Production, Microbiological and Proximate Analysis of *Akamu* Produced from different Varieties of Maize

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Abstract: *Akamu*, is produced by spontaneous fermentation of maize grains by mixed microbial activities. It is consumed by adults and children as breakfast meals and also serves as a weaning diet especially for the low income earners. This work aimed at producing *akamu* from White and Yellow maize grains determining the effect of processing methods (Changed and unchanged steep water) on the nutrient status of the *akamu*. White and yellow varieties of maize grains purchased from local sellers at Ubani Main Market, Umuahia, Abia State were sorted, washed and steeped in rain water in two sets for spontaneous fermentation for 48 hrs. For one set, the steep water was not changed throughout the period of fermentation while for the other set, the steep water was changed every 24 hrs. 1ml of the steep water from each set-up was serially diluted every 24 hrs and 0.1 ml aliquots of appropriate dilution was inoculated by spread plate method on suitable media. Plates for the isolation of bacteria were incubated at at 35^{o}C for 24 hrs while the plates for isolation of fungi were incubated at 22^{o}C for 5 days. The isolates were characterized and identified using standard procedures. The pH, Titratable acidity and temperature were also determined every 24 hrs. The proximate composition namely moisture, crude protein, crude fat, crude fibre, ash and carbohydrate were determined using standard methods. The bacterial isolates are *Lactobacillus* species, *L. plantarum*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aurues* while the fungal isolates include *Saccharomyces cerevisiae*, *Mucor alternaria* and *Aspergillus flavus.* At the end of the fermentation, only the bacteria: *Lactobacillus* species, *L. plantarum*, *Bacillus subtilis* and the yeast: *Saccharomyces cerevisiae* were isolated from the *akamu*. The crude protein (9.25 and 9.23), carbohydrate (75.30 and 74.80) and fibre (3.15 and 3.22) of the *akamu* produced from the unchanged water samples were significantly higher than those from the changed water samples for both yellow and white maize samples respectively. This research shows that *akamu* produced from unchanged steep water has higher carbohydrate, protein and fibre contents than that made from changed steep water. It's also higher in acidity than its counterpart. No significant difference in the microbial and proximate composition of *akamu* was recorded between the two varieties of maize used.

Key words: *Akamu*, maize varieties, fermentation, microorganisms, proximate composition

INTRODUCTION

Maize (*Zea mays*) which belongs to the family: Poaceae is one of the most important crops in the word and preferred staple food for more than 1 billion people in sub Saharan Africa and Latin America (Gupta *et al*., 2009). Maize is a multipurpose crop, providing food and fuel for human beings, feed for animals, poultry and livestock. Its grains have great nutritional value and are used as raw material for manufacturing many industrial products (Afzal *et al*., 2009). Its grains are important for the production of oil, starch and glucose (Niaz and Dawar, 2009). Moreover, Food composition data is important in nutritional planning and provides data for epidemiological studies (Ali *et al*., 2015). However, there is limited information about the nutritional composition of the different maize varieties growing in Nigeria.

Development of maize cultivars with high productivity coupled with enhanced sugar and starch content in the kernels may cater to their enhanced use in human consumption and industrial usage. Therefore, a more detailed knowledge of nutritional properties of maize genotypes will be beneficial in the production of maize food with improved nutritional quality.

Akamu, also known as *ogi is* a fermented product usually from maize which is grown in all parts of Nigeria. It is known that the traditional method of processing *akamu* results in loss of nutrients (Galitsky *et al*., 2003).

Akamu is consumed by adults and children as breakfast meals, and it also serves as a weaning diet (Ashaye *et al.,* 2001; Amusa *et al.,* 2005). After 5–6 months, breast-feeding is no longer sufficient to satisfy the nutritional requirements of the growing infant. Beginning from this period, the child needs solid foods to meet increasing nutritional needs (Onofiok and Nnanyelugo, 2008). This period is the weaning period and in Nigeria, *akamu* is introduced gradually to the child's diet to supplement nutrition. Fermented maize is very widely utilized as food in African countries and in fact cereals account for as much as 77% of total caloric consumption (Osungbaro, 2009). Maize is rich in carbohydrates and minerals, including potassium and magnesium. It contains trace amounts of lysine and tryptophan, contributing to the low content of protein, and trace amounts of B-vitamins.

Traditionally, *akamu* is produced after maize grains are steeped in water continuously for three days. Some people do change the steep water during the two days of fermentation. So, this work was aimed at producing *akamu* from White and Yellow maize grains by the traditional fermentation. The effect of processing method (changing and not changing the steep water) on the nutrient status of the *akamu* so produced is thus investigated.

MATERIALS AND METHODS

Collection of samples

Grains of White and Yellow Maize (*Zea mays* L.) varieties were purchased at Ubani Market, Umuahia, Abia State and taken to CESLAB, Umudike, Abia State for analyses. The two varieties of maize grains were sorted to remove bad ones, stones and chaff.

Production of *akamu*

One hundred grams (100 g) each of the Yellow and White Maize types were steeped in rain water in a 400 ml transparent plastic bucket for three days. Each of the maize grain variety was steeped in duplicate and the buckets were labelled accordingly. The White and Yellow Maize grains were grouped into two to give "Changed Water" and "Unchanged Water". For the "Changed Water" groups, the steep water was changed after every 24 hrs while the steep water for the "Unchanged Water" was not changed till spontaneous fermentation by chance inoculated microorganisms was over after 72 hr (3 days). This design gave rise to four types of *akamu* products: two "Changed Water" and two "Unchanged Water" *akamu* Samples. After fermentation, the grains now swollen were rinsed in clean water, wet milled and sieved with a clean sterile muslin cloth. The filtrate was allowed to settle and the supernatant drained off. The resulting slurry paste was the *akamu* (Rooney, 2001).

Microbiological Analyses

One gram of each of the four *akamu* samples and 1 ml of the steep water from each of the samples were serially diluted separately in 9 ml peptone water. 0.1 ml aliquot of appropriate dilution was inoculated by spread plate method in duplicates on Nutrient, MacConkey, De Mann Rogosa Sharpe (MRS) Agars and incubated at 35oC for 48 hrs for the isolation of bacterial species. The inoculated plates were incubated aerobically at 37^{o}C for 48 hours. Same aliquots was inoculated on sterile plates Sabourand Dextrose Agar (SDA) for the isolation of fungi and incubated at 22^{o}C for 5 days. The Temperature, pH and Titratable Acidity of the fermenting maize grains were determined while the microbial success of the fermenting maize grains was determined every 24 hrs till the fermentation ended.

Identification of bacterial isolates from fermenting maize grains

After incubation, the cultures were observed for their morphologies and then sub-cultured and stored in agar slants in the refrigerator at 4^{o}C for further use.

Total viable counts of the isolates were also determined on the various culture plates. The bacterial cultures were Gram stained and subjected to biochemical and sugar fermentation tests (Harley and Prescott, 2001).

Identification of fungal isolates from fermenting maize grains

Fungal mycelia were stained with Lactophenol Cotton Blue, mounted on the slides and observed under 40X Objective Lens of the microscope and colony colour, growth pattern on plates, details of philiades and spores were also used as identification parameters (Domesch and Anderson, 2007).

Determination of Proximate Compositions of *Akamu*

Proximate analyses of the samples were performed to determine the moisture (Lee *et al*., 2007); fats (James, 1995); crude fibre (James, 1995); protein (James 2005); total ash and carbohydrate contents (AOAC, 2000) respectively.

Moisture content determination

This was done using the gravimetric method. A measured weight of the sample (5g) was dispensed into a previously weighed moisture can. The sample in the can was dried in the oven at 105^0C for four (4) hours and cooled in a desiccator and weighed. It was returned to the oven at the same temperature for further drying during which it cooled and weighed at hourly interval until no further reduction in its weight (constant weight) was attained. The formula below was used to calculate the moisture content.

Calculation:

$$\% \text{ moisture} = \frac{w2 - w3}{w_2 - w_1} \times 100$$

Where:

W1= initial weight if the empty crucible

W2= weight of crucible + sample before drying

W3= final weight of crucible + sample after drying

Total solid = 100 - % moisture

Crude fibre content determination

Crude fibre was determined as the quantity of residue left after the removal of acid and alkali labile materials, fats and ash. The Gravimetric method of Onwuka (2018) was employed. A measured weight, 5g, of the sample was boiled in 150ml of 1.25% H_2SO_4 solution under reflux for 30minutes. It was washed repeatedly with hot distilled water using a two-fold Muslin cloth to retain the particles. Carefully, the washed sample was transferred quantitatively back into the flask and boiled for another 30minutes in 1.25% NaOH solution. Thereafter, it was washed again thoroughly in hot distilled water and flushed with normal hexane to remove any traces of fat and dried in the oven for 30minutes 100^0C, cooled in a desiccator and weighed. The crude fibre contained in the sample was burnt out in the furnace (as in Ash determination) leaving the ash in the crucible which was cooled and weighed.

The formula below was used to calculate the crude fibre content.

$$\% \text{ crude fibre} = \frac{W2-W3}{W} \times 100$$

Where :

W = Weight of sample analysed

W_2 = Weight of sample and crucible after boiling, washing and drying

W_3 = Weight of crucible and sample after burning washed dried sample in furnace

Protein content determination

The protein content was determined by Kjeldahl method in which the nitrogen content is determined and multiplied with the factor 6.25 to obtain the protein content (Onwuka, 2018). One gram of the sample was weighed into a filter paper and transferred into a neat dried Kjeldahl flask. Exacted 10ml of concentrated sulphuric acid was added to the flask in the presence of a pinch of selenium catalyst.

The flask was heated gently in a fume cupboard using a heating mantle in an inclined position and allowed to digest (digest is complete when the liquid is clear and free from black or brown color). The flask was allowed to cool and was diluted with 100ml of distilled water. A distillation apparatus consisting of 500 ml flask with stopper carrying a dropping funnel and a splash head adaptor and a vertical condenser in which a straight delivery tube is attached was used. Approximately 50 ml of boric acid solution was measured into 500ml Erlenmeyer titration flask and a few drops of screened methyl red indicator were added and the Erlenmeyer flask placed on the receiving end of the delivery tube dipping just below the level of the boric level. Some anti-bumping agent granules and 75 ml of NaOH solution were added to the distillation flask. Exactly 50ml of distilled water was added and was gently shaken to ensure mixing of contents. The flask was connected to distillation bulb and boiled vigorously until about 100ml of the distillate was obtained. The distillate was titrated with 0.1ml HCI till first trace of pink color

% Protein = % N2 x 6.25

$$\% N2 = \frac{100}{W} \times \frac{14 \times 0.02 \times (B-T) \times Vf}{1000 \quad Va}$$

Where W= weight of sample in grams
B = Titre value of reagent blank
T = Titre value of sample
Vf = Total value of digest
Va = volume of digest distilled

Fat content determination

This was determined using the continuous solvent extraction gravimetric method. Accordingly, a measured weight of the sample (5g) was wrapped tightly in a previously weighed 15 cm porous paper (Whatman No 1 paper). The wrapped sample was put in a porous thimble and placed in a soxhlet reflux flask. The reflux flask was mounted onto an oil extraction flask containing about 200 ml of n-hexane. The upper end of the reflux flask was coupled to a condenser. The solvent in the flask was heated to vaporize and condense into the reflux flask enveloping the wrapped sample (extracting the oil in it). When the reflux flask is filled up, the oil extracted siphons into the extraction flask and the cycle continues. The processes of vaporization, condensation, extraction and reflux was allowed to go on repeatedly up to fourteen (14) refluxes. The extracted wrapped samples were removed with the aid of a long pair of forceps and dried while the solvent was measured.

The fat content was calculated as the percentage weight of the oil extracted from the sample using the formula below

Calculation:

$$\% \text{ crude fat content} = \frac{W2-W3}{W_2 - W_1} \times 100$$

Where:
W_1 = weight of the empty filter paper
W_2 = weight of the paper and sample before extraction
W_3 = weight of the paper and sample after fat extraction and drying.

Ash content determination

Ash content was determined by the ash gravimetric method. Exactly 5g of the sample was weighed into a previously weighed crucible and burnt to ashes in a muffle furnace at 500-550^{0}C for about 2hours (until a grey ash material was seen). The crucible and its content was cooled in a desiccator and weighed.

By difference in weight, the weight of the Ash was determined and expressed as a percentage weight of the sample using the formula below.

$$\% \text{ Ash} = \frac{W2 - W1}{W} \times 100$$

Where:
W = weight of sample analyzed
W_1 = weight of empty crucible
W_2 = weight of crucible and ash

Carbohydrate content determination

The nitrogen free extract method was used. Carbohydrate was calculated as weight difference between 100 and the summation of other proximate components as Nitrogen Free Extract (NFE).

Calculation:

% N F E =100- % (a + b + c +d +e)

Where: A= protein; B= fat content; C= ash content; D= fibre content; E= moisture content.

RESULTS

In Table 1, the bacterial isolates from the fermented maize samples are presented. They include *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactobacillus* spp and *L. plantarum.*

Fungal isolates from fermented maize samples are *Aspergillus flavus, Mucor alternaria* and *Saccharomyces cerevisiae* (Table 2).

From the Microbial Succession study, result shows that for the White Maize variety, *S. aureus* and *E. coli* were isolated during the early period of the fermentation from both the Changed and Unchanged Water Buckets, but were eliminated from both setups at the end of the fermentation. For both set-ups too, *Lactobacillus* spp and *B. subtilis* persisted till the end of the fermentation. For the White Maize variety too, *M. alternaria* and *A. flavus* were recovered from both the Changed and Unchanged Water samples for the first 24 hrs, but both fungi were not isolated from the *akamu* at the end of the fermentation. However, only *S. cerevisiae* was isolated from the *akamu* made from the Changed and Unchanged water maize samples. For the Yellow Variety of Maize grains, *S. aureus* and *E. coli* were also isolated during the early period of the fermentation from both the Changed and Unchanged Water Buckets, but were eliminated from both setups at the end of the fermentation. For both set-ups too, only *Lactobacillus* spp and *B. subtilis* were isolated from the *akamu* at the end of the fermentation. For the Yellow Maize variety also, *M. alternaria* and *A. flavus* were recovered from both the Changed and Unchanged Water samples at the early part of the fermentation but were eliminated from the *akamu* at the end of the fermentation. Only *S. cerevisiae* was isolated from the *akamu* made from the Changed and Unchanged water maize samples. Table 3.

For the bacteriological analyses of the Changed water samples, The Total Heterotrophic Plate Count (THPC) had a maximum value of 4.85 x 10^6 CFU/mL from White Maize at 0 hr of fermentation while the lowest value recorded was 2.8 x 10^6 CFU/mL from White Maize sample at 24 hr of fermentation. For the Total Coliform Plate count (TCPC), the maximum for the unchanged Water Samples was 3.3 x 10^6 CFU/mL from White Maize at 24 hr of fermentation while the lowest value was 0 CFU/mL from both white and Yellow Maize samples after 48 hr of fermentation. The Total Lactic Acid Bacterial Count (TLABPC) was 5.85 x 10^6 CFU/mL at 48 hr of fermentation and from Yellow Maize sample while the lowest value was 1.20 x 10^6 CFU/mL from Yellow Maize at 0 hr of fermentation.

For the bacteriological analyses of the unchanged water samples, the THPC had a maximum value of 5.35 x 10^6 CFU/mL from white Maize and a minimum value of 1.7 x 10^6 CFU/mL at 24 hr. The highest TCPC was 4.3 x 10^6 CFU/mL from Yellow Maize at 0 hr of fermentation while the lowest value was 0 CFU/mL for both maize varieties. For the TLABPC, the highest value was 7.85 x 10^6 CFU/mL while the lowest value was 1.65 x 10^6 CFU/mL from white Maize after 24 hr of fermentation (Table 4).

The result of Total fungal Plate Count (TFPC) of the fungal isolates is presented on Table 5. For the Changed Water samples, the highest TFPL was 5.55 x 10^6 CFU/mL while the lowest value was 2.85 x 10^6 CFU/mL from White Maize at 0 hour of fermentation.

For the Unchanged Water samples, the highest TFPC was 4.50 x 10^6 CFU/mL at 48 hr from Yellow Maize while the lowest value was 3 x 10^6 CFU/mL was obtained at 0 hr of fermentation from White Maize.

The pH, Temperature and Titratable acid (T. A) of the fermenting maize samples are indicated on Table 6. For the Unchanged Water samples, the lowest pH recorded was 3.5 from Yellow Maize at 48 hr of fermentation while the highest pH (6.5) was recorded at 0 hr of fermentation from both steep waters. The highest temperature recorded for the Changed water was 27.0 from White Maize at 48 hr of fermentation and the lowest was 26.3 from Yellow maize at 0 hr of fermentation. The highest T. A for the Changed water maize samples was 0.43 from Yellow Maize at 48 hr of fermentation while the lowest value was 0.25 from White Maize at 0 hr of fermentation.

For the Unchanged Water maize samples, the lowest pH recorded was 3.5 from Yellow Maize at 48 hr of fermentation while the highest pH (6.5) was recorded at 0 hr of fermentation from White Maize sample. The highest temperature recorded was 27.5 from White Maize at 48 hr of fermentation the lowest was 26.3 from Yellow maize at 0 hr of fermentation. The highest T. A was 0.43 from Yellow Maize at 48 hr of fermentation and lowest was 0.25 from Yellow Maize at 0 hr of fermentation.

Result of proximate analyses (Table 7) show that the carbohydrate, crude protein, moisture and fibre contents of the *akamu* from the unchanged Water samples were significantly different from the *akamu* from changed water. However, the fat and ash values were not statistically different from the values of the same parameters for the unchanged water Maize sample. Similar result was recorded for the same parameters of *akamu* produced from for the Changed and unchanged Yellow Maize samples.

Table 1: Bacteria Isolates From Fermented Maize Samples

Colonial Features	Gram Reaction	Cell Arrangement	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	Sugar test	Probable Isolates
White	+	Short	-	+	+	+	+	-	+	-	+	*Bacillus subtilis*
Moistened,	+	Rod									+	
Pink coloured		Short	-	-	-	-	+	+	+	-	-	*Escherichia coli*
Golden	-	Rods										
Pigment		Cocci	+	-	+	-	+	-	+	-	+	*Staphylococcus aureus*
Yellow	+	Group										
Creamy		Long	-	-	-	-	-	-	+	-	+	*Lactobacillus* spp.
White	+	Rod										
Creamy		Long	-	-	-	-	-	-	+	-	-	*L. plantarum*

Table 2: Fungal Isolates From Fermented Maize Samples

Suspected Yeast/ Fungal	Morphological Characterization	Microscopically Characterization
Saccharomyces cerevisiae	Small white to creamy Circular convex colonies With thick surface	Actively budding yeast Form pseudomycellum
Mucor alternaria	White woolly growth resembling Cotton candy	Mucor has broad hyphae Non-separate and long
Aspergillus flavus	Yellow-green with With white mycelia	Conidiophores and conidia columnar Conidia heads were radiate to columnar with loosely packed phialid

Table 3: Microbial Succession during the Fermentation of White and Yellow Maize grains (hr)

Isolates	White *Akamu* Changed Water			White *Akamu* Unchanged Water			Yellow *Akamu* Changed Water			Yellow *akamu* Unchanged water		
	0	24	48	0	24	48	0	24	48	0	24	48
Bacteria												
Staphylococcus aureus	+	-	-	+	-	-	+	+	-	+	-	-
Lactobacilus spp.	-	+	+	-	+	+	-	+	+	+	+	+
Lactobacilus plantarum	-	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli	+	-	-	+	-	-	+	-	-	+	-	-
Bacillus subtilis	+	+	+	+	+	+	-	+	+	-	+	+
Mold and Yeast												
Mucor alternaria	-	+	-	+	+	-	-	+	-	-	+	-
Aspergillus flavus	-	+	-	-	+	-	-	+	-	-	+	-
Saccharomyces cerevisiae	-	+	+	-	+	+	+	+	+	+	+	+

Table 4: Total Viable Bacterial Counts from Two Varieties of Maize Samples

Fermentation Time (Hr)	Sample	THPC (cfµ/ml)	TCPC (cfµ/ml)	TLABC (cfµ/ml)
Changed Water				
0	White	4.85×10^6	2.7×10^6	3.8×10^6
	Yellow	3.75×10^6	2.25×10^6	1.20×10^6
24	White	2.8×10^6	3.3×10^6	2.95×10^6
	Yellow	3.95×10^6	1.5×10^6	5.65×10^6
48	White	2.8×10^6	0	4.9×10^6
	Yellow	2.9×10^6	0	5.85×10^6
Unchanged Water				
0	White	4.85×10^6	3.4×10^6	4.5×10^6
	Yellow	4.3×10^6	6.3×10^6	3.35×10^6
24	White	1.7×10^6	2.7×10^6	1.65×10^6
	Yellow	4.4×10^6	2.45×10^6	3.65×10^6
48	White	5.35×10^6	0	6.92×10^6
	Yellow	5.15×10^6	0	7.85×10^6

Key; THPC Total Heterotrophic plate count, TCPC Total Coliform plate count, TLABC Total Lactic Acetic Bacterial plate count, Sample W-White *akamu*, sample Y-Yellow *akamu*

Table 5: Total Fungal Plate Counts (TFPC) from Two Varieties of Maize Samples

Fermentation Time (Hours)	Sample	TFPC
Changed Water		
0	White	2.85×10^6
	Yellow	3.95×10^6
24	White	3.0×10^6
	Yellow	5.55×10^6
48	White	4.75×10^6
	Yellow	4.05×10^6
Unchanged Water		
0	White	3×10^6
	Yellow	3.8×10^6
24	White	4.25×10^6
	Yellow	4.45×10^6
48	White	5.37×10^6
	Yellow	5.50×10^6

Table 6: Temperature, pH and Titratable Acidity of Maize Samples During Fermentation

Period fermentation	Sample	pH	Temperature (^{0}C)	Titratable Acid (%)
Changed Water				
0	White	6.5	26.5	0.24
	Yellow	6.5	26.3	0.29
24	White	6.0	26.9	0.30
	Yellow	6.1	26.5	0.38
48	White	5.5	27.0	0.35
	Yellow	5.3	26.9	0.41
Unchanged Water				
0	White	6.5	26.3	0.25
	Yellow	6.5	26.1	0.28
24	White	5.2	26.8	0.36
	Yellow	4.9	27.0	0.38
48	White	3.6	27.5	0.45
	Yellow	3.5	27.5	0.43

Table 7: Proximate Composition of Fermented Yellow and White *Akamu* Samples (%)

	Maize samples varieties			
Parameters	**White *Akamu* (Changed Water)**	**White *Akamu* (Unchanged Water)**	**Yellow *Akamu* (Changed Water)**	**Yellow *Akamu* (Unchanged Water)**
Moisture Content	$9.13^a \pm 0.80$	$9.60^b \pm 0.90$	$9.04^a \pm 0.70$	$10.3^b \pm 0.10$
Crude Protein	$7.03^a \pm 0.90$	$9.23^b \pm 0.90$	$7.24^a \pm 0.90$	$9.25^b \pm 0.90$
Crude fat	$3.56^a \pm 0.09$	$3.50^a \pm 0.09$	$3.59^a \pm 0.10$	$3.58^a \pm 0.09$
Crude fibre	$2.033^a \pm 0.09$	$3.22^b \pm 0.08$	$2.32^a \pm 0.08$	$3.15^b \pm 0.08$
Ash	$1.70^a \pm 0.04$	$1.65^a \pm 0.04$	$1.61^a \pm 0.04$	$1.55^a \pm 0.03$
Carbohydrate	$72.05^a \pm 0.18$	$74.80^b \pm 0.16$	$72.23^a \pm 0.19$	$75.30^b \pm 0.14$

Values with the same letter(s) along the same column do not differ significantly at 95% level. Values are means of triplicate results

DISCUSSION

The bacterial isolates from this work (*Bacillus subtilis, Escherichia coli, Staphylococci aureus, Lactobacillus* species and *L. plantarum*) are similar to result obtained by Ogodo *et al.* (2018) who studied the Bacteriological Quality of Commercially Prepared Fermented *Akamu* sold in some parts of South Eastern Nigeria. He reported the presence of the isolate *E. coli, Staphylococci* species and *Lactobacillus* species among other isolates. Similar bacterial isolates were reported by Omemu *et al*. (2018) and Karami *et al.* (2017). This indicates that the fermentation of maize grains for the production of *akamu* is carried out by many microorganisms some of which come into the process as contaminants through water, equipment, handlers and air.

The isolation of *Aspergillus flavus, Mucor alternaria* and *Saccharomyces cerevisiae* during the fermentation process. This implies that molds and yeasts are involved in the fermentation processes. Omemu *et al.* (2018) reported the presence of *Aspergillus flavus Mucor racemosus* and *Saccharomyces cerevisiae* during the production of *akamu.* The indigenous natural fermentation is known to takes place in a mixed colony of microorganisms such as moulds, bacteria and yeasts (Antony and Chandra, 1997). Thus, fermentation products in food substrates are based on the microorganisms involved in the fermentation. Some of the compounds formed during fermentation include organic acids (e.g., palmitic, pyruvic, lactic, acetic, propionic and butyric acids), alcohols (mainly ethanol) aldehydes and ketones (acetaldehyde, acetoin, 2-methyl butanol) (Campbell-Platt, 1994).

For the fermentation using changed water procedure, the five bacteria were isolated within the first 24 hours of fermentation, but at the end of the fermentation only the LAB and *Bacillus subtilis* were isolated from the produced *akamu.* The trend is the same with the fermentation of the two varieties of maize in which the steep water was not changed during the period of fermentation. *Staphylococcus aureus* and *E. coli* which are food borne pathogens were eliminated from the fermented sample 48 hours of fermentation. This result shows that gram positive bacteria were present till the end of the fermentation although *Staphylococcus aureus* was absent at the last day of fermentation while the gram negative isolates did not get to the end of the fermentation. *E. coli* and *Staphylococcus aureus* are capable of causing food-borne infections (Ogueke *et al.*, 2010). The elimination of these pathogens are attributed to the production of anti-microbial substances like organic acids, bacteriocins, diacetyl and CO_2 and by LAB. Bacteriocins are peptides that elicit antimicrobial activity against food spoilage organisms and food borne pathogens, but do not affect the producing organisms. LAB also synthesize other antimicrobial compounds such as hydrogen peroxide, reuterin, and reutericyclin (Leroy and De Vuyst, 2004).

Food borne fungal pathogens and spoilers were within the first 24 hr of the fermentation. The presence of *Aspergillus niger* on the surfaces of raw maize grains and during the early stage of fermentation has been reported (Wagara *et al.*, 2014). They are mostly likely part of the grains surface microflora that is undesirable in many foods because of their mycotoxin producing potentials (Jonsyn, 1989: Jespersen *et al.*, 1994). However, they were not isolated from the akamu after 48 hrs. *S. cerevisiae* was the only fungus isolated from the akamu. This yeast is known to produce ethanol during fermentation and together with other anti-microbial substances produced by Lactic acid which have been reported to have antifungal effects (Schnürer and Magnusson, 2005; Huwig *et al.*, 2001) eliminated the fungal pathogens and food spoilers from the final product. The involvement of several species of fungi in traditional food fermentation as reported in this work is in agreement with the findings of

Omemu *et al*. (2007a). The reduction in the pH of the *akamu* to 3.5 could have been possible only through acid production by LAB and this inhibited the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease (Ananou *et al.,* 2007). By doing this, the shelf life of fermented food is prolonged. This is because the sheer overgrowth of desirable edible bacteria in food outcompetes the other non-desirable food spoilage bacteria (Schnürer and Magnusson, 2005). One of the arguments supporting the use of LAB fermentation to prevent diarrheal iseases is because they modify the composition of intestinal microorganisms, and by this, act as deterrents for pathogenic enteric bacteria. Thus, LAB are applied as a hurdle against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment (Ananou *et al.*, 2007).

The result further shows a significant difference in microbial count of change and unchanged *akamu* water at all fermentation periods among the two maize varieties. The population of the microbes in the unchanged *akamu* water was very high compared to the changed *akamu* water. This is because the population of the microbes in the change water are reduced whenever the water change is done while that of the unchanged water kept increasing. This would have led to better hydrolysis of food complexes into simple non-toxic products with desirable textures, aroma that makes them palatable for consumption (Steinkraus, 1997).

The result of the proximate composition of the *akamu* from both maize varieties showed no significant difference in the crude fat and ash contents for both the changed and unchanged water samples. However, significant differences were observed in the carbohydrate, protein and Crude fibre of the changed and unchanged water samples with higher values from *akamu* made from unchanged water samples. When the water samples were not changed, there was a buildup in microbial loads of the starter cultures leading to better enzymatic hydrolysis of the carbohydrate. Protein and crude fibre of the maize grains. This was not so in the changed water sample as the change in steep water interrupted the microbial buildup and metabolic activities of the started cultures. Hence, a poor modification of the carbohydrate, protein and crude fibre.

The increase in the protein content of the *akamu* from unchanged water samples could have been from LAB which are single cell proteins. This is similar to result reported by Ndukwe, *et al.* (2015) who studied the varietal differences in some nutritional composition of ten maize varieties grown in Nigeria. This study showed that *akamu* is rich in carbohydrate and but relatively low protein. This is why *akamu* is supplemented with protein rich substances in order to boost its protein content which is essential for the growth of children (Enujiugha 2006).

CONCLUSION

This study has shown that *akamu* produced from unchanged steep water has higher carbohydrate, protein and fibre contents that that made from changed steep water, although the *akamu* is relatively low in protein content. It is also higher in acidity than its counterpart. This study showed no significant difference in the microbial and proximate composition of *akamu* from the two varieties of maize used, indicating that both yellow and white maize have similar nutrient composition.

RECOMMENDATION

We recommend that people should not change the steep water during the production of *akamu* because the *akamu* produced this way has better nutritional and higher acidity contents. This higher acidity has better effect in eliminating pathogens and spoilage organisms.

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