EFFECTS OF SOME SELECTED STARTERS ON THE NUTRITIONAL COMPOSITION OF A NIGERIAN FERMENTED BEVERAGE, 'AGADAGIDI'

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Abstract
''Agadagidi,'' a cloudy sweet-sour taste typical African traditional alcoholic beverage is made from overripe bananas or plantains through fermentation and it is mostly consumed by the rural communities in the south western part of Nigeria. The aim of this work is to use selected microorganism\s predominant in this beverage both singly and mixed to assess the nutritional composition of ''Agadagidi''. Chemical analysis was carried out to confirm the proximate, anti nutrient and mineral content of this fermented beverage. Generally there were reduction in the pH from 6.4 to 3.4 and increase in the total titratable acid from 0.04 to 1.1% after 72 hr of fermentation. The highest value of protein content with significant difference (2.34 ±0.05%) and lowest carbohydrate content was observed with the ''Agadagidi'' containing L. mesenteroides at 2.29 ± 0.25%. The anti-nutrient composition of ''Agadagidi' was found to have the lowest phytate and oxalate values of 2.2 ± 0.12 mg/100g and 0.09 ± 0.00 mg/100g with the addition of L. mesenteroides while the mineral composition of ''Agadagidi'' showed no increase with any of the starters as compared with the spontaneous fermentation. This work has been able to enlighten us that Leuconostoc mesenteroides used as starter culture amongst others had the highest nutritional parameters in ''Agadagidi'' beverage.

Key words: ''Agadagidi**''**, Proximate composition, Fermentation, Starter culture, alcoholic beverage.

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1. INTRODUCTION
''Agadagidi'' are made from over ripped bananas and plantains and have the opaque sweet-sour nature typical of African traditional alcoholic beverages and it is common in south-western part of Nigeria (Omojasola *et al*., 2012, Oriola *et al*., 2017). The fermentation of over ripe plantain to produce 'Agadagidi' is a waste prevention processing of plantain, a perishable crop which has much less value when it is over-ripe; hence it is used for wine or beer production (Sanni *et al*., 1999; Chia and Huggin, 2003; Areola *et al*., 2011). In several African countries, traditional fermented beverages are consumed because of their high nutritional value. They are as a source of energy providing important nutrients to contribute to the diet of the population (Achi, 2005; Abdoul-Latif *et al*., 2013).

Starter culture is a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process (Rakib *et al*., 2017). The use of starter cultures has been
U7sed as an approach to alleviate the problems of nutritional quality and microbiological stability observed in African traditional fermented beverages (Holzapfel, 1997; Achi, 2005; Viera-Dalodé *et al*., 2007).

Currently, there is no information on the use of starter cultures for ''Agadagidi'' fermentation. However, few investigations have been carried out on the microbiology of traditionally fermented plantain (Oriola *et al*, 2017). The predominant microorganisms isolated from this traditionally fermented ''Agadagidi'' should be developed into starter cultures that could be used to produce fermented beverage of consistent quality and improve the macro and micronutrient content of this beverage. Thus, it should be possible to improve the nutritional composition of ''Agadagidi'' through using starter culture.

The objective of the present study is therefore to investigate the potential microorganism\s isolated from spontaneously

fermented ‘‘Agadagidi’’ in view of their nutritional relevance as starter cultures in its production.

2. MATERIAL AND METHODS

Preparation of ‘‘Agadagidi’’

The production of ‘‘Agadagidi’’ was done in the laboratory by applying indigenous method. Overripe plantain pulps were washed in tap water to remove debris and dirt. They were peeled and their pulps were crushed in portable water at a ratio of 1:5 (w/v) in a clean transparent container, covered and left to ferment for 2 days. Samples were withdrawn from the semi-liquid crushed overripe plantain pulps at 0 h, 24 h and 48 h of fermentation. The fermented liquid was filtered with clean muslin to remove the plantain mashes. The liquid then served as “Agadagidi”.

Over ripe plantain pulp
↓
Wash in clean tap water and remove peel
↓
Mashing plantain pulp
↓ (Addition of water)
Soaking/ fermentation (2 days)
↓
Filter
↓
‘‘Agadagidi’’

Fig.1: Flow chart for the production of ‘‘Agadagidi’’

Analysis for Acidity of “Otika” and “Agadagidi” Samples

Determination of pH

Each sample (20 mL) was poured into beaker and pH was determined using pH meter (Hannah Model 3051 UK) after standardization with buffer solutions having pH 4 and 7 according to the Owuamanam *et al*. (2011).

Determination of Total Titratable Acidity (TTA)

The percent titratable acidity (TTA) was determined following the method described by Owuamanam *et al*. (2011). Ten millimeter of the sample was poured in beaker and allowed to stand for 10 min. The solution was filtered with Whatman filter paper (Grade 1, Qualitative Filter Paper, Standard Grade, 25 mm). Twenty five milliliters of the filtrate was titrated against 0.1 M NaOH using phenolphthalein as indicator and the acid was expressed as % lactic acid. The mean of TTA was obtained from triplicate determinations and calculated as follows:

TTA (%) = [(average titre value × 0.1M × 0.009008) / Weight of sample] × 100

Preparation of Starter Cultures

The cultures of the LAB, *Lactococcus lactic*, *Leuconostoc mesenteroides* and yeast (*Saccharomyces cerevisiae*) isolated from the fermented beverages were used because of their predominance and exhibition of antimicrobial substances against pathogenic and spoilage microbes (previous work). LAB was cultivated by streaking them on MRS agar and incubating them at 37°C for 24 hr. A colony was picked and grown in MRS broth at 37°C for 24 hr. Ten milliliters of the grown culture was centrifuged at 5000 rpm for 15 min. The cells pellet was washed in sterile distilled water and centrifuged again. It was diluted to a culture containing 10^9cfu/mL. Pure culture of *Saccharomyces cerevisiae* was cultivated by streaking on Malt Extract agar, incubated at 28°C for 48 hr. A colony was then inoculated into 10 mL of Malt extract broth and incubated at 28°C for 24 hr. The culture was centrifuged and washed as described above. It was diluted to 10^7cfu/mL. Ten mL portions of the respective suspensions of each culture and the combination of both starter cultures were used as innocula for 250 mL each of the prepared beverage. Traditional production of the beverage (without starter culture) served as control. Fermentation was done at room temperature (Asmahan and Mustafa, 2009).

Proximate Composition Determination

Proximate composition was determined for ‘‘Agadagidi’’ with starter cultures both singly and in mixture. These are explained below according to A. O. A. C. (2000).

Determination of Moisture Content

Ten milliliters each of samples was

transferred to a pre-weighed cleaned petri dish and dried in the oven at 105°C for 3 hr. After drying, the petri dish and its content were kept in the desiccator to cool and weighed. The sample was further dried, cooled and re-weighed at interval of 30 minutes until a constant weight was obtained. The moisture content was calculated using the formula stated below and expressed on dry weight basis:

% MC = $(W_1 – W_2) / (W_1 – W_0)$

Where: W_0 = weight of empty Petri dish

W_1 = Weight of Petri dish + sample before drying

W_2 = Weight of Petri dish + sample after drying.

Determination of Crude Fat Content

Each sample (10 mL) was put into an already weighed flat bottom flask with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample was carefully transferred into the thimble. Extraction was carried out using petroleum ether (BP 40-60°C), the thimble was plugged with cotton wool, fully dropped into the extractor and the extraction was carried out continuously for 8 hr. The solvent was removed by evaporation in a water bath and the remaining part of the flask was dried at 80°C for 30 minutes in a hot air oven to remove excess of the solvent. The flask was cooled in a desiccator, reweighed and the percentage fat was calculated as:

Percentage = (Weight of extracted fat) / (Weight of sample) ×100

Determination of Total Ash Content

The weight of the crucible dish was taken. The sample (20 mL) was weighed into the crucible and placed inside an already weighed muffle furnace rack and the temperature was set to 500°C for 16 hours until the sample was completely ashed. The ash in the crucible dish was removed and kept in a desiccator to cool before it was weighed and the percentage ash was calculated thus:

Percentage ash = (Total weight of ash) / (Weight of sample) × 100

Determination of Crude Protein Content

Twenty milliliter of each sample was weighed into 500 mL Kjeldahl flask and two tablets of the catalyst mixture and 20 mL of concentrated sulphuric acid (H_2SO_4) were added, stoppered and swirled. The flask and its content were heated gently in a fume cupboard in an inclined position and swirled occasionally until the liquid was clear and free from black or brown colour. The digested mixture was allowed to cool and made up to 100 mL with distilled water. Twenty-five milliliters of 1% (w/v) boric acid was put into a 250 mL conical flask, 2 drops of mix indicator, 92% (w/v) Bromocessol green and 2% (w/v) methyl red were added and placed under the collection spigot of the distillation apparatus. Ten milliliters of the digest was pipetted into the stopper portion of the condenser and 20 mL of 40% (w/v) sodium hydroxide solution was added. The solution was allowed to distil for 15 minutes or when the volume of ammonia collected into boric acid in the receiver flask was 50 mL and when the purple solution had turned to green. The distillate was then titrated against 1.0 M Hydrochloric acid (HCl) to a pink color point. A blank titration was carried out using 20 mL of 1% (w/v) boric acid. Percentage crude protein was calculated as follows:

Gram Nitrogen (% g N) content = [0.014 × (A-B) × molarity of HCL × dilution × 100] / (Weight of sample)

Percentage crude protein = % g N ×6.25

Where A = volume of titre for the test sample, and B = volume of titre for blank

Determination of Crude Fibre Content

The sample (200 mL) was measured into a 500 mL beaker and boiled in 200 mL H_2SO_4 10% (v/v) for 30 minutes. The suspension was filtered and the residue was washed vigorously with boiling water until it was no longer acidic. The residue was boiled in 200 mL of 0.313M NaOH for 30 minutes, filtered, washed with ethanol and diethyl ether. The residue obtained was transferred into a pre-weighed crucible in a hot air oven for 30 minutes. The dried residue was cooled in a desiccator and reweighed. The sample was then ashed in a furnace at 550°C, cooled and weighed. Amount of the crude fibre in the sample was calculated as thus acid

insoluble ash (%).
Fibre content (%) = [(Weight of crucible + fibre -Weight of crucible with ash) / (Weigh of sample)] × 100

Determination of Total Carbohydrate Content

The carbohydrate was calculated by subtracting the sum of ash, protein, fat and fibre from 100. Carbohydrate (CHO) =100 - (ash+ protein+ fat+ fibre).

Minerial Composition Determination

Quantification of Mineral Elements

Potassium, calcium, iron, zinc, sodium, phosphorus and magnesium contents were medium heat under perchloric acid fume hood and heating continued until dense white fume appeared. It was allowed to cool followed by the addition of distilled water (50 mL). The solution was filtered completely and washed into a Pyrex volumetric flask and was made up with distilled water. Absorbance of the solution was read in the Atomic Absorption Spectrophotometer in triplicate.

Quantification of Antinutrients

Determination of Phytate Content

Twenty five mL of the filtrate was placed in a 100 mL conical flask and 5 mL of 0.03% (w/v) NH_4 SCN solution was the added as indicator. Distilled water (50 mL) was added to give it the proper acidity. This was titrated against ferric chloride solution which contained about 0.005 mg of Fe^{2+} per $FeCl_3$. was used, the equivalent was obtained and from this, the phytate content in mg/100 g was calculated.

Iron equivalent = titre value × 1.95
:. Phytic acid = titre value × 1.95 × 1.19 × 3.55 mg/phytic acid = 8.24
:. % phytic acid = (α × 8.24) /1000 × 100 / (weight of sample)
Where α = titre value.

Determinationof Tannin Content

Fifty milliliters of each sample was poured into a sample bottle. Ten milliliters of 70% (v/v) aqueous acetone was added and point when a faint colour appeared that persisted for at least 30 seconds.

Oxalate (mg/g) = V_T × 0.904

Where, V_T = Titre value

determined using Atomic Absorption Spectrophotometer (Thermo scientific S series Model GE 712354) after digestion with a perchloric-nitric acid mixture (A. O. A. C., 2000). Phosphorus content was determined by Vanado molybdate method using spectrophotometer. Prior to digestion, 10ml of each sample was measured into 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated HNO_3 (25 mL) and concentrated H_2SO4 acid (2 mL) under a fume hood. The contents were mixed and heated gently on a hot plate in a digester at low temperature to properly covered. The bottles were put in an ice bath shaker and shaken for 2 hours at 30°C. Each solution was then centrifuged and the supernatant was stored on ice. Each solution (0.2 mL) was pipetted into a test tube and 0.8 mL of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml stock and the solution was made up to 1 mL with distilled water.

Folin reagents (0.5 mL) were added to the test sample and the standards prepared followed by addition of 2.5 mL of 20% (w/v) Na_2CO_3. These solutions were then vortexed and incubated for 40 minutes at room temperature of 28°C ± 2°C). The absorbance was read at 725nm against the reagent blank concentration of the sample. From the optical density of the tannic acid, standard curve was plotted. The tannin content of the samples was obtained from the standard curve.

Determination of Oxalate Content

Ten milliliters of each sample was weighed into 100 mL conical flask. Then 75 mL of 3 M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 hour. It was filtered using Watman N0.1 filter paper. Sample filtrate (25 mL) (extract) was collected and titrated hot (80-90°C) against 0.1M $KMnO_4$ solution to the end

Determination of Total Phenolic Content

Ten mL of diluted sample was added to 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent was added, followed by the addition of 2 mL of

7.5% (w/v) Na2CO3, then mixed well on a vortex vibrator for 5 min and incubated in the dark at ambient temperature (29°C) for 1 h prior to measuring the absorbance at 765 nm. Gallic acid was used as a calibration curve and the results were expressed as mg gallic acid equivalents per 100 mL sample.

Statistical Analysis

Numerical data obtained from this study were subjected to analysis of variance; (ANOVA) and the means were separated by using New Duncan's Multiple Range Tests in SPSS 16.0 computer-aided programme. Errors were calculated as standard error.

3. RESULTS AND DISCUSSION

The chemical analysis of on "Agadagidi" showed a decrease in pH from the 0 hr of fermentation (6.4) to 72 hr of fermentation (3.4) while the TTA increased as the fermentation progressed from 0 hour of fermentation (0.04%) to 72 hr of fermentation (1.1%) (Fig.1). Fermentation was found to cause a gradual reduction in a pH and increase in total titratable acidity with time. The change in pH of "Agadagidi" decreased as well the fermentation period increased, this is due to the breakdown of sugars present in the over ripe plantain pulp into organic acids thereby increasing the acidity and automatically reducing the pH, this work correlate with the work of Akinleye (2014) whose fermented beverage pH ranged between 4.5 to 3.9.

Analysis of proximate composition provided information on the basic chemical and nutritional composition of the beverage. The compositions were moisture, ash, crude fat, protein, crude fibre, and carbohydrate. These constituents are essential to the assessment of the nutritive quality of the food being analysed. The moisture content is very vital for life maintenance and analysis of it is one of the most widely used measurements which determine the way the food will be processed and its shelf life. The ash composition of food samples is very important in determining mineral contents. Protein is needed for normal body growth, repairs and maintenance. A relatively high amount of protein is therefore required for functional foods and nutraceuticals, because they are used for supplementation.

Table 1 shows the proximate composition of laboratory prepared ''Agadagidi'' with various starters. The highest value 2.34 ± 0.05 of protein content with significant difference was found with 'Agadagidi' prepared with *L. mesenteroides*. The increase in protein with the addition of *Leuconostoc mesenteroides* in "Agadagidi" might be due to its microbial growth that resulted to an increase in microbial biomass during the fermentation process and could be also due to the action of extracellular enzymes such as protease produced by the fermenting microorganism compared to the one without any addition of microorganism.

It could also be due to conversion of plant proteins or other nitrogenous compounds into microbial protein. Adebawo *et al*. (2000) also reported fermented cereal gruels with higher lysine, arginine and methionine contents. The highest value of ash content (0.21 ± 0.01%) was found with the sample containing *S. cerevisiae* while the lowest carbohydrate content was also found with the sample containing *L. mesenteroides* at 2.29 ± 0.25%.

The reduction in carbohydrate content of "Agadagidi" inoculated with *Leuconostoc mesenteroides* is probably due to the breakdown of some starch and sugar components by increased activity of alpha-amylase which hydrolyses starch to simple sugar.

The anti-nutrient composition of ''Agadagidi'' produced in the laboratory with different starters was found to have the lowest phytate and oxalate values of 2.2 ± 0.12 mg/100g and 0.09 ± 0.00 mg/100g with *L. mesenteroides* while tannin and total phenol were found to have the least values of 0.46 ± 0.0 and 0.76 ± 0.0 mg/100g in the spontaneous fermentation of 'Agadagidi'.

The anti-nutrients are known to precipitate nutrients thereby reducing the nutrients meant to be absorbed by the body.

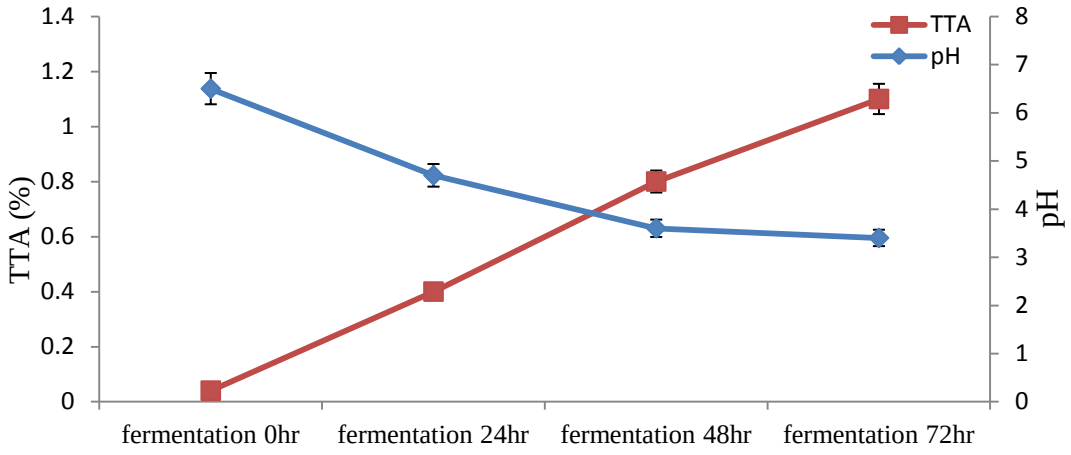

Figure 2: pH and TTA during the production of 'Agadagidi'

TABLE 1: Proximate composition of 'Agadagidi' produced from laboratory with various isolates

Sample	Proximate composition (%)					
	Ash	Moisture	Fat	Fibre	Crude Protein	Carbohydrate
Natural Fermentation	0.20±0.02^{d}	95.44±0.08^{a}	0.00±0.00	0.00±0.00	1.94±0.01^{c}	2.42±0.06^{a}
S.cerevisiae	0.21±0.01^{e}	94.50±0.66^{a}	0.00±0.00	0.00±0.00	1.71±0.03^{b}	3.58±0.69^{a}
L.mesenteriodes	0.05±0.01^{a}	95.56±0.03^{a}	0.00±0.00	0.00±0.00	2.34±0.05^{e}	2.29±0.25^{a}
Mixed cultures	0.16±0.02^{c}	95.36±0.10^{a}	0.00±0.00	0.00±0.00	2.10±0.00^{d}	2.38±0.11^{a}

Legend: Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).
Mixed cultures = *L. lactis* + *S.cerevisiae* + *L. mesenteroide*

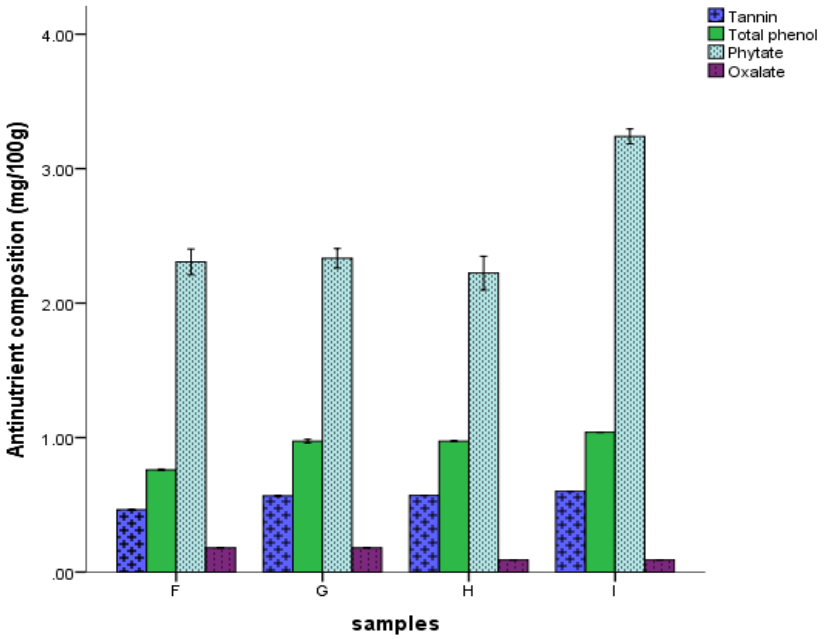

Figure 3: Antinutrient composition of ''Agadagidi'' produced in the laboratory with various starters.
Legend: F = Natural fermentation, G = *S.cerevisiae*, H = *L.mesenteroides*, I = *L.lactis* + *S.cerevisiae* + *L.mesenteroides*

TABLE 2: Mineral composition of "Agadagidi" produced with various isolates

Sample	Mineral composition (mg/100g)						
	Na	K	P	Ca	Mg	Zn	Fe
Natural Fermentation	47.90± 0.05^{d}	25.41± 0.01^{e}	1.89±0.02^{b}	1.12±0. 01^{e}	0.88±0.06^{d}	0.07±0. 01^{c}	0.62±0. 07^{c}
S.cerevisiae	40.33± 0.33^{c}	14.81± 0.03^{c}	0.00±0.00^{a}	0.12±0. 03^{c}	0.35±0.01^{b}	0.01±0. 00^{a}	0.03±0. 01^{a}
L. mesenteroides	38.08± 0.01^{b}	9.19±0. 01^{b}	0.00±0.00^{a}	0.08±0. 01^{b}	0.12±0.01^{a}	0.00±0. 00^{a}	0.00±0. 00^{a}
Mixed cultures	33.90± 0.05^{a}	8.40±0. 00^{a}	0.00±0.00^{a}	0.03±0. 01^{a}	0.02±0.01^{a}	0.00±0. 00^{a}	0.00±0. 00^{a}

Legend: Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).
Mixed cultures = *L.lactis* + *S.cerevisiae* + *L. mesenteroides.*

As earlier stated, there were reduction in the phytate and oxalate content with *Leuconostoc mesenteroides* added as starter. It has been observed it might be as a result of the enzymatic activities of the microorganisms. It has been also been documented that fermentation also reduces the antinutrient contents.

Shown in table 2 is the mineral composition of ''Agadagidi'' produced in the laboratory with various starters, there was no increase with any of the starters compared with the spontaneous fermentation (without starter culture\s) which had the highest values in all the mineral contents. The lowest of the mineral contents were found with the mixed starters containing *L. mesenteroides*, *L. lactis* and *S. cerevisiae*. The reduction in mineral content of the Agadagidi added with starters might due to their ability to metabolize and utilize the minerals. This work correlate with Oladunmoye, 2007 who discovered that there was reduction in ascorbic acid after the fermentation of locust beans with starter cultures.

5. CONCLUSIONS

In this study, fermentation on its own enhanced mineral contents of the prepared ''Agadagidi''. The use of *Leuconostoc mesenteroides* amongst others as starter culture was also able to increase other nutritional content of 'Agadagidi', a fermented plantain beverage.

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