b}	83.32^{g}
9	10.76^{d}	$1.78^{d,e}$	1.12^{g}	1.84^{d}	84.50^{b}
10	11.14^{a}	1.80^{e}	$1.09^{d,g}$	$1.89^{a,c}$	84.08^{a}
11	11.81^{e}	1.92^{f}	1.04^{f}	2.07^{e}	83.16^{e}
12	12.79^{b}	2.17^{g}	$1.05^{e,f}$	2.11^{e}	81.88^{d}
13	11.77^{e}	1.88^{h}	1.04^{f}	1.76^{f}	83.55^{f}
LSD	0.31	0.05	0.03	0.04	0.36

a,b,c…… values on the same column with the same superscript are not significantly different (p>0.05).

Table 4 shows the functional properties of the gari samples. Sample 12 had the highest value for water absorption capacity (4.17g/g) while the least value was 3.58g/g obtained in sample 1. Sample 12 also had the highest values for swelling index and bulk density which were 3.62 ml/ml and 0.4945g/g respectively. The least value for swelling index was obtained in samples 5 and 10 (3.36 ml/ml) while the least value for bulk density (0.4685 g/g) was obtained in sample 5.

Table 4: Functional properties of the various gari samples

Sample/Run	Bulk density (g/ml)	Water absorption capacity (g/g)	Swelling index (ml/ml)
1	0.4826^{a}	3.58^{a}	$3.38^{a,e}$
2	0.4729^{b}	3.79^{b}	$3.45^{b,e}$
3	0.4831^{a}	$3.77^{b,e}$	$3.48^{b,f}$
4	$0.4852^{a,d}$	$3.80^{b,e}$	$3.45^{b,c,e}$
5	0.4685^{c}	3.65^{a}	3.36^{a}
6	$0.4761^{b,f}$	3.61^{a}	$3.43^{c,e}$
7	0.4875^{d}	$3.94^{c,e}$	$3.45^{b,c,e}$
8	0.4828^{a}	4.08^{d}	3.56^{d}
9	$0.4762^{b,f}$	3.75^{b}	3.41^{e}
10	0.4728^{b}	3.75^{b}	3.36^{a}
11	0.4929^{e}	4.16^{d}	3.56^{d}
12	0.4945^{e}	4.17^{d}	3.62^{g}
13	0.4784^{f}	3.85^{e}	3.51^{f}
LSD	0.0036	0.09	0.04

a,b,c…… values on the same column with the same superscript are not significantly different (p>0.05).

Table 5 shows the results obtained for the chemical properties of the gari samples. The least Total Titrable Acidity (TTA) value was obtained in samples 8 and 12 (0.34%) while the highest value (0.52%) was observed in sample 10. The highest HCN value was obtained in sample 10 (7.87 mg/kg) while the least value (4.31 mg/kg) was obtained in sample 11.

Table 5: Chemical properties of the various gari samples

Sample/Run	Total titrable acidity (%)	HCN (mg/kg)
1	$0.42^{a,g}$	5.79^{a}
2	$0.41^{a,g}$	$5.11^{b,f}$
3	0.47^{b}	$5.27^{a,b,f}$
4	0.45^{b}	$5.28^{a,b,f}$
5	$0.44^{a,b}$	7.27^{c}
6	$0.49^{c,e}$	7.11^{c}
7	0.35^{d}	4.53^{d}
8	0.34^{d}	$4.82^{d,f}$
9	0.45^{b}	7.12^{c}
10	0.52^{e}	7.87^{e}
11	0.39^{f}	4.31^{d}
12	0.34^{d}	$4.77^{b,d,f}$
13	0.40^{g}	5.18^{f}
LSD	0.03	0.55

a,b,c…… values on the same column with the same superscript are not significantly different (p>0.05).

Figure 1 shows the pH values obtained during the fermentation of grated cassava mash for gari production. The values decreased with increase in fermentation time. The least pH was obtained in sample 12 (4.52). Samples fermented for 96h had generally lower pH values while samles fermented for 48h had higher pH values.

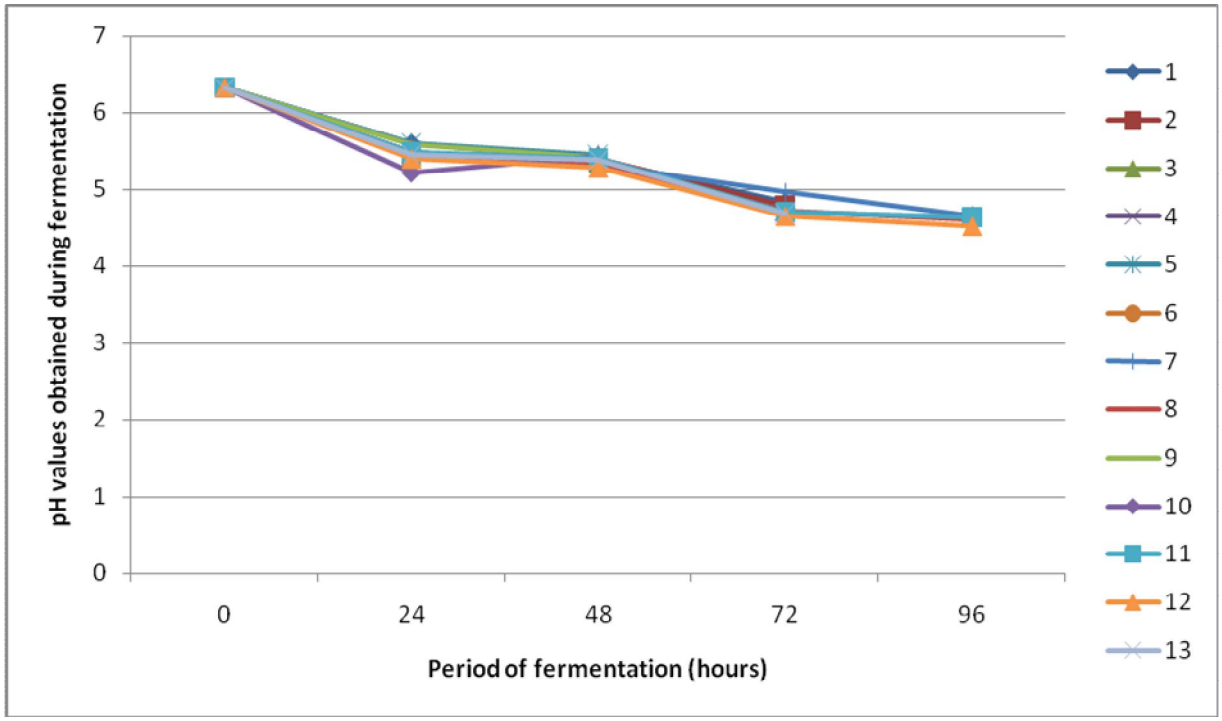

Figure 1: pH values during the fermentation of cassava mash for gari production

Figure 2 shows the HCN values obtained during the fermentation. The valuesdecreased as fermentation time increased. The samples fermented for 96h had the least values with sample 12 having the lowest of all the values (42.76 mg/kg).

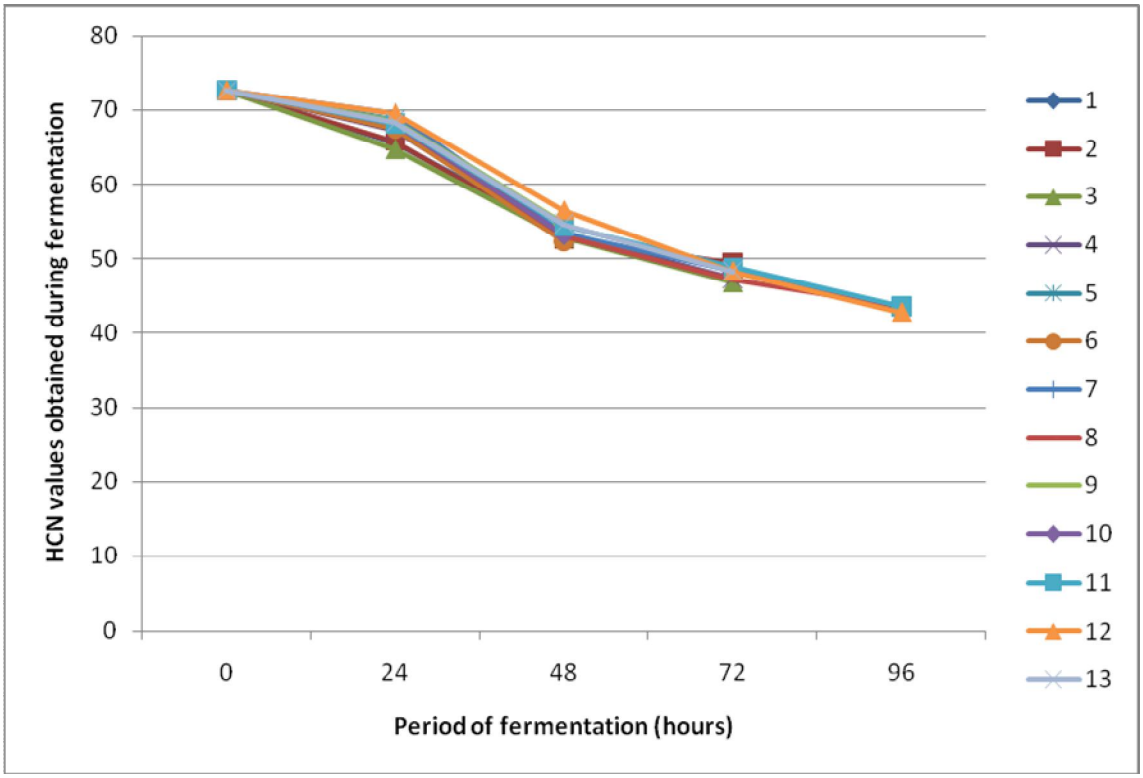

Figure 2: HCN values obtained during fermentation of grated cassava for gari production

Close observation of the results from the study seem to reveal that the conditions for the fermentation of sample 12 (temperature 40^{O}C, relative humidity 85% and time 96h.) were best for gari fermentation. The sample had the highest protein content of 2.11%, ash (2.17%) and swelling index (3.62 ml/ml). Statistical analysis showed that the values obtained for these parameters were significantly different ($p<0.05$) from the values obtained from the other samples. This still buttress the fact that sample 12 may be the best product in terms of the nutritional quality and eatability. Although it did not produce a product with the least HCN content the value obtained (4.77 mg/kg) was very low and it did not differ significantly ($p>0.05$) with the least HCN value (4.31 mg/kg).

A critical look on the results also indicated that the length of fermentation may have had the most significant effect on most of the parameters monitored. Samples fermented for 96h. had least values for HCN. The least pH values were also obtained in 96h fermented samples. The problem with the consumption of cassava based products such as gari is the unusually high level of HCN in the product (Bokanga, 1994; Ernesto *et al*., 2002). Thus reduction in the HCN level in these samples is of great significance.

They also had the highest values for protein, ash, bulk density, water absorption capacity and swelling index. Despite being a cheap source of food calories cassava is nutritionally deficient in proteins. The high consumption pattern of cassava diets (including gari) as witnessed in developing countries without adequate protein supplementation exposes the vulnerable group (children, pregnant and lactating mothers) to incidence of protein – energy malnutrition (FAO, 1984; Ahaotu *et al.*, 2011). The higher protein content of sample 12 is therefore note worthy. Thus fermentation of grated cassava mash at 40^{O}C, 85% relative humidity and for 96h will help increase the protein content of the final product. This will help reduce incidence of protein – energy malnutrition. The higher yield of protein in the sample could be attributed to the activities of the microorganisms involved in the fermentation. Microorganisms usually convert carbohydrates to proteins during fermentation (Gregory *et al*., 1976). It could be that the combination of variables favoured the increased production of proteins. Of all the variables it seemed that temperature had the least effect on the fermentation parameters monitored.

The sour taste has been taken as an index of gari quality (Achinewhu and Owuamanam, 2001).The lower pH values observed in the 96h fermented samples therefore indicate that the products would be of good quality and sample 12 having the least pH value of all the samples could be taken to be the best of all the samples. Oni *et al.* (2008) also observed a correlation between functional properties and sensory properties of cassava products. Thus sample 12 with the highest value for swelling index, one of the functional properties of food materials, still indicates that may be the best of all the samples.

The pasting properties of the samples are shown in table 6. Sample 11 had the highest peak viscosity (276.33 cp). This was followed by sample 5 (273.08 cp) while sample 3 had the least value (161.92 cp). Peak viscosity indicates the water binding capacity of starch grains and the ability of the starch to swell freely before their physical breakdown. It is an indication of the extent of starch damage or ease of cooking of the starch fraction in the gari (Balagoplan *et al*., 1988; Ikegwu *et al*., 2005). Thus sample 11 could be said to have the best water binding capacity and its starch may be one of the easiest samples to cook (gelatinize). Its gelatinization time was 5.00 min. This corresponds with the result obtained for its (sample 11) water absorption capacity which was high (4.16 g/g).

Table 6: Pasting properties of gari samples

Sample/Run	Peak 1 (CP)	Trough 1 (CP)	Breakdown viscosity (CP)	Final viscosity (CP)	Setback viscosity (CP)	Peak time (min)	Pasting Temperature (^{O}C)
1	254.17	249.92	8.25	377.17	131.25	4.87	80.12
2	244.92	150.67	94.25	371.00	220.33	5.00	80.66
3	161.92	100.57	-61.35	232.92	71.00	4.58	81.33
4	164.92	100.33	-64.59	227.33	62.41	4.77	80.33
5	273.08	228.25	44.83	341.50	113.25	5.20	80.66
6	249.00	218.17	30.83	368.33	150.17	4.93	80.46
7	247.75	216.17	2.58	327.17	111.00	5.00	82.55
8	270.17	233.20	36.25	372.42	138.50	4.87	80.43
9	268.17	205.83	62.33	338.92	133.08	5.07	81.42
10	165.30	108.08	-57.22	236.42	71.12	4.36	80.20
11	276.33	24.33	36.00	318.30	151.50	5.00	80.43
12	234.33	205.00	29.42	311.92	106.92	5.13	80.78
13	169.42	109.08	-60.34	230.57	61.15	5.02	80.47

The breakdown viscosity of the samples was variable, with sample 2 having the highest value (94.25 cp). Breakdown viscosity reflects the ability of the sample to withstand shear stress and heating during cooking. Thus samples 3, 4, 10 and 13 with values -61.35 cp, -64.59 cp, -57.22 cp and -60.34 cp respectively may not withstand cooking.

The highest final viscosity (377.17 cp) was obtained from sample 1 while the least value (227.33 cp) was obtained from sample 4. Final viscosity indicates the change in viscosity after the sample was held at 50^{O}C. It shows the ability of the starch to form stable and viscous paste after cooking (Maziya Dixon *et al*., 2007). Gari is prepared for eating by stirring some quantity of gari granules in hot water to form a thick paste or dough ('eba'). The dough is moulded with the palm, deeped in soup or stew and swallowed (Achinewhu and Owuamanam, 2001). The mouldability of the dough influences the consumer acceptability of he gari, which is directly related to the final viscosity of the paste.

Gari can also be taken as a snack by soaking in water and drinking it with roasted ground nut or dried fish. Sample 11 which had the highest value for peak viscosity (276.33 cp) could be said to be best suited for drinking. The results obtained in the study therefore indicate that different fermentation conditions are required for producing gari that could be used for different purposes, may be for eating with soup or stew, or for drinking.

Samples 3, 4, 10 and 13 had the least values for setback viscosity (71.00 cp, 62.41 cp, 71.12 cp and 61.15 cp respectively). Setback viscosity of starch paste is an indication of resistance of the starch to retrograde (Sanni *et al*., 2001). Peak time and pasting temperature correspond to gelatinization time and temperature. It is the time and temperature required for the gari to form a thick paste. Thus sample 10 had the least peak time (4.36 min) while sample 1 had the least pasting temperature (80.12^{O}C).

The results from the study are of significance since they could be used as a guide for quality index of gari meant for different uses.

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