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ORIGINAL RESEARCH

Variation in physicochemical water quality parameters during fermentation of maize for *Ogi* production

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ABSTRACT
This study evaluated the variation in *in-situ* physicochemical water quality parameters during fermentation of maize for ogi production. Triplicate sample of yellow maize was purchased from Rumuomasi, Port Harcourt, Rivers state, Nigeria. The maize was fermented for 0 – 96 hours. 2ml of the fermentation medium water was aseptically collected for microbial count enumeration using standard microbiological procedure. Similarly, the physicochemical parameters including salinity, temperature, turbidity, conductivity, pH and total dissolved solid were analyzed using *in-situ* meter kits. Results showed that microbial counts were 7.11 Log cfu/ml (at 0 hours) and 11.17 Log cfu/ml after 96 hours of fermentation (for total heterotrophic bacteria), 5.35 Log cfu/ml (at 0 hours) and 11.77 Log cfu/ml after 96 hours (for Lactic acid bacteria), 4.16 Log cfu/ml (at 0 hours) and 6.52Log cfu/ml after 96 hours (for yeasts/mould) and 4.12 Log cfu/ml (at 0 hours) and 0.00 Log cfu/ml after 96 hours (for the *Enterobacteriaceace*). Parameters increased from 114.67 to 1663.33ppm (salinity); 230.00 to 2903.33 µS/cm (conductivity); 161.33 to 2076.73 mg/l (total dissolved solid) and 65.17 to 473.33 NTU (turbidity) with fermentation; while temperature and pH values overlaps showing non-uniform trend, however they increased from 28.47 to 29.57ºC (temperature) and 3.83 to 6.37 (pH) indicating decreasing acidity as fermentation progressed. Analysis of variance showed that there were significance differences ($P<0.05$) among the various fermentation periods. The implications of the variation in the physicochemical and biological parameters during the fermentation process were discussed.

KEY WORDS: *Variation, In-situ water quality, Microorganisms, Fermentation*

Introduction

Water is an indispensable resource needed for the survival of life (Agedah *et al*., 2015). Water serve as home for diversity of life including plants, animals, microbes and humans (Ogamba *et al*., 2015a). In a developing country like Nigeria, potable water and water used for domestic purposes are met by groundwater (borehole and well), surface water (rivers, creek, creeklets, stream, lakes and ponds) and rain water (Izah *et al*., 2016a; Izah and Srivastav, 2015). Water is essential in agriculture, construction and manufacturing sector. In many food processing sector such as palm oil processing from fruit bunch of oil palm and ogi production from maize, large quantity of water is used.

Water frequently becomes contaminated by environmental phenomenon such as microbes. In a review by Izah and Ineyougha (2015), potable water (including surface water-rivers, stream, lake, creek; groundwater – borehole, well; and rainwater) which is also used for domestic purposes often contain an array of bacteria mostly of the genera *Staphylococcus*, *Escherichia, Alcaligenes, Proteus, Pseudomonas, Enterobacter, Salmonella, Klebsiella, Bacillus*, *Aeromonas, Micrococcus, Citrobacter, Streptococcus, Vibrio, Shigella, Enterococcus, Flavobacterium, and Chromobacterium.* In a case study,

Oyelana and Coker (2012) reported that water used in ogi production are often contaminated by fungal species including *Aspergillus flavus, Aspergillus niger, Penicillin oxalicum, Fusarium oxysprium* and *Rhizopus stolonifer* in Mowe rural community in southwest Nigeria. Other water quality parameters that could be altered during fermentation include general physicochemistry characteristics (such as pH, temperature, conductivity, turbidity, total dissolved solid, salinity, dissolved oxygen) and laboratory based parameters (such as chemical oxygen demand and biological oxygen demand).

Typically, cereals such as maize belong to the grass family (*Poceace*) which is cultivated mainly because of their grains which are edible. They contain vitamins, minerals, carbohydrates, fats, oils etc (Adegbehingbe, 2013). Similarly, Kohajdová and Karovičová (2007), Adebayo *et al*. (2010) referred to cereal grains as world essential sources of dietary proteins (low concentration), carbohydrates, vitamins, minerals and fibre. Specifically, cereal account for about 80% of average diets in India and Africa (Adebayo *et al*., 2010). In Nigeria cereal mainly cultivated include maize, guinea corn, rice, millet and sorghum. These cereals have several economic importances. For instance, maize (*Zea mays*), guinea corn (*Sorghum bicolor*) and millet (*Pennisetum typhodenum*) is used for the production of *Ogi* (a fermented cereal porridge) (Adesokan *et al*., 2010; Omemu, 2011; Farinde, 2015; Bolaji *et al*., 2014). *Ogi* is used as weaning food for infants as well as a dietary food staple for adults in West Africa (Wakil and Kazeem, 2012; Bolaji *et al*., 2014; Adegbehingbe, 2013; Abioye and Aka, 2015) including the elderly and sick.

The processing of maize for *Ogi* production typically requires fermentation. Authors have variously reported steeping period of 1 – 3 days in clean water (Wakil and Daodu, 2011; Iwuoha and Eke, 1996; Adebukunola *et al*., 2015; Ijabadeniyi, 2007; Abioye and Aka, 2015; Farinde, 2015; Badmos *et al*., 2014; Bolaji *et al*., 2015). Generally, the fermentation of food generally involves the activity of microorganisms and their enzymes for the improvement of foods with specific attributes (Badmos *et al*., 2014). Kohajdová and Karovičová (2007) also described fermentation as a biochemical process which involves alteration of primary food matrix, which is brought about by microbes and their associated enzymes. This change often enhance the shelf life, texture, taste and aroma, nutritional value and digestibility and considerably reduces anti-nutritional qualities. Akinleye *et al*. (2014) reported that fermented foods are of immense importance because they supply and preserve enormous quantities of nutritious foods with regard to flavours, aromas and textures which enrich the human diet. For instance, Nwokoro and Chukwu (2012) reported enrichment with protein content in fermented akamu.

The biochemical, growth, survival dynamics of microbes involved in the fermentation of food such as cereals are the effect of stress reactions in response to the changing of the physical and chemical conditions into the food micro-environment (Wakil and Daodu, 2011). Water and microorganisms are major factors that aid in fermentation of the maize. Hence it's ideal that analysis be carried out at the collection/sampling point. During maize fermentation, unstable parameters such as pH decreases (tending towards acidity) as fermentation progresses (Wakil and Daodu, 2011; Adegbehingbe, 2013). This may have an influence in microbial composition of the fermentation medium. Hence, this study focuses on the variation in microbial and in-situ water quality parameter during the fermentation of maize for Ogi production.

Materials and Methods

Field Sampling

Dried yellow maize samples were purchased from Rumuomasi market, Port Harcourt, Rivers state, Nigeria from three maize sellers. Sterile Ziploc bags were used to package the maize prior to transportation to the laboratory for the study.

Sample preparation

About 650g of the maize samples were added to the 1000 ml sterile conical flask and sterile water was aseptically added up to 950 ml level mark in the container. The control was set up (sterile water without maize). The cap of the container was loosely covered. Thereafter, 2ml of the ferment water samples from both medium (medium with maize and sterile

water) was collected after shaking and the medium was used for microbial assessment at 0, 24, 48, 72 and 96 hours. Similarly, the in-situ parameters (temperature, pH, conductivity, salinity and total dissolved solid) were determined under aseptic condition directly by dipping the calibrated probe into the ferment water. Similarly, 10ml of ferment water was collected for turbidity analysis).

***In-situ* Analysis**

All the in-situ parameters were carried out following manufactures guide. The pH was determined in–situ by using pH meter (Extech DO700; a multipurpose meter) following 3-point calibration (7.00pH, 4.00 pH and 10.01pH). The turbidity was measured using turbidity meter (Extech Model TB400). While total dissolved solid, conductivity, salinity and temperature was determined using a multipurpose meter (Extech EC400).

Enumeration of microbial counts

Nutrient Agar (for total heterotrophic bacteria count), MacConkey Agar (for the enumeration of *Enterobacteriaceae* family), Potato dextrose agar (for mould and Yeast), DeMan, Rogosa and Sharpe Agar i.e. MRS Agar (for Lactic acid bacteria) were the media used in this study. The four media were prepared according the manufacturers' instruction. Pour plate techniques described by Pepper and Gerba (2005) and Benson (2002) were used. About 0.1 ml of the serial diluted samples was plated in the various media. Agar plate for total heterotrophic bacteria count was incubated for 24 – 48 hours at 37°C; mould and yeast were incubated for 3-4 days at 30°C; bacteria of the *Enterobacteriaceae* family was incubated for 24hours at 37ºC; Lactic acid bacteria were incubated at 37°C for 3-4 days under anaerobic condition using MRS Agar containing 10mg/ml cycloheximide. The colonies that grew on the various media were counted and expressed as colony forming units (cfu)/ml of the maize fermentation water.

Statistical Analysis

Statistical analysis was carried out using SPSS software on logarithm transformed microbial counts and *in-situ* water quality parameters. Data were expressed as Mean ± standard error (n=3). A one-way analysis of variance was carried out at $\alpha = 0.05$ and Post hoc was carried out using Duncan multiple range test statistics. Pearson's correlation matrix was used to identify the relationship between the in-situ parameters of the water samples (control and maize fermentation medium).

Results and Discussion

Table 1 presents the microbial counts in maize fermentation water used for ogi production.

The microbial counts were 7.11 Log cfu/ml (0 hours) and 11.17 Log cfu/ml after 96 hours of fermentation (total heterotrophic bacteria), 5.35Log cfu/ml (0 hours) and 11.77Log cfu/ml after 96 hours (Lactic acid bacteria), 4.16Log cfu/ml (0 hours) and 6.52Log cfu/ml after 96 hours (yeasts/mould) and 4.12Log cfu/ml (0 hours) and 0.00 Log cfu/ml after 96 hours (bacteria of the *Enterobacteriaceae* family). Typically, there was significant difference ($p<0.05$) among the various fermentation hours. The microbial counts were least in 0 hours and highest after 96 hours of fermentation apart from bacteria of the *Enterobacteriaceae* family. The variation could be due to release of biochemical constituents that favours proliferation of a particular microbes. This is because as fermentation progresses the microbial consortia decreases and favours yeast (*Saccharomyces cerevisiae*) and lactic acid bacteria (*Lactobacillus* species) (Adegbehingbe, 2013). As population of yeast and Lactobacillus species increases, the mould, bacteria of *Enterobacteriaceae* and total heterotrophic counts reduced with regard to diversity. With reduction in diversity, the density or population increased. Hence, lactic acid bacteria and yeasts in fermentation often co-exist during fermentation of food product especially cereals. However, yeast and moulds associated with ogi have been documented by Omemu *et al*. (2007a), while the significance of yeast in fermentation of maize for ogi production has been reported by Omemu *et al*. (2007b). The microbial counts across the various days showed similar trend with the work of Wakil and Daodu (2011), Ijabadeniyi, (2007), Nwokoro and Chukwu (2012), Akinleye *et al*. (2012), Adegbehingbe (2013) and Adesokan *et al*. (2010).

Table 2 presents the *in-situ* maize fermentation water quality parameters between 0 – 96 hours. Table 2 and 3 also presents Pearson's correlation coefficient (r) matrices for the

Table 1: Microbial counts of maize fermented water for ogi production

Fermentation duration, hours	Total Heterotrophic Bacteria, Log cfu/ml	Lactic acid bacteria counts, Log cfu/ml	Bacterial of *Enterobacteriaceae* family, Log cfu/ml	Mould and Yeast counts, Log cfu/ml
0	7.11±0.51a	5.35±0.53a	4.12±0.37c	4.16±0.54a
24	7.75±0.75a	6.96±0.85ab	2.68±0.39b	4.82±0.39ab
48	8.52±0.38ab	8.23±0.55b	1.84±0.11b	5.06±0.44ab
72	9.59±0.35bc	10.33±0.56c	0.56±0.56a	6.21±0.58b
96	11.17±0.45c	11.77±0.50c	0.00±0.00a	6.52±0.55b

Each value is expressed as mean ± standard error (n = 3); Different letters along the column is significantly difference at *P*<0.05 according to Duncan Multiple range test Statistics

in-situ parameters for maize medium fermentation and control (sterile water) respectively. The maize medium fermentation water were least at the beginning (i.e. 0 hours) and highest at the end (96 hours) for all the parameter apart from temperature and pH. pH showed a decline with regard to fermentation, with least and highest value obtained at 96 hours and 0 hours. Generally, the maize fermentation water with regard to salinity ranged from 114.67 - 1663.33ppm, being significantly different (P<0.05) among the various fermentation hours. Significant variation (P<0.05) also exists between the fermentation maize medium and the control (sterile water). This study showed that as fermentation progresses the water becomes more saline. This suggests that as fermentation duration increases, the concentration of most ions in the water increases.

However, in maize medium, salinity significantly correlate with total dissolved solid, conductivity and turbidity and negatively correlate with pH (P < 0.01) (Table 3), while in control water salinity significantly correlate with total dissolved solid and conductivity (P<0.01) (Table 4). Typically, salinity is a measure of salt content in the fermentation medium.

Table 2: In-situ water quality parameter during maize fermentation for ogi production

Fermentation duration, hours	medium	Salinity, ppm	Conductivity, µS/cm	Total dissolved solid, mg/l	Turbidity, NTU	Temperature, ºC	pH
0	Maize	114.67±3.48a	230.00±1.00a	161.33±5.36a	65.17±11.26b	28.87±0.09bc	6.37±0.03d
	sterile water	84.27±0.95a	168.57±1.69a	117.93±1.27a	0.60±0.50a	28.47±012a	6.57±0.03d
24	Maize	509.00±28.01b	686.33±286.42b	711.67±37.82b	207.33±50.78c	29.27±0.03d	5.70±0.06c
	sterile water	85.40±0.92a	170.93±1.95a	119.53±1.31a	0.15±0.03a	29.50±0.00ef	6.57±0.09d
48	Maize	827.67±54.05c	1663.33±108.60c	1162.33±74.40c	318.67±10.86d	29.57±0.03f	4.83±0.20b
	sterile water	84.87±1.02a	169.77±1.97a	118.80±1.35a	1.40±0.53a	29.33±0.03de	6.53±0.03d
72	Maize	1246.73±118.93d	2223.33±158.36d	1550.03±110.15d	386.67±23.78e	28.83±0.03bc	4.00±0.12a
	sterile water	85.90±0.89a	171.93±1.79a	120.30±1.25a	0.36±0.05a	28.70±0.00b	6.47±0.03d
96	Maize	1663.33±293.84e	2903.33±161.49e	2026.73±113.48e	473.33±17.29f	28.97±0.09c	3.83±0.12a
	sterile water	85.57±0.88a	171.23±1.68a	119.77±1.19a	0.70±0.13a	28.87±0.03bc	6.50±0.06d

Each value is expressed as mean ± standard error (n = 3); Different letters along the column is significantly difference at *P*<0.05 according to Duncan Multiple range test Statistics

Table 3: Pearson's correlation matrix of the *in-situ* water quality parameter during maize fermentation for ogi production

Parameters	Salinity, ppm	Conductivity, µS/cm	TDS, mg/l	Turbidity, NTU	Temperature, ºC	pH
Salinity, ppm	1					
Conductivity, µS/cm	0.934**	1				
TDS, mg/l	0.961**	0.970**	1			
Turbidity, NTU	0.909**	0.901**	0.970**	1		
Temperature, ºC	-0.174	-0.119	-0.079	0.020	1	
pH	-0.884**	-0.937**	-0.948**	-0.921**	0.109	1

**. Correlation is significant at the 0.01 level (2-tailed).
N= 15, n = 3

The conductivity level ranged from 230.00 - 2903.33 µS/cm. There is significant difference (P<0.05) among the various fermentation hours. Also significant variation (P<0.05) exists between the fermentation maize medium and the control (sterile water). Nevertheless, the conductivity of the maize medium significantly correlate total dissolved solid and turbidity and negatively correlate with pH (P < 0.01) (Table 3). While the control water sample showed positive correlation with total dissolved solid (P<0.01) (Table 4). As the microorganisms ferment the maize medium, substances are released. Typically, conductivity of water is a measure of the ability of the water to allow the passage of electrical current, which are direct relationship with concentration or number of ions in the water.

The total dissolved solid concentration of maize medium fermentation water ranged from 161.33 – 2076.73 mg/l. Basically, there is significant variation (P<0.05) among the various fermentation hours. Similarly, significance difference (P<0.05) exists between the fermentation maize medium and the control. The total dissolved solid of the maize medium showed positive significant correlation with turbidity and showed negative correlation with pH (P < 0.01) (Table 3). Typically, total dissolved Solids are solids found in water that can penetrate through filter. Hence, as fermentation duration increases the total dissolved solid increases. Total dissolved solid is a measure of the amount of material dissolved in water including ions and electrolytes.

The turbidity level of maize medium fermentation water ranged from 65.17 – 473.33 NTU, being significantly different (P<0.05) among the various fermentation hours. Significance difference (P<0.05) exists between the fermentation maize medium and the control. Turbidity of the maize medium showed negative relationships with pH (P<0.01) (Table 3). Turbidity is a measure of water clarity. As fermentation progresses, the water becomes more cloudy, thereby affecting the physical nature of the water. Hence, the variation in turbidity also suggests changes in the total suspended solids level of the fermentation medium.

Table 4: Pearson's correlation matrix of the control (sterile water)

Parameters	Salinity, ppm	Conductivity, µS/cm	TDS, mg/l	Turbidity, NTU	Temperature, ºC	pH
Salinity, ppm	1					
Conductivity, µS/cm	0.998**	1				
TDS, mg/l	0.999**	0.998**	1			
Turbidity, NTU	-0.160	-0.159	-0.160	1		
Temperature, ºC	0.153	0.151	0.152	0.080	1	
pH	0.060	0.092	0.053	0.175	0.080	1

**. Correlation is significant at the 0.01 level (2-tailed).
N= 15, n = 3

Like, total dissolved solid, conductivity, salinity and turbidity, temperature showed that there is significance difference (P<0.05) among the various fermentation hours and between the maize medium and the control sample. However, the concentration ranged from 28.47 – 29.57ºC. Typically, spatial spreading of temperature over the water is prejudiced by amount of insulation received and nature of surface (Ogamba *et al*., 2015b). Hence, the cause of variation suggested that temperature is a highly unstable parameter and changes could occur even at holding temperature.

The pH of the maize medium fermentation for ogi production were 6.37 (0 hours) and 3.83 (96 hours), being significantly different (P<0.05) among the various fermentation period and between maize fermentation medium and the control sample. As pH decreases, *Lactobacillus* and *Saccharomyces cerevisiae* counts increases (Izah *et al*., 2016b). This suggested that *Lactobacillus* species and *Saccharomyces cerevisiae* are the predominant microbes that cause acidification during maize fermentation for *Ogi* production. These microbes have been implicated as major microbes causing acidification in kunu (a cereal based food drink) (Adebayo *et al*., 2010)

4.0 Conclusion

Fermentation of food using indigenous microorganisms by natural spontaneous process is as old as man. Fermentation typically brings about changes in the quality attributes of food being fermented. During fermentation several variations occur. Hence this study was discerned to assess the variation in *in-situ* water quality parameter during maize fermentation for *Ogi* production. The study revealed that the microbial counts/density and in-situ water quality such as salinity, turbidity, conductivity, total dissolved solid typically increases as fermentation progresses; while pH decreases as fermentation proceeded while temperature fluctuates. This describes the unstable nature of temperature even at holding condition.

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