Global Scientific JOURNALS

GSJ: Volume 8, Issue 6, June 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

Physico-chemical and Nutritional Assessment of *Obiolor*, a Nigerian Fermented Cereal-based Beverage

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Abstract

Two types of processing methods for *Obiolor* produced from sorghum and millet grains were investigated namely: wet-milled and flour-milled prepared *Obiolor*. Study of their physico-chemical, nutritional and antinutritional components were evaluated for improved production and nutritional enhancement. Proximate parameters determined were percentage moisture, ash, fat, crude fibre, crude protein and carbohydrate. Antinutritional contents such as phytic acid and trypsin inhibitor were evaluated as well as physico-chemical analysis such as pH, total titratable acidity (TTA), total soluble solids (TSS) and specific gravity. *Obiolor* produced from wet-milled process had 74.41±0.15% moisture, 2.86±0.00% ash, 5.20±0.02% fat, 1.75±0.25% crude fibre, 3.89±0.04% crude protein and 11.10±0.22% carbohydrate. *Obiolor* from flour-milled process had moisture content of 71.79±0.00%, ash content of 1.42±0.00%, fat content of 5.38±0.12%, crude fibre of 3.50±0.50%, crude protein of 4.40±0.01% and carbohydrate content of 13.52±0.60%. Antinutritional component in the traditional fermented beverages showed that phytic acid was 1.20 mg⁄100g and 1.00 mg⁄100g while trypsin inhibitor had values of 3.426 mg⁄100g and 3.488 mg⁄100g for both wet and flour milled prepared beverage. There was a fall in pH with a corresponding increase in total titratable acidity during the fermentation period. Total soluble solids and specific gravity were also determined. The various studied parameters indicated that flour-milled prepared *Obiolor* is of a better nutritional and physico-chemical attributes as compared to wet-milled prepared *Obiolor.*

Key words:

Beverage, Nutritional content, Antinutritional content, *Obiolor*, Proximate analysis, Wet-milled, Flour-milled.

Introduction

Cereal grains form a major source of dietetic nutrients for all people, predominantly those in the developing countries and could be taken in form of food, feed or beverage (Slavin 2010). A beverage is any fluid other then water which can be consumed in a bid to quench thirst (Sunday and Enobong, 2017). Some may however be consumed as an alternative in filling human nutritional deficit as well as a source of stimulant (Sunday and Enobong, 2017). A variety of beverages are consumed world wide ranging from exotic beverage to the native beverage which may be alcoholic or non-alcoholic (Sunday and Enobong, 2017). *Obiolor* is a non-alcoholic drink produced from fermented sorghum and millet malts in Nigeria. It *is* consumed daily by people of Igala tribe in Nigeria, prepared on a small scale basis and extremely associated with good health (Solange *et al*., 2014). Indeed, spontaneous fermentations enhance the dietary value of beverages that contributes extensively to the diet of consumers. They also increase sensory quality which is very germane in beverage acceptance. Non-alcoholic beverages are consumed by all population particularly children, pregnant women, sick and old people and are used for weaning infants while alcoholic beverages are usually preferred by men. These beverages have diverse names in different countries and regions where they are produced. Their production varies from one region to another, but essentially includes malting, brewing and fermentation feedstock for millet, maize and mostly sorghum (Solange *et al*., 2014). Sorghum contains anti-nutritional factors such as tannins and phytates. They can form complexes with proteins, vitamins and minerals and therefore diminish the bioavailability of nutrients. To improve its nutritional quality, germination, fermentation, cooking (Dicko *et al.*, 2006) and drying methods are used. Also, the cereal-based beverage could get wasted due to spoilage as a consequence of preparing excess quantity. This could be avoided by dry-milling the germinated grains and using appropriate quantity of the flour preferred by the consumer at a given period. Although, there are literatures on the traditional preparation of *Obiolor*, there is scanty information about its production from flour and its effect on nutritional, antinutritional and physico-chemical quality. Hence, the study therefore seeks to compare the physico-chemical properties, proximate composition and antinutritional content of *Obiolor* when produced from wet-milled and flour-milled processes.

Materials and Methods

Sample Collection

Sorghum and Millet grains used in this study was purchased at a retail market in Abeokuta, Nigeria, conveyed in a polythene bag to the laboratory in the Department of Microbiology, Federal University of Agriculture, Abeokuta and stored at room temperature in a dry place.

Germination of Cereal Grains

Sorghum and millet grains were cleaned by manual sorting to get rid of deformed, small, broken and immature kernels, dust, sand, stones, and other foreign materials. 80 g of sorghum and 20 g of millet grains were rinsed in water and drained and steeped in tap water for 8 h. Water was drained and germination was carried out by spreading the grains on a tray at temperature of 28±2°C in a room. Seeds were sprayed from time to time with water. The germinated grains were recovered when the radical was about 1.5 mm in length (Oluwajoba *et al*., 2013).

Spontaneous Fermentation of *Obiolor* from Wet-milled Process

The germinated grains were wet milled along side with sweet potatoes and prepared into slurry by mixing in water. The slurry was then mixed in boiled water (ratio 1:4 v/v) and stirred. The mash was cooled, filtered and the residue discarded, while the filtrate was concentrated by boiling for 30 mins with constant stirring. The resulting gruel was cooled rapidly and allowed to spontaneously ferment for 24 h at ambient temperature (Bonno, 2011).

Spontaneous Fermentation of *Obiolor* from Dried-milled Process

The germinated grain was mixed with of sweet potatoes and sun dried. The dried sample was milled into flour and sieved through 450 µm aperture sieve. The flour was boiled for about 30 mins and cooled. It was then filtered using muslin cloth to obtain a clear filterate after which it was concentrated by boiling for 30 mins before fermenting for 24 h at ambient temperature.

Physical and Chemical Analysis

pH Determination

The pH of the samples obtained at regular interval during fermentation was determined (El-Faki and Eisa, 2010) employing a pH meter (Hanna instrument). The fermenting gruel was homogenized and diluted 10 folds and the pH of the resulting solution was measured.

Determination of Titratable Acidity

The total titratable acidity in the fermenting samples was carried by titration against 0.1N NaOH, using phenolphthalein (1%) as indicator. Each ml of 0.1 N NaOH is equal to 90.08 mg of lactic acid (A.O.A.C., 1990)

Total Soluble Solid (TSS)

This was determined using a hand refractometer. The TSS value was read directly from the calibrated scale of the refractometer. It was expressed as (%) sucrose or degree brix (El-Faki and Eisa, 2010).

Specific Gravity

This was obtained using an hydrometer. It is expressed mathematically as

$$\frac{\rho_{wort}}{\rho_{water}}$$

Where ρwort is the density of the wort and ρwater is the density of water (Fellows, 2005)

Determination of Nutritional Content of Natural Fermented *Obiolor*

Moisture Determination

Ten ml of sample was measured in a clean crucible using sensitive balance (Ekanem *et al*., 2018). The samples were put into a moisture extraction oven at 105^0C and heated for 3 h. The dried samples were put into desiccators, allowed to cool and reweighed. The procedure was repeated until steady weight was obtained.

Ash Determination

The ash content was determined from the loss in weight that occurred during incineration of the evaporated sample at a temperature high enough to permit all organic matter to be burnt off without allowing appreciable breakdown of the ash constituent. Ashing was carried out in a muffle furnace subjected to heat at 550°C for 6 h (A. O. A. C., 2005).

Crude fibre Determination

Two grams of the sample was transferred into a 1L conical flask. One hundred millilitres of sulfuric acid (0.255 mol/L) was heated to boiling and then introduced into the conical flask containing the sample. The contents were then boiled for 30 mins, ensuring that the level of the acid was maintained by the addition of distilled water. After 30 mins, the contents were then filtered through a muslin cloth held in a funnel. The residue was rinsed thoroughly until its washing was no longer acidic to litmus. The residue was then transferred into a conical flask. One hundred milliliters of sodium hydroxide (0.313 mol/L) was then brought to boil and then introduced into the conical flask containing the sample. The contents were then boiled for 30 mins, ensuring that the level of the acid was maintained by the addition of distilled water. After 30 mins, the contents were then filtered through a Whatman 125 mm filter paper held in a funnel. The residue was rinsed thoroughly until its washing was no longer alkali. The residue was then introduced into an already dried crucible and ashed at 550°C (Oluwajoba *et al*., 2013).

Fat Content Determination

Two grams of the sample was loosely wrapped with a filter paper and put into the thimble which was fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120 ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5 h. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was received as mass of fat and is expressed in percentage of the sample (Nwosu *et al*., 2011**)**.

Crude Protein Determination

The micro kjeldahl method described by A.O.A.C (1990) was used. Two grams of each of the samples was mixed with 10 ml of concentrated H_2SO_4 in a heating tube. One tablet of selenium catalyst was added to the tube and the mixture was heated inside a fume cupboard. The digest was transferred into distilled water. Ten millimeter portion of the digest was mixed with equal volume of 45% NaOH solution and poured into a kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid solution containing drops of methyl red indicator. A total of 50 ml distillate was collected and titrated as well. The Nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content.

Determination of Carbohydrate

This was determined as the difference obtained after subtracting total organic nitrogen (protein), Lipid, Ash, Moisture and Fibre from the total dry matter (AOAC., 2005).

Determination of Anti-nutritional Content of Natural Fermented *Obiolor*

Determination of Phytate

The method described by AOAC (2002) was used for phytate determination. Sample (2 g) was weighed into 250 ml conical flask. 100 ml of 2% concentrated hydrochloric acid was used to soak each of the samples in conical flasks for 3 h and then filtered through a double layer filter paper. 50 ml of each of the sample filtrates was placed in 250 ml beakers and 100 ml of distilled water was added to each of the samples to improve proper acidity. 10 ml of 0.3% Ammonium Thiocyanate solution was added to each sample solution as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per ml. The end point was signified by brownish-yellow coloration that persisted for 5 mins (Adelekan *et al*., 2013).

Determination of Trypsin Inhibitor

Samples (0.2 g) were weighed into screw cap centrifuge tubes. 10 ml of 0.1 M phosphate buffers were added into each and contents were shaken at room temperature for 1 h on stuart orbital shaker. The suspension obtained was

thereafter centrifuged at 500 rpm for 5 mins and filtered through Whatman 125 mm filter paper. The volumes of each were adjusted with 2 ml phosphate buffer and the test tubes were placed in water bath maintained at 37°C. 6 mls of 5% Trichloroacetic acid (TCA) solution was added to an empty tube to serve as the blank. 2 mls of casein solution was added to each of the tubes initially placed in the water bath before incubation for 20 mins. 6 mls of TCA solution was added into the sample tubes 20 minutes after (so as to stop the reaction) and shaken. The reaction was allowed to proceed for 1 hour at room temperature and the mixture was filtered through Whatman 125 mm filter paper. The absorbance of sample filtrates and trypsin standard solutions were then read at 280 nm (Adelekan *et al*., 2013)

Statistical Analysis

Analysis of variance (ANOVA) was used to determine statistically significant differences among treatments using SPSS statistical soft-ware, version 17.

Results and Discussion

The pH of *Obiolor* samples ranged from 4.80-3.80 for wet-milled sample (Figure 1) while pH for flour-milled prepared *Obiolor* ranged from 4.70-4.00 from 6 to 24 h of fermentation period respectively. There was a reduction in pH during the natural fermentation of sorghum and millet for *Obiolor* production. The TTA of the *Obiolor* beverage during fermentation is illustrated in Figure 2. There was a steady increase in the TTA in the range of 0.12% to 0.17% during 24 h fermentation period for wet-milled prepared *Obiolor* while the flour-milled *Obiolor* had its values in the range of 0.14%-0.22% from 6-24 h fermentation time. This trend has been reported in other African fermented drinks such as the fermentation of millet-acha based *kunun-zaki* beverage (Ayo, 2004) and fermentation of maize grains for *Ogi* production (Oyedeji *et al*., 2013) amongst several others. The reduction in pH and increase in TTA could be attributed to the actions of lactic acid bacteria (Ojokoh *et al*., 2015). It is a beneficial factor for the fermenting organisms in which only acid tolerant microorganisms will thrive at the end of fermentation (Muyanja *et al*., 2010).

Regardless of the significant rise in acidity, total soluble solids remained stable. Total Soluble Solids of the *Obiolor* samples is shown in Table 1. TSS was 9.00 °brix throughout the period of natural fermentation for *Obiolor* production. TSS was also reported to have total soluble solids of 8 to 9 °brix in *Tchapalo,* an Ivorian sorghum beverage (Djè *et al*., 2008). The reduced amount of TSS contents during *Obiolor* fermentation could be attributed to the quick consumption of available solids carried out by yeast. It could also be as a result of action of fermentative microbes on the carbohydrate of the filtrate as the solid has been partially gelatinized (Danbaba *et al*., 2014**)**.

The specific gravity of the beverage is shown in Table 2. Wet-milled prepared *Obiolor* had a specific gravity of 1.009 while flour-milled prepared sample had a specific gravity of 1.008. These values remain constant throughout the period of fermentation. This result is related to what was obtained by Odibo *et al*. (2007), who found the specific gravity of beverage from two sorghum varieties to be 1.008 and 1.003 respectively.

Nutritional Analysis

The proximate composition of *Obiolor* is presented in Table 3. Proximate analysis is a vital tool in the assessment of nutritional status of food and food products (Ajiboye *et al*., 2014). Moisture content of naturally fermented *Obiolor* had high values in the range of 74.41±0.15 to 71.79±0.00% (P<0.05) for both wet and flour-milled *Obiolor* (Table 5). *Kunu-zaki,* another cereal non-alcoholic beverage was found to be in the range of 82.0±0.15% to 90.70±0.15%. The high levels of moisture content are expected because it is a liquid based beverage (Ajiboye *et al*., 2014)**.** The ash content of wet-milled prepared *Obiolor* is 2.86±0.00% while the ash content for flour-milled prepared *Obiolor* is 1.42±0.00%. This value is in close range with 2.40±0.03 obtained by Ajiboye *et al*. (2014), however, values are lower than what was obtained by Ekanem *et al*. (2018) who worked on *Kunu* drink, a non alcoholic cereal beverage. Fat content in this study was also not significantly different (P>0.05) in *Obiolor* prepared from wet milled process (5.20±0.20%) and flour- milled process (5.38±0.12%). These values are higher than 0.39±0.01% obtained by Ajiboye *et al*. (2014) but lower than 5.5% obtained by Ekanem *et al*. (2018). Studies have reported that germination reduces fat content (Jan *et al*., 2017) due to the break down and consumption of fats as an energy source for biochemical reactions during germination (Jan *et al*., 2017). The protein content for wet and flour-milled prepared beverages were 4.40±0.01% and 3.89±0.04% respectively. Essien *et al*. (2011) reported that loss of protein during processing of the drinks may be responsible for the low content observed. Ofudje *et al*. (2016) reported that the protein content of *Kunu-zaki*, a non-alcoholic local beverage was revealed to be in the range of 2.18±0.02 to 8.40±0.23%. The consequence of fermentation on protein has yielded inconsistent results possibly due to diverse experimental designs, study durations and variation in the initial protein content of foods. A number of studies had reported increase (Doudu *et al*., 2003; Pranoto *et al*., 2013) while others decrease (Osman, 2011; Pranoto *et al*., 2013) in protein value upon fermentation. It seems that most of these effect caused relative changes due to loss of dry matter as a result of microorganisms hydrolysing and metabolizing carbohydrates as source of energy (Smith *et al*., 2018). Carbohydrate content of *Obiolor* observed in this study was 11.10±0.22 and 13.52±0.60 (P<0.05) for *Obiolor* prepared from both wet and flour-milled process respectively. The

carbohydrate reduction during fermentation shows the beverage as an ideal substrate for microorganisms. The main carbohydrate in cereal beverages is starch which provides the most calories in developing countries (Chaves-Lopez *et al*., 2014). The quantity of available carbohydrate in the beverage could serve as a source of energy from adenosine triphosphate (Ajiboye *et al*., 2014). There was no significant difference in the crude fibre for both wet and flour-milled *Obiolor* which was 1.75±0.25% and 3.50±0.50% respectively. These values are higher than 0.30±0.01 obtained from sorghum-millet beverage (Ajiboye *et al*. 2014) and 1.001 obtained from *kunu drink* (Ekanem *et al*. 2018).

Antinutritional Analysis

The phytic acid and trypsin inhibitor of *Obiolor* were recorded after fermentation as showed on Table 6. The phytic acid concentration of wet-milled prepared *Obiolor* was 1.20 mg⁄100 g while *Obiolor* from flour-milled process had a phytic acid concentration of 1.00 mg⁄100 g. These values have higher phytic acid concentration than malted soy *Kunu-zaki* (Adelekan *et al*., 2013) which had a lower value to be 1.00 mg/100 g in flour-milled prepared beverage. However, a different trend was observed where the concentration of wet-milled prepared beverage had trypsin inhibitor concentration of 3.426 mg⁄100 g which was lower than the value of 3.488 mg⁄100 g obtained for flour-milled prepared beverage. Germination has been reported to diminish the concentration of antinutritional factors in grains. Fermentation also enhances bioavailability of calcium, phosphorus and iron likely due to degradation of oxalates and phytates that combine with minerals thereby reducing their bioavailability hence, making them free and more accessible (Sripriya *et al.,* 1997).

Conclusion

In this study, *Obiolor* was produced from sorghum and millet grains using two different methods: wet-milled and flour-milled prepared *Obiolor*. The results were slightly different from each other except for total soluble solids which had the same values for both wet and flour-milled beverages. However, proximate analysis revealed that flour-milled *Obiolor* had more protein, carbohydrate, crude fibre and fat content, indicating that it is of more nutritive value than wet-milled produced *Obiolor* which is commonly practised at household level. The use of sorghum-millet flour also allows for preservation of the grains for production of *Obiolor.* Further studies need to be conducted to investigate the effect of different steeping and germination conditions for *Obiolor* production.

Acknowledgment

The authors would like to appreciate members of staff of the central science laboratory, Bowen University for the assistance rendered during the course of this study.

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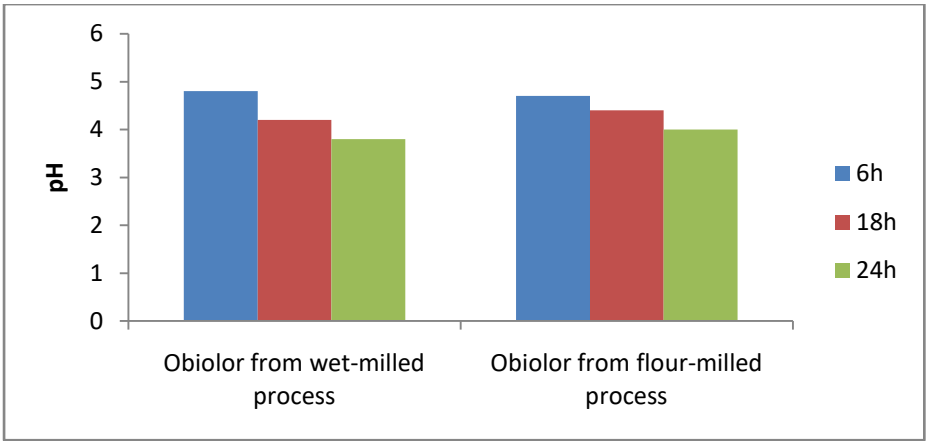

Figure 1: pH of Sorghum and Millet during Natural Fermentation of *Obiolor*

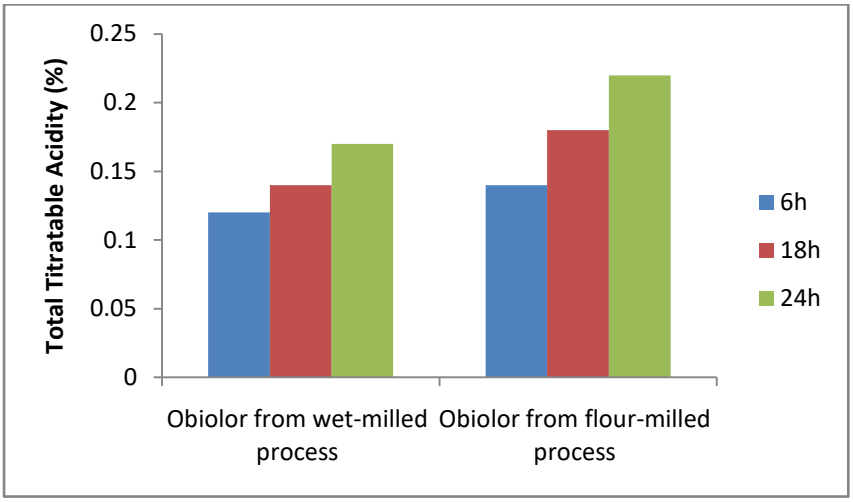

Figure 2: Total titratable acidity of Sorghum and Millet during Natural Fermentation of *Obiolor*

Table 1: Total Soluble Solids of Naturally Fermented *Obiolor*

Sorghum/millet Beverage	°Brix
Obiolor from wet-milled process	9.00
Obiolor from dried-milled process	9.00

Values are mean ± SD, where n=3. Mean with different superscript across the same row are significantly different ($p<0.05$).

Table 2: Specific Gravity of Naturally Fermented *Obiolor*

Sorghum/millet Beverage	Specific Gravity
Obiolor from wet-milled process	1.009
Obiolor from flour-milled process	1.008

Values are mean ± SD, where n=3. Mean with different superscript across the same row are significantly different ($p<0.05$).

Table 3: Nutritional components of Naturally Fermented *Obiolor*

Sorghum and Millet Beverage	%Moisture	%Ash	%Fat	% Crude Fibre	% Crude Protein	%CHO
Obiolor from wet-milled process	74.41±0.15[a]	2.86±0.00	5.20±0.02[a]	1.75±0.25[a]	3.89±0.04[a]	11.10±0.22[a]
Obiolor from flour-milled Process	71.79±0.00[b]	1.42±0.00	5.38±0.12[a]	3.50±0.50[a]	4.40±0.01[b]	13.52±0.60[a]

Values are mean ± SD, where n=3. Mean with different superscript across the same row are significantly different ($p<0.05$).
Key: CHO – Carbohydrate

Table 4: Antinutritional Component of Naturally Fermented *Obiolor*

Sorghum/millet Beverage	Phytic acid (mg⁄100 g)	Trypsin Inhibitor (mg⁄100 g)
Obiolor from wet-milled process	1.20	3.426
Obiolor from flour-milled process	1.00	3.488

Values are mean ± SD, where n=3. Mean with different superscript across the same row are significantly different ($p<0.05$).