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Research Article

Evaluation of sugar and free amino acid during fermentation of *ogi* from maize, *acha* and sorghum

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Abstract

This research investigated effect of fermentation time and cereal type on the total reducing sugar (TRS), total sugar (TSS), and total free amino acid (TFA) during the production of *ogi*. The result showed that TFA generally increased with increase in fermentation time (7.916 – 17.596 mg/g). Maize, *acha* and sorghum *ogi* had the lowest total reducing sugar (TRS) at 0 h (16.927 glucose mg/g), 12 h (16.655 glucose mg/g) and 48 h (18.212 glucose mg/g) respectively and TSS was lowest in *acha ogi* from 12 h to 48 h (33.191 - 34.370 glucose mg/g). Principal component analysis and Agglomerative hierarchical clustering were used to evaluate the variability in sugar and amino acid contents and ranked the contributions of the variables. The factors were divided into four principal components with cumulative variance contribution rate of 87.47%. The result showed that *acha* and sorghum *ogi* had lower sugar content than maize *ogi* during fermentation. This research suggested that maize, *acha* and sorghum can be used in the production of cereal based *ogi* for weaning food at 48 h due to high free amino acid content, and also advanced the use of *acha* in production of *ogi* for diabetic patients due to its low total sugar content.

Keywords: *Acha*, *ogi*, sugar, amino acid, sorghum, maize

Abbreviations: A - *acha*; M - maize; S – sorghum.

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Introduction

Ogi is a fermented cereal gruel or porridge made from maize (*Zea mays*) or corn; sorghum (*Sorghum vulgare*) or millet (*Pennisetum typhoideum*). The choice of grain depends on preference and ethnicity of the producer (Ohenhen and Ikennebomeh 2007). *Ogi*, usually called pap, *akamu* and *koko* by the people of West Africa can be processed into a slurry paste by heating in boiling water under constant stirring (Adeshokan et al. 2010). It is produced by steeping cereal in water for 72 h at ambient temperature and wet milling (Ohenhen and Ikennebomeh 2007). Fermentation and fermentation period after wet milling depend on individual preference. *Ogi* slurry usually has a smooth texture, a sour flavour resembling that of yoghurt and a characteristic aroma that differentiates it from starch and flour (Omemu 2011).

Acha (*Digitaria exilis*) also known as Fonio, Findi, Funde, Pom, and Kabug is a highly nutritious cereal crop of West African origin belonging to the family Graminaea (Oyetayo and Agbaje 2012; Malomo et al. 2018) is a lesser-known cereal rich in vitamins, minerals, fiber, carbohydrate, protein, amino acids. It is important because it is high in methionine and cysteine lacking in wheat, rice, maize and other cereal crops and also have low glycemic index which could be an advantage in type II diabetic condition (Alegbejo et al. 2011; Ukeyima 2019).

Fermentation of cereals has been reported to increase acidity, total free amino acids and their derivatives by proteolysis and/or by metabolic synthesis. This process reduces the pH thereby inhibiting pathogenic organisms (Kohajdová and Karovičová 2007; Malomo et al. 2018) and also improves the rheological properties, acidification, taste and flavour of fermented foods (Malomo et al. 2019). Starch digestibility also increases during fermentation and this could be due to enzymatic properties of fermenting microflora that brings about the breakdown of starch into simple sugar (Mugula et al, 2003; Malomo et al, 2019). The microorganisms involved in fermentation of food increases palatability and improve the quality of food by increasing the availability of proteins and vitamins (Ogodo et al, 2019). Many researchers have worked on *ogi* (Omemu et al, 2011; Olaniran et al, 2019) but there is dearth of information on effect of fermentation on sugars and free amino

acid. Fermentation may have direct effect on the taste, sugars, total free amino acid and other properties of *ogi*. *Ogi* intended for weaning food should be high in free amino acid and those intended for diabetic patient supposed to have lower sugar content. Therefore, this present study was carried out to determine the effect fermentation time and different cereal “maize (*Zea mays*), *acha* (*Digitaria exilis*) and sorghum (*Sorghum bicolor*)” on the total sugar, total reducing sugar and total free amino acid of *ogi*.

Materials and Methods

Procurement of materials. Quality protein maize (ART/98/SW06/OB/W) was obtained from the Institute of Agricultural Research and Training (I.A.R.T.), Ibadan, Nigeria. Sorghum (red variety) was purchased from a local market in Ile-Ife, Osun State, Nigeria. *Acha* grain was obtained from a local market in Zaria, Nigeria and the identity were ascertained at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Chemicals used for analysis were obtained from Sigma-Aldrich and were of analytical grade.

Production of *ogi*. The cereals (maize, *acha* and sorghum) were sorted, weighed and steeped separately for 72 h. The grains were drained and wet-milled into slurry using an attrition mill. The *ogi* (100 ml) obtained was dispensed into sterile plastics and fermented for 48 h (Olaniran et al. 2019).

Extraction of sugar from *Ogi* samples. *Ogi* samples from maize, *acha* and sorghum were picked at six-hour interval for extraction. The samples were dried in Gallenkamp oven at 45°C for 10 h, ground in a blender (USHA MG 2053 N, India) and sieved using 50 µm. *Ogi* sample (5 g) was weighed and thoroughly mixed in a conical flask containing 50 ml of 80 % ethanol v/v and 10 ml of petroleum ether was added. The ethanol-petroleum ether suspension was stirred at room temperature for 30 min in magnetic stirrer (Lab-line, Model No 1580-1, U.S.A.) and mixture was transferred into centrifuge tubes and centrifuged (Bosch Model No TDL-5, Germany) at 5000 rpm for 30 min. The petroleum ether phase was discarded and the clear ethanol phase was kept in the refrigerator for further analysis (Malomo et al. 2019).

Determination of total reducing sugar. Ethanolic extract (1 ml) was measured into each test tube; 2 ml of Dinitrosalicylic acid reagent was added and boiled for 5 min at 100°C in Gallenkamp water bath (Gallenkomp, HH-S6, England) and cooled thoroughly under running water. Distilled water (7 ml) was added and the absorbance was read against reagent blank at 540 nm in a UV Spectrophotometer (Spectrumlab 752S, YM1206PHB2, China). The amount of reducing sugar in the samples was extrapolated from a standard curve of known concentrations of glucose (0-1000 µg/ml) (Adepoju et al. 2016).

Determination of total sugar. Total sugar was determined using the anthrone reagent method of Morris (1948) described by Malomo et al. (2019). Ethanolic extract (1 ml) was added to 4 ml of anthrone reagent, heated in boiling water bath (Gallenkomp, HH-S6, England) for 10 min and rapidly cooled. Absorbance was read at 620 nm against a reference blank in spectrophotometer (Spectrumlab 752S, YM1206PHB2, China) and the amount of sugar liberated was obtained from the standard curve based on known concentrations of glucose (10-100mg/l)

Determination of total free amino acid. *Ogi* samples (5 g) were weighed into 250 ml conical flask and 50 ml of 80 % ethanol v/v was added. The suspension was mixed properly and 10 ml of petroleum ether was added. The ethanol-petroleum ether suspension was stirred at room temperature for 30 min using a magnetic stirrer (Lab-line, Model No 1580-1, U.S.A) and centrifuged at 5000 rpm for 30 min. The petroleum ether phase was discarded and the clear ethanol phase was used as the sample extract (Malomo et al. 2019). The ninhydrin method described by Rosen (1957) was used for determination of free amino acid. Cyanide acetate buffer (0.5 ml at pH 5.4) and 0.5 ml of 3.0 % ninhydrin solution (3 g of ninhydrin in 100 ml of 2-methyl ethanol) was added to the extract (1.0 ml) in test tube and heated in boiling water bath (Gallenkomp, HH-S6, England) for 15 min. Isopropyl-alcohol water mixture (10 ml) at ratio 1:1 was added rapidly and the solution was allowed to cool to room temperature (27±2°C). The absorbance was read at 570 nm using spectrophotometer (Spectrumlab 752S, YM1206PHB2, China). Total

free amino acid in the samples was obtained from a standard curve of known concentrations of glycine (10-100µm/ml) (concentrations of glucose (10-100mg/l) (Omafuvbe 2000).

Statistical analysis. Data obtained were subjected to descriptive and inferential statistics using SPSS (version, SPSS, Inc., USA). Means of samples were separated using Duncan Multiple range Test (SAS Institute 1985). Principal component and clustered analysis were carried out on the data obtained using XLSTAT 2016 (Addinsoft Inc. USA).

Results and Discussion

Total free amino acid of *ogi* powder at different stages of fermentation. The total free amino acid (Table 1) generally increased from the beginning of fermentation to 36 h and slightly decreased from 36 h to 48 h in maize and sorghum *ogi*. It was significantly higher ($p < 0.05$) in *ogi* produced from maize (9.788 - 17.600 mg/g) than sorghum (8.474 - 16.610mg/g) and *acha* (7.916 - 16.607mg/g) from 0 h to 24 h but there was no significant difference ($p > 0.05$) from 36 h to 48 h. There was no significant difference ($p > 0.05$) between *ogi* produced from sorghum and *acha* from 12 h to 48 h of fermentation. The total free amino acid of *ogi* produced from sorghum and *acha* had similar total free amino acid content throughout the period of fermentation. Malomo et al. (2019) also reported increase in total free amino acid during fermentation of *acha* and maize and attributed it to the proteolytic activities of fermenting organisms.

Total reducing sugar *ogi* at different stages of fermentation. The total reducing sugar was highest in maize *ogi* from 12 h to 36 h (20.544 - 23.591 glucose mg/g) and was not significantly ($p > 0.05$) different from *acha ogi* at 48 h. It was higher in sorghum *ogi* than *acha ogi* from 0 h to 12 h while *acha* had significantly higher ($p < 0.05$) than sorghum *ogi* from 36 h to 48 h of fermentation. Malomo et al. (2019) also reported that the total reducing sugar of fermenting maize was higher than that of *acha* during fermentation. The range of result of this work is in agreement with the report of Olaniran et al. (2019).

Table 1. Total free amino acid of *ogi* powder at different stages of fermentation, mg/g

Samples	Fermentation time, h				
	0	12	24	36	48
A	7.916 ^b ±0.114	13.607 ^b ±0.211	16.607 ^b ±0.284	17.344 ^a ±0.890	17.560 ^a ±0.060
M	9.788 ^a ±0.414	14.609 ^a ±0.200	17.600 ^a ±0.092	17.632 ^a ±0.100	17.596 ^a ±0.100
S	8.474 ^{ab} ±0.150	13.607 ^b ±0.241	16.610 ^b ±0.217	17.686 ^a ±0.200	17.524 ^a ±0.040

Values are means of three replicates ± standard error.

Means followed by different superscript in the same column are significantly different at $p < 0.05$.

Total reducing sugar *ogi* at different stages of fermentation. The total reducing sugar was highest in maize *ogi* from 12 h to 36 h (20.544 - 23.591 glucose mg/g) and was not significantly ($p > 0.05$) different from *acha ogi* at 48 h. It was higher in sorghum *ogi* than *acha ogi* from 0 h to 12 h while *acha* had significantly higher ($p < 0.05$) than

sorghum *ogi* from 36 h to 48 h of fermentation. [Malomo et al. \(2019\)](#) also reported that the total reducing sugar of fermenting maize was higher than that of *acha* during fermentation. The range of result of this work is in agreement with the report of [Olaniran et al. \(2019\)](#).

Table 2. Total reducing sugar *ogi* at different stages of fermentation, glucose mg/g

Samples	Fermentation time, h				
	0	12	24	36	48
A	17.689 ^b ±0.200	16.655 ^c ±0.304	20.540 ^a ±0.210	20.925 ^b ±0.445	19.164 ^a ±0.164
M	16.927 ^c ±0.100	20.544 ^a ±0.100	23.448 ^a ±0.200	23.591 ^a ±0.009	19.069 ^a ±0.069
S	18.355 ^b ±0.945	19.162 ^b ±0.520	20.449 ^a ±0.002	19.164 ^c ±0.091	18.212 ^b ±0.244

Values are means of three replicates ± standard error.

Means followed by different superscript in the same column are significantly different at $p < 0.05$.

Table 3. Total sugar in *ogi* at different stages of fermentation, glucose mg/g

Samples	Fermentation time, h				
	0	12	24	36	48
A	39.335 ^a ±0.300	36.767 ^b ±0.112	33.191 ^b ±0.297	32.191 ^c ±0.410	34.370 ^c ±0.112
M	39.049 ^a ±0.490	40.018 ^a ±0.231	39.085 ^a ±0.2130	40.086 ^b ±0.311	37.156 ^b ±0.128
S	30.333 ^b ±0.210	32.543 ^c ±0.102	33.262 ^c ±0.381	37.159 ^a ±0.094	38.764 ^a ±0.086

Values are means of three replicates ± standard error.

Means followed by different superscript in the same column are significantly different at $p < 0.05$.

Total sugar in *ogi* at different stages of fermentation. The total sugar content of *ogi* was highest in *ogi* produced from *acha* (39.335 glucose mg/g) and lowest in *ogi* produced from sorghum (30.333 glucose mg/g) at the beginning of fermentation. It was lowest in *achaogi* between 24 h and 48 h (32.191 and 34.370 glucose mg/g). *Ogi* produced from maize had the highest total sugar content from the beginning of fermentation to the 36

h (37.156 - 40.086 glucose mg/g). The breaking down of complex carbohydrate into simple sugar by fermenting microorganism and utilization of these sugars as carbon source could be responsible for fluctuation in total sugar ([Oyarekua and Adeyeye 2009](#); [Adepoju et al. 2016](#)). The highest total sugar content in maize *ogi* could be due to high amount of digestible starch. [Jideani and Podgorski \(2009\)](#) and [Jideani and Jideani \(2011\)](#) reported the digestible

starches (DS) of maize and *acha* to be 43.7 and 41.4 respectively. This suggest that *acha* could be consumed as food with low glycemic index.

PCA was applied to pooled measurements in order to describe the group of physical data, to establish the relationships between the different physical variables, and to detect the most important factors of variability.

Table 4. Eigenvalues of *ogi* samples

	PC1	PC2	PC3
Eigenvalue	1.623	1.002	0.376
Variability, %	54.086	33.384	12.530
Cumulative, %	54.086	87.470	100.000

The PCA with Eigenvalue more than 1 were selected. Table 4 showed that component PC1 and PC2 best represent the samples with Eigenvalues of 1.623 and 1.002 respectively. These components account for 87.470 % of the total variance with PC1 having 54.086 % and PC2 with 33.384 % (Table 4). Total reducing sugar (0.901) and Total free amino acid (0.889) were best represented in component 1 while the total sugar (0.987) was represented on component 2. Total reducing sugar (TRS) and total free amino acid have strong positive correlation as shown in Figure 1.

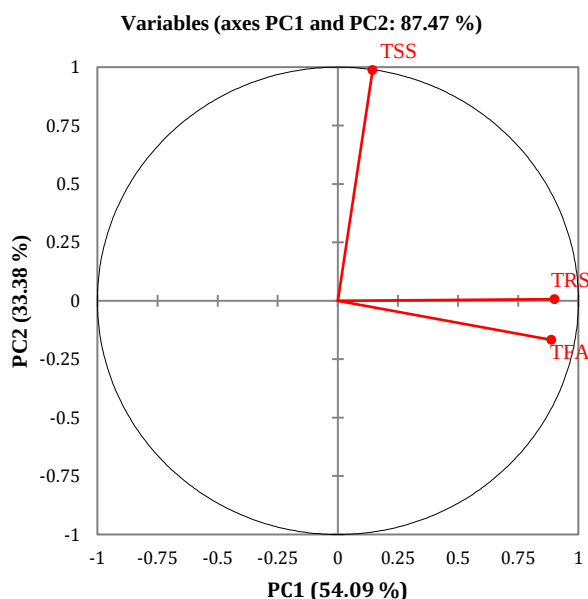


Figure 1. The plot showing relationship between variables

Total sugar has weak positive correlation with Total reducing sugar and negative correlation with free amino acid. This was an indication that the decrease in the total sugar led to increase in total free amino acid.

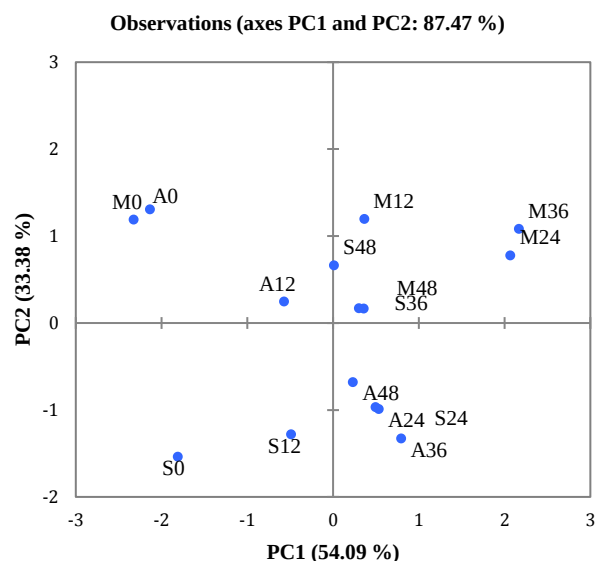


Figure 2. The plot showing relationship between observations

Samples were grouped according to the hour of fermentation (Fig. 2). *Ogi* produced from maize (M0), *acha* (A0), sorghum (S0) are represented at the negative side of PC1 at the beginning of fermentation. *Ogi* produced from *acha* (A12) at 12 h, was also represented on the negative axis of PC2. *Ogi* produced from maize at 24 h (M24), 36 h (M36), 48 h (M48) and sorghum at 36 h (S36), are grouped together on the positive axis of component 1. *Acha* fermented for 24h (A24) and 36 h (A36), and (A48) are grouped together at the negative axis of component 2 likewise *Ogi* produced from sorghum at 12 h (S12) and 24 h (S24) while *ogi* maize fermented for 12 h (M12) and sorghum for 48 h (S48) were grouped on the positive axis of component 2.

Figure 3 showed that maize *ogi* had the highest total sugar and starch but were both higher in samples fermented at 12 and 48 h than at 0, 24 and 36 h. The Biplot (Fig. 3.) showed that total sugar (TSS) was high in maize *ogi* at 12 h, 24, 36 and 48 h and in sorghum at 36 and 48 h.

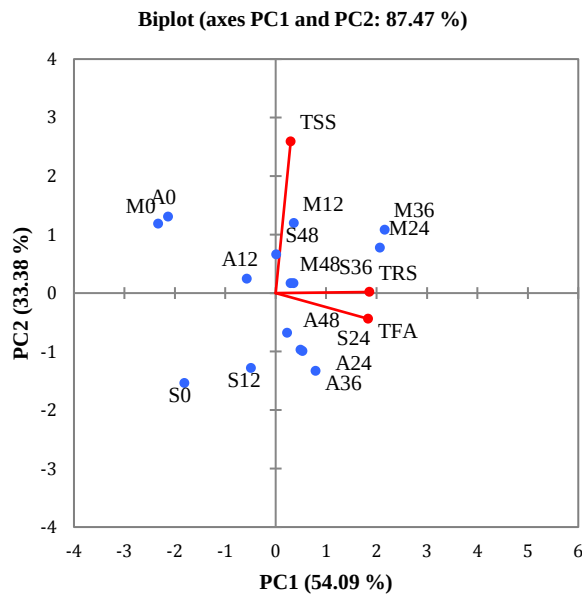


Figure 3. The Biplot showing relationship between variables and observations

Ogi produced from *acha* had negative correlation with total sugar from 0 hour to 12 h of fermentation. This showed the longer the fermentation time, the lower the total sugar in *acha*. It also has negative correlation with sorghum at the beginning of

fermentation and at 12 h indicating that TSS increased in sorghum with increase in time of fermentation. Total free amino was low in all *ogi* powder samples at 0 h (A0, M0 and S0) and only had weak positive correlation at 12 h in maize *ogi* (M12). It was highest in sorghum at 36 h, followed by maize *ogi* at 48 h. It also had strong correlation with *achaogi* fermented between 24 and 48 h and maize *ogi* fermented for 24 and 36 h. Increase in fermentation time led to increase in total free amino acid (TFA) in all *ogi* samples produced. Total reducing sugar also increased with increase in fermentation time and was highest in *ogi* produced from maize.

Agglomerative hierarchical clustering showed that *acha ogi* (A0) and maize *Ogi* (M0) are related at the beginning of fermentation and are grouped in class one (Figure. 4). *Ogi* maize fermented between 12 and 48 h was related to *acha ogi* at 12 h in class two. *Acha ogi* fermented between 24 to 48 hours is related with sorghum *ogi* at 24 h in class 3. Sorghum *ogi* fermented for 0 h and 12 h was grouped separately in class 4. This shows that *ogi* from *acha* is more related to maize *ogi* than sorghum *ogi* in terms of total reducing sugar, total sugars and total free amino acid during fermentation of the slurry from these three cereals.

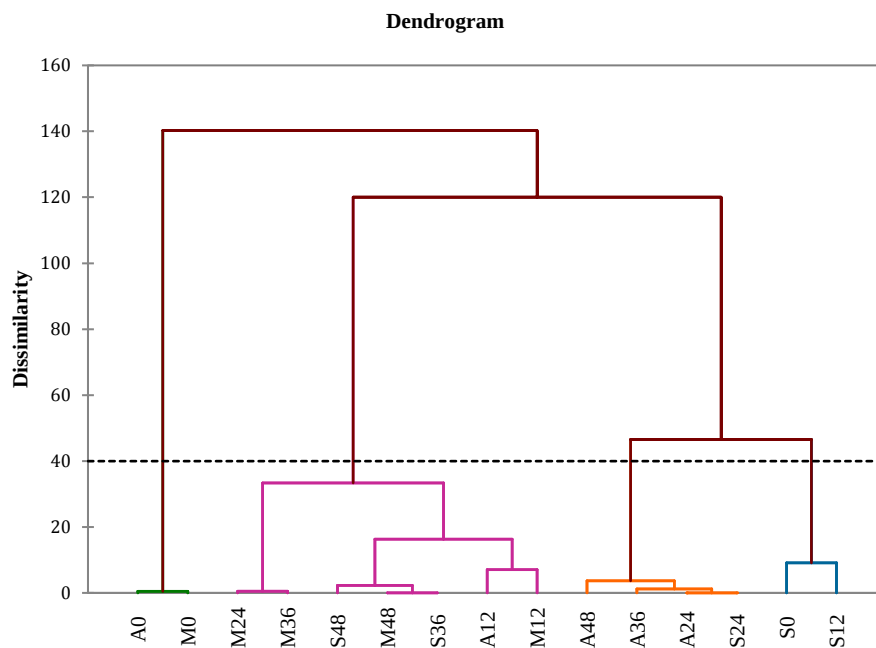


Figure 4. Agglomerative hierarchical clustering of *ogi* from maize, sorghum and *acha*

Conclusions

The study showed that fermentation time and type of cereal affected the quantity of total sugar, total reducing sugar and total free amino acid available in *ogi*. Maize had the highest total sugar, total free amino acid and total reducing sugar. Though the initial total sugar of sorghum sample was low, it increased towards the end of fermentation. The choice of sorghum or *acha* for *ogi* with low glycemic index depends on the time of fermentation. It is suggested that the cereals meant for weaning should be fermented for 48 h because total free amino acid was highest at that point. *Ogi* produced from *acha* at 48 h is recommended for people living with Type II diabetes because it had the lowest sugar content and high total free amino acid which was not significantly different from maize and sorghum *ogi*.

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