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USE OF PRINCIPAL COMPONENT ANALYSIS AND AGGLOMERATIVE HIERARCHY CLUSTERING TO STUDY THE RELATIONSHIP BETWEEN ALPHA AMYLASE AND STARCH DURING FERMENTATION OF *OGI* FROM MAIZE, SORGHUM AND *ACHA*

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ABSTRACT

Ogi, a popular breakfast cereal and weaning food in West Africa was produced from maize, acha and sorghum. *Ogi* was produced from the three cereals and the gruels obtained were subjected to fermentation. The total starch content and alpha amylase activity during the period of fermentation of the *ogi* slurries were evaluated using standard methods. Total starch content was determined at 0, 12, 24, 36 and 48 h and α-amylase activity was also determined at 10 ºC, 20 ºC, 30 ºC, 40 ºC at 0, 12, 24, 36 and 48 h. The outcome of the research showed that α-amylase activity increased with increase in temperature and was highest in maize *ogi* (1.507 – 4.458 E. U) while acha *ogi* (0.497 – 3.219 E. U) had the lowest α-amylase activity . The starch content decreased with increase in time of fermentation time and was highest in maize *ogi* while acha *ogi* had the lowest. This shows that the higher the temperature of fermentation and longer fermentation time can increase the breakdown of starch by α-amylase in *ogi* slurry. The result of the Principal component analysis (PCA) showed distinct similarity in maize and acha. Agglomerative hierarchical clustering grouped acha into two groups (groups one and two) but grouped all maize and sorghum *ogi* samples together into group three. Due to the low starch content and low α-amylase activity observed in acha, it could be utilized in the production of gluten-free *ogi* with low glycemic index especially for vulnerable groups.

Keywords: alpha-amylase, starch, fermentation, maize, sorghum, acha, *ogi*, PCA, hierarchy, clustering, relationship

INTRODUCTION

Ogi is a smooth, free flowing thin porridge obtained from wet milled, fermented maize and sorghum in the southern part of Nigeria. It serves as a major weaning food for infants, a breakfast meal for both children and adults in West Africa **(Abioye and Aka, 2015; Ukeyima *et al*., 2019)**. *Ogi* can be produced from cereals such as maize, sorghum, millet and recently acha. The choice of grain depends on preference and ethnicity **(Ohenhen and Ikenebomeh, 2007)**.
Presently, researchers are studying how resistant starches can reduce the glycemic and insulin response **(Deepa *et al*. 2010; Jideani and Jideani, 2011)**. The in-vitro starch digestibility and glycemic property of acha and maize porridge has been investigated.The in-vitro digestibility and glycemic property of maize and acha showed the digestible starches (DS) in maize and acha were 43.7 and 41.4 **(Jideani and Podgorski 2009)**. Acha also contains resistant starches which has been shown to be promising in the management or prevention of certain diseases or health conditions **(Deepa *et al*. 2010; Jideani and jideani, 2011)**.
Fermentation improves absorption of nutrient, especially from agricultural produce, by enzymatic splitting of cellulose, hemicellulose, and related polymers that are not digestible by humans into simpler sugars and sugar derivatives **(Parker, 2018; Malomo *et al*., 2019)**. Most of these enzymes are naturally present in cereal grains but at low levels **(Malomo *et al*., 2019)**. α-Amylases are hydrolytic enzymes which specifically break the α-1, 4-glucosidic bonds in starch. Possible sources of α-amylase in plants, animals and microbes have been reported **(Aiyer, 2005; Asante *et al*., 2013)**. The effect of controlled fermentation of *ogi* has been reported **(Ohenhen and Ikenebomeh, 2007)**. The volatile compounds and associated microbes during fermentation of *ogi* slurry from maize have been identified by **Bolaji *et al*. (2020)**. There are several articles on the use of acha but there is a dearth of information on the effect of fermentation on α-amylase activity and starch during fermentation of *ogi* from acha, sorghum and maize, hence this study.

MATERIALS AND METHODS

White Quality protein maize variety (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 grains were purchased from a local market in Ebonyi State, Nigeria.
Ogi was produced from the cereals using the method described by **Olaniran *et al*. (2019)**. Slurries of *ogi* obtained from these cereals were further fermented for 48 h. Samples were taken from the fermenting slurries at 0, 12, 24, 36 and 48 h. Samples obtained were then used for further studies.

Determination of α-amylase activity of *ogi* samples

Alpha-amylase activity of the fermenting *ogi* samples were determined as described by **Malomo *et al*. (2019)**. Each *ogi* sample (5 g) was weighed at 0, 12, 18, 24, 36 and 48 h of fermentation and homogenised in 50 ml of 0.2 M sodium acetate buffer (pH 4.0). The homogenate was transferred into a conical flask and was mechanically shaken at 150 rpm for 10 min at room temperature in a Gallenkamp orbit shaker (3597 C2-2, England). The suspensions were transferred to centrifuge tubes and centrifuged at 5000 rpm for 30 min in centrifuge (Bosch Model No TDL-5, Germany) and the supernatant was used as crude enzyme for enzyme assay. The substrate for assay was 0.5 ml of 0.5 % soluble starch, buffered with 0.2 ml of 0.2 M sodium acetate (pH 5.6). Crude enzyme extract (0.3 ml) was added to the mixture, mixed and incubated at 40 °C for 30 min in Gallenkamp water bath (HH-S6, England). The reaction was terminated by the addition of 2 ml of 3, 5-Dinitrosalicyclic acid (DNSA) and boiled for 5 min in the water bath. The mixture was cooled under running water and 7 ml of distilled water was added. Blank that consisted of 0.3 ml distilled water, 0.5 ml of 0.5 % soluble starch and 0.2 ml of buffer was treated in similar way. The absorbance of the resultant solution was read at 540 nm in UV Spectrophotometer (Spectrumlab 752S, YM1206PHB2, China). Reducing sugar in the samples was estimated from a standard curve of known concentrations of maltose (0-1000 µg/ml). One unit of α-amylase was defined as the amount of enzyme required to produce 1 microgram of reducing sugar equivalents per minute measured as maltose from soluble starch under the experimental conditions. 1 enzyme unit (E.U.) =1 µg of maltose produced/min

Extraction and estimation of starch from *ogi* samples

Modified method of **Kiran and Sandeep (2016)** was used for determination of starch by using anthrone method. *Ogi* samples (5 g) was homogenized in 80% ethanol to remove sugars present in the samples. Residue was retained after centrifugation at 5000 X g for 15 min. The starch was extracted by 52% perchloric acid at 0 ºC for 20 min. One millilitre (1 ml) of extract fromeach sample was taken into testtubes and 4 ml of anthrone was added. The tubes were kept for boiling for 5 min. The colour intensity was measured at 620 nm in spectrophotometer (Spectrumlab 752S, YM1206PHB2, China). Starch concentration was calculated from standard curve of known concentration glucose (0 – 10000µg).

RESULTS AND DISCUSSION

Alpha amylase activity during fermentation of *ogi* samples

The α-amylase activity increased with increase in temperature (Fig. 1-4). It was generally higher in *ogi* samples produced from maize (1.507- 4.458 E. U) than sorghum (1.070 – 3.019 E. U) and lowest in *ogi* produced from acha (0.497 – 3.219E. U) throughout the period of fermentation.It was within the range of 0.497 – 3.281 E. U at 10 ºC, 0.643 – 3.453 E. U at 20 ºC, 0.931 – 3.531 at 30 ºC and 1.875 – 4.458 E. U at 40 ºC. Acha was reported to have lower α-amylase activity during fermentation of *the ogi* slurries and relatively evoke low sugar on consumption, an advantage for diabetics **(Malomo *et al.*, 2019)**. Amylolysis susceptibility of maize starch is higher, because of the presence of surface pores and channels that facilitate enzymatic diffusion **(Zhang *et al*. 2006)**.

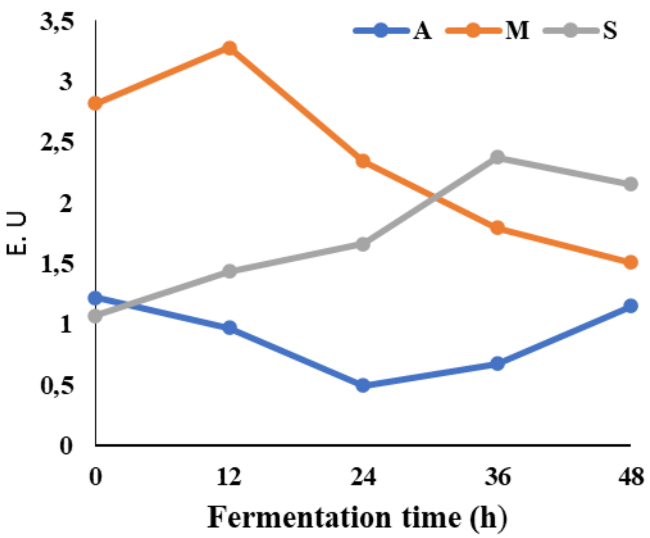

Figure 1 Alpha amylase activity of *ogi* samples at 10 ºC

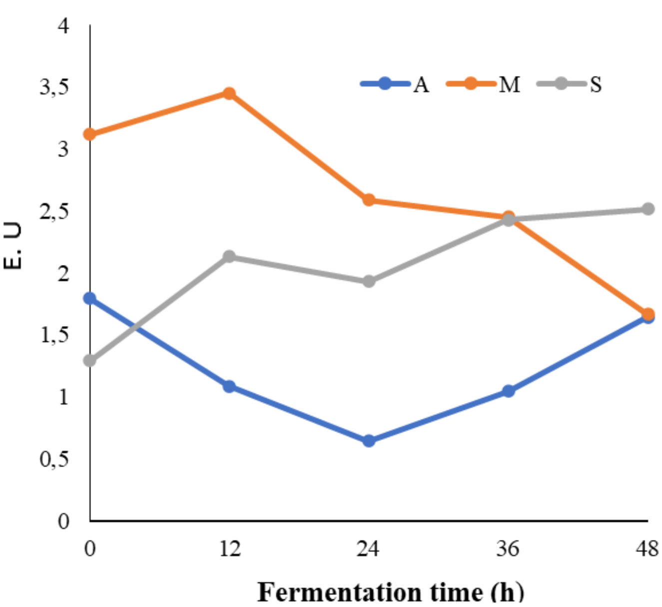

Figure 2 Alpha amylase activity of *ogi* samples at 20 ºC

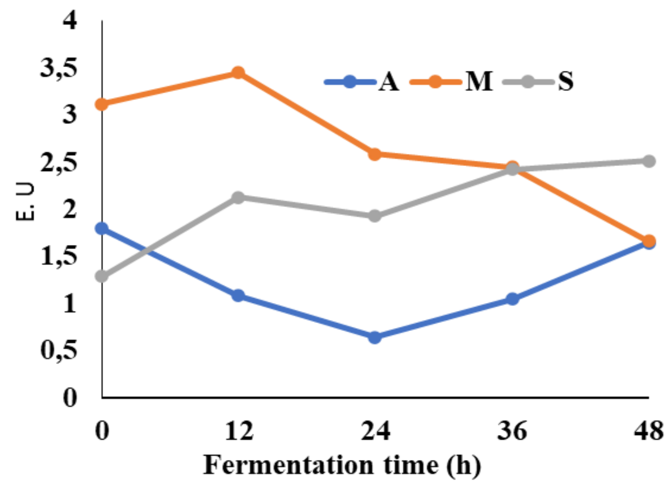

Figure 3 Alpha amylase activity of *ogi* samples at 30 ºC

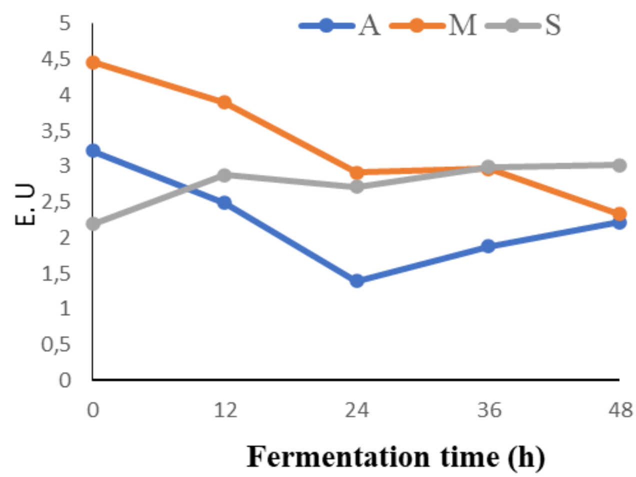

Figure 4 Alpha amylase activity of *ogi* samples at 40 ºC

Total starch content of *ogi* samples

Ogi samples produced from maize had the highest starch content while (61.20 – 71.55 %) and lowest in acha *ogi* samples (33.31 – 51.86 %) (Table 1).There was significant difference ($p < 0.05$) in the starch content of all the cereals from 0 – 36 h of fermentation but there was no significant difference ($p > 0.05$) between *ogi* from maize and sorghum *ogi* at 48 h of fermentation. Starch is the main constituent of maize kernels, about 72–73% of the total weight (Paraginskia *et al*. 2014). Acha (68 %) is reported to have lower starch content than sorghum (73.8 %) **(Ballogou *et al*., 2013)**.

Table 1 Changes in total starch content of *ogi* samples

Sample	Fermentation time (h)				
	0	12	24	36	48
A	$51.86^{c}\pm1.200$	$51.32^{b}\pm1.11$	$47.06^{c}\pm2.01$	$42.47^{c}\pm1.78$	$33.31^{b}\pm0.70$
M	$71.55^{b}\pm1.00$	$69.16^{b}\pm0.08$	$68.45^{b}\pm0.65$	$66.58^{b}\pm1.03$	$61.20^{a}\pm 1.50$
S	$73.23^{a}\pm0.56$	$72.96^{a}\pm0.74$	$72.11^{a}\pm1.02$	$70.99^{a}\pm0.98$	$60.00^{a}\pm0.02$

Keys: A- *Ogi* from Acha; M- *Ogi* from Maize; S- *Ogi* from Sorghum

The principal component analysis (PCA) was used to determine the relationship between temperature and α-amylase. Also, to evaluate the effect of α-amylase on starch during fermentation of *ogi* produced from maize, acha and sorghum. The principal components were grouped into five components and PC 1 and PC 2 best represent the samples.

Starch (TS) had positive correlation (Fig. 4) with all the variables but had strongest correlation with α-amylase activity at 10 ºC (0.616) and reduced to 0.544 at 40 ºC. This showed that as the temperature increased, the starch production decreased. The temperature at 10 ºC had strongest positive correlation at 20 ºC (0.969) and also reduced progressively to 0.658 at 40 ºC.

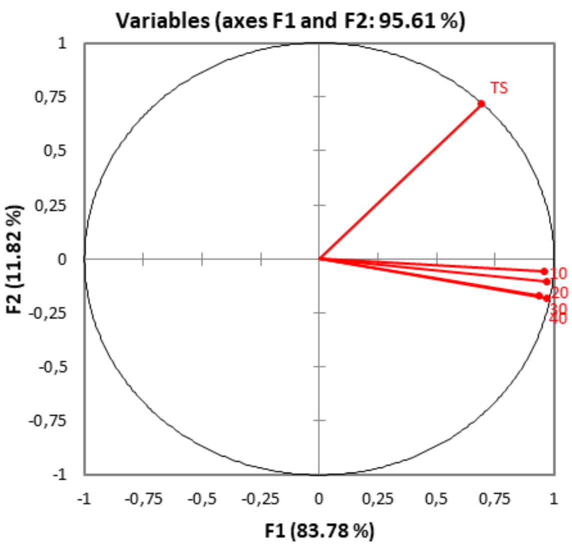

Figure 5 Principal component analysis (PCA) for parameters-projection of variables in the factor-plane, considering two factors.

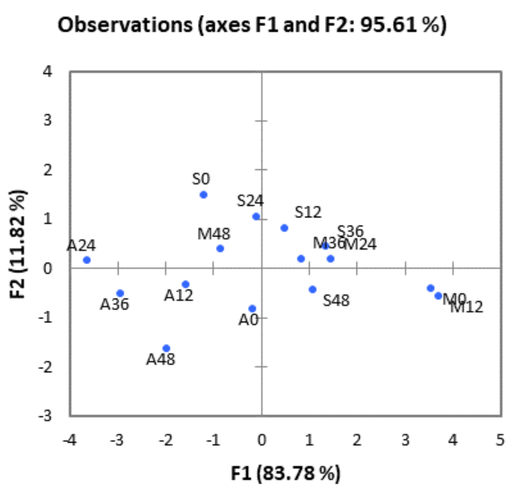

Figure 6 Principal component analysis (PCA) for parameters-projection of observation in the factor-plane, considering two factors.

The relationship between the samples are shown in Fig. 6. All acha samples were grouped separately on the negative side of PCA 1 except A0 at the negative side of PCA 2. Maize *ogi* fermented for 0 to 36 h were represented on the positive axis of PCA 1 and M48 on the positive side of PCA 2. Sorghum was represented on the positive axis of PCA 2 at 0 – 24 h and on the positive side of PCA 1 from 36 to 48 h. This showed that there is similarity in the pattern of α-amylase activity in both maize and sorghum *ogi* during fermentation.

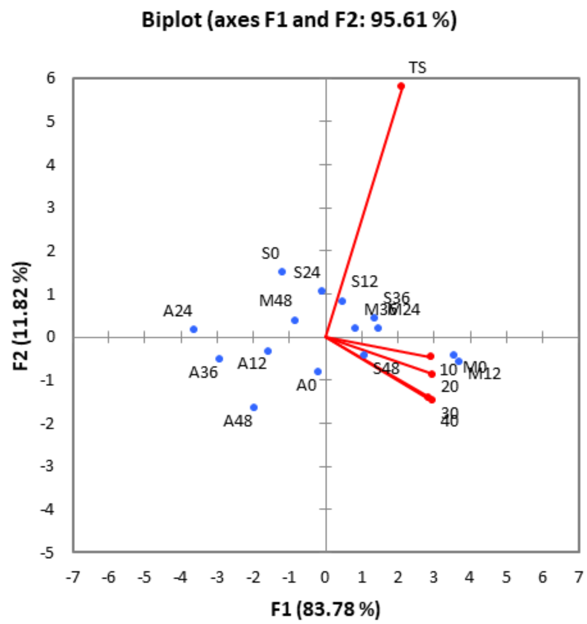

Figure 7 Principal component analysis (PCA) for biplot in the factor-plane, considering two factors.

The biplot (Fig 7) showed that the α-amylase activity on maize *ogi* and sorghum *ogi* fermented from 0 to 24 h had positive correlation with TS while all *ogi* samples produced from acha had negative correlation with TS which means that *ogi* from acha had the lowest TS. The lower the temperature, the stronger the correlation with starch. This showed that increase in α-amylase activity led to decrease in starch.

Agglomerative hierarchical clustering (AHC) (Fig. 8) S grouped *ogi* samples into three. Acha *ogi* fermented from 0 to 36 h were grouped in class 1 while at 48 h, acha *ogi* was grouped in class 2. Maize *ogi* and sorghum *ogi* were grouped in class 3 showing that there are similarities between alpha amylase activity and starch content of maize *ogi* and sorghum *ogi*.

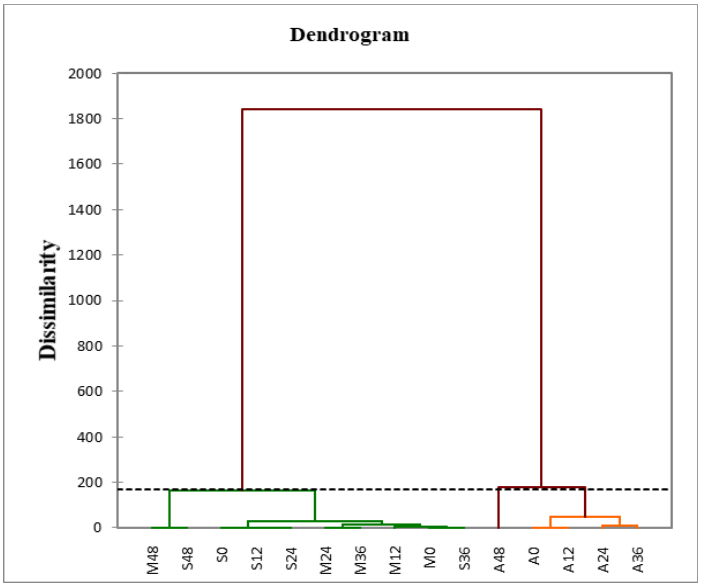

Figure 8 Agglomerative hierarchical clustering

CONCLUSION

The α-amylase activity generally decreased with increase in temperature. It was highest in maize *ogi* and lowest in acha *ogi*. Total starch content was also highest in maize *ogi* and lowest in acha *ogi*. PCA showed more similarity in maize *ogi* and sorghum *ogi* than acha *ogi*. The AHC grouped *ogi* produced from acha in different and grouped acha *ogi* separately. It is therefore suggested that *ogi* with low glycemic index could be produced from this rich non-glutenous cereal "acha".

REFERENCES

Abioye, V. F. & Aka, M. O. (2015). Proximate composition and sensory properties of moringa fortified maize-*ogi*. *Journal of Nutrition and Food Science*, 12,1-4. https://doi.org/10.4172/2155-9600.s12-001

Asante, E., Adjaottor, A. A. & Woode, M. Y. (2013). Isolation of α-amylase from malted rice (Wita 7) extract using cassava starch column procedure. *African Journal of Biotechnology*, 12(23), 3738-3744.

Aiyer, P. V. (2005). Review on amylases and their applications. *African Journal of Technology*, 4(13), 1525-1529

Ballogou, V. Y., Soumanou M. M., Toukourou, F. & Hounhouigan, J. D. Structure and nutritional composition of Fonio (*Digitaria exilis*) grains :A Review. (2013). *International Resource Journal of Biology and Science*, 2(1), 73-79.

Bolaji, O. T., Apotiola, Z. O., Ojo, T. I., Abdussalaam, R. B., Akoro, S. M. **&** Ogunsola, O. A. (2020).Volatile Compounds Determination by GC-MS and Identification of Dominant Microorganism at Varying Fermentation Period of Ogi Slurry. *Journal of Food Stability*, 187. https://doi.org/10.36400/j.food.stab.3.1.2020-0033

Deepa, G., Sing, V. & Naidu, K. A. (2010). A comparative study on starch digestibility, glycemic index and resistant starch of pigmented ("Njavara" and "Jyothi") and a non-pigmented ('IR64') rice. Journal of Food Science and Technology, 47(6), 644–649. https://doi.org/10.1007/s13197-010-0106-1

Jideani, A. & Jideani,V. A. (2011). Developments on the cereal grains *Digitaria exilis* (acha) and *Digitaria iburua* (iburu). Journal of Food Science and Technology, 48(3), 251-259. https://doi.org/10.1007/s13197-010-0208-9

Jideani, V. A. & Podgorski, S. C. (2009). In-vitro starch digestibility and glycemic property of acha (*Digitaria exilis*) porridge. Cereal Foods for World Supplement, 54, A48.

Kiran, K. M. & Sandeep, B.V. (2016). Comparative study of proximate composition and total antioxidant activity in leaves and seeds of *Oryza sativa* and *Myriostachya wightiana*. *International Journal of Advanced Resources*, 4(2), 842-852.

Malomo, A. A., Abiose, S. H. & Adeniran, H. A. (2019). Effects of substitution of acha and soybean on alpha amylase activity, sugars and total free amino acid during production of maize *masa*. *Journal of Microbiology, Biotechnology and Food Science*, 9(3), 534–538. https://doi.org/10.15414/jmbfs.2019/20.9.3.534-538

Ohenhen, R. E. & Ikenebomeh, M. J. (2007). Shelf Stability and Enzyme Activity Studies of Ogi: A Corn Meal Fermented Product. *Journal of American Science*, 3(1), 38 -42.

Olaniran, A. F., Abiose, S. H. & Gbadamosi, S. O. (2019). Nutritional quality and acceptability evaluation of *ogi* flour biofortified with garlic and ginger. *Journal of Health Sciences*, 7, 101-109 https://doi.org/10.17265/2328-7136/2019.02.005

Paraginskia, R. T., Vaniera, N. L., Moomand, K., de Oliveiraa, M., Zavarezea, E. R., Silvad,R. M., Ferreiraa, C. D. & Eliasa, M. C. (2014). Characteristics of starch isolated from maize as a function of grain storage temperature. *Carbohydrate Polymer*, 102, 88– 94. https://doi.org/10.1016/j.carbpol.2013.11.019

Ukeyima, M. T., Acham, I. O. & Awojide, C. T. (2019). Quality evaluation of *ogi* from acha (*Digitaria exilis*), soybean (*Glycine max*) and carrot (*Daucus carota* L.) composite flour. Asian *Journal of Biotechnology, Bioresources and Technology*, 5(2), 1-11. https://doi.org/10.9734/ajb2t/2019/v5i230054

Zhang, G. Y., Ao, Z. H. & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecule*, 7, 3252–3258. https://doi.org/10.1021/bm060342i