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Original Article

Nutritional Properties of Some Selected Microorganisms on 'Otika', a Nigerian Fermented Beverage

Oriola, Olasunkanmi Bukola

Department of Microbiology, Atiba University, Oyo State, Nigeria.

checklobebe@yahoo.co.uk

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Abstract - "Otika" is a fermented grain beverage made in the Southwestern region of Nigeria using locally developed fermentation methods. On the third day of fermentation, it has a nice flavor that is sweet, somewhat sour, and has an opaque brown color. This work aims to assess the nutritional properties of this beverage by using a few chosen predominant microorganisms, both in a mixed and single form. An analysis of this fermented beverage's proximate, antinutrient and mineral content was done. After 72 hours of fermentation, the pH dropped from 7.2 to 4.9, and the total titratable acid increased from 0.04% to 0.1%. When compared to other isolates, L. plantarum had the greatest protein content (1.33 ± 0.05%). Wesiella cibaria had the lowest carbohydrate amount (8.24 ±0.6%), whereas S. cerevisiae had the greatest carbohydrate content (11.02 ± 0.02%). The W. cibaria culture yielded the lowest levels of tannin and total phenol content (1.9± 0.0 mg/100g and 2.5± 0.00 mg/100g, respectively). The values of potassium, magnesium, iron, and zinc with the Otika bought from a local producer were the highest at 18.26 ± 0.02, 2.03 ±0.11, 0.42 ±0.14, and 2.02 ± 0.01 mg/100g, respectively. Overall, the use of the W.cibaria strain as a single starter culture in the fermentation process of "Otika" helped to raise the nutritive value among other microorganisms that served as starter cultures.

Keywords - Mineral content, Proximate composition, Fermentation, Starter culture, Anti nutrient.

1. Introduction

Otika' is a popular Indigenous beverage with a vinegar-like flavour consumed in the southwestern region of Nigeria [1]. It is mainly produced from the grains of guinea corn (Sorghum vulgare and Sorghum bicolor). The traditional process of preparing 'Otika' involves steeping sorghum grains in water overnight, malting, mashing, fermentation and maturation [2-4]. It has a good flavour; it is sweet, slightly sour with a brownish-opaque colour and has a pleasant taste during the third day of fermentation [3, 5]. Microorganisms are used for the production of specific metabolites such as acids, alcohols, enzymes, antibiotics, and carbohydrates in food fermentation. Major fermentation microorganisms include Lactic Acid Bacteria (LAB), moulds, and yeasts, which sometimes form complex populations that act in concert [6]. All of the traditional fermented beverages are originally fermented by natural microorganisms, which have been transferred from generation to generation. Hence, preparation procedures for these beverages are still traditional arts, and the fermentation is, by and large, uncontrolled and unpredictable [7]. The natural microflora of the raw material is inefficient, uncontrollable, and unexpected or is eradicated by the heat treatments given to the food. This poses problems for the end product of the fermented food because of the inconsistency in nutrient and organoleptic quality.

A starter culture is a microbial preparation made up of a significant percentage of cells from at least one microbe that is introduced to a raw material to increase the activity of fermentation and produce a fermented food [8]. The use of starter cultures is a good approach for the control and optimization of the fermentation process to alleviate the problems of variations in organoleptic quality and microbiological stability. Few or no investigations have been carried out on the use of starter cultures for fermenting "Otika". The predominant microorganisms isolated from this traditionally fermented "Otika" could be developed into starter cultures that could be used to produce a fermented improved nutritional beverage with consistent quality. Thus, it should be possible to modify the nutritional composition of "Otika" through the use of starter cultures. The objective of this study is, therefore, designed to examine the influence of the predominant microorganisms on the proximate, antinutrient, and mineral contents of this fermented beverage.

2. Material and Methods

2.1. "Otika" Preparation

In a ratio of 1:3 (w/v), Sorghum grains and water were mixed and left for two days at room temperature to steep. It was spread on a moistened fresh banana and left for 3 days with regular wetting. After the germinated grains were dried for 2 days and milled using the grinding instrument, the wort was removed from the milled malted sorghum grains by cooking (100°C) for 7 hours. It was cooled at room temperature and poured into a bowl. It was left to ferment for 3 days naturally. This was done in the laboratory using the indigenous method.

Sorghum Grains
↓ (Addition of Water at 1:3 v/w)
Steeping (48 hr)
↓ (Spread on the Tray at Room Temperature)
Germinating (24-48 hr.)
↓ (Remove Rootlet after Drying)
Milling
↓ (Filter)
Cooking the Wort (100°C) 7 hrs
↓
Left to Ferment for 72 hrs

Fig. 1 Flow chart in the production of "Otika"

2.2. pH Determination

A volume of twenty milliliters of the sample was poured into a beaker. The pH of the solution was ascertained using a pH meter (Hannah Model 3051 UK). Prior to measuring the pH, the pH meter was standardized with buffer solutions of pH 7 and 4.

2.3. Total Titratable Acidity (TTA) Determination

Fifty milliliters of the solution was filtered using Whatman filter paper, specifically Grade 1, which is a qualitative filter paper of standard grade with a diameter of 25 mm. After filtration, twenty-five milliliters of the resulting filtrate were subjected to titration. This titration was performed using 0.1 M NaOH (sodium hydroxide) and phenolphthalein as an indicator. The purpose of this titration was to determine the amount of acid present in the sample, which was expressed as a percentage of lactic acid.

To obtain accurate results, triplicate determinations were conducted, and the mean of the Titratable Acidity (TTA) was calculated using the values obtained from these three determinations:

$$\text{TTA (\%)} = \frac{\text{Average Titre Value} \times 0.1\text{M} \times 0.009008}{\text{Weight of Sample}} \times 100$$

2.4. Starter Cultures Preparation

The cultures of Weisellia cibaria, Lactobacillus plantarum and yeast (Saccharomyces cerevisiae), which were isolated from the otika, were used due to their exhibition of antimicrobial substances against pathogenic and spoilage microbes and predominancy. The lactic acid bacteria were cultivated by growing them on MRS agar and incubating them at 37°C for 24 hr. A colony was transferred into MRS broth at 37°C for 24 hr. Ten milliltres of this grown culture was centrifuged for 15 min at 5000 rpm. The cell pellets were washed with sterile distilled water and recentrifuged. It was diluted to have a culture containing 109cfu/mL. The 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 prepared as described previously. It was diluted to a concentration of 107cfu/mL. Ten mL portions of the respective suspensions of each culture, as well as a combination of both starter cultures, were used as inocula for 250 mL of the prepared beverages. The traditional production of beverages without any starter culture served as the control [9].

2.5. Determination of *Proximate Composition*

Proximate analysis was determined for the fermented beverage using the starter cultures as both a single and a mixture. These are discussed below, according to A.O.A.C. [10].

2.5.1. Moisture Content Determination

First, 10 milliliters of each sample were poured into a new pre-weighed petri dish and placed in an oven at a temperature of 105°C for a period of 3 hours to dry the samples. After this, the petri dish and its contents are placed in a desiccator to cool down. Once cooled, they are weighed again. The sample is then subjected to further drying, cooling, and weighing at 30-minute intervals until a constant weight is achieved. This indicates that the sample has reached a state of constant dryness. The moisture content of the sample is calculated using a specific formula and expressed on a dry-weight basis:

$$\%\ \text{MC} = \frac{P_1 - P_2}{P_1 - P_0}$$

Where,

P_0 = Weight of empty Petri dish

P_1 = Weight of Petri dish + sample before drying

P_2 = Weight of Petri dish + sample after drying

2.5.2. Crude Fat Determination

A 10 mL sample is placed in a pre-weighed flat bottom flask with an extractor attached to it. The thimble is positioned halfway into the extractor, and the sample is carefully transferred into the thimble. Extraction is performed using petroleum ether with a boiling point range of 40-60°C. The thimble is sealed with cotton wool fully inserted into the extractor, and the extraction process is carried out continuously for 8 hours. After extraction, the solvent is removed by evaporation in a water bath. The remaining part of the flask is then dried at 80°C for 30 minutes in a hot air oven to eliminate any excess solvent. Once cooled in a desiccator, the flask is reweighed to determine the weight of the extracted fat. The percentage of fat in the sample is calculated by,

$$\text{Percentage} = \frac{\text{Weight of Extracted Fat}}{\text{Sample Weight}} \times 100$$

2.5.3. Total Ash Determination

The weight of the crucible dish is measured. Then, a 20 mL sample is added to the crucible and placed in a muffle furnace rack that has also been weighed. The temperature of the furnace is set to 500°C, and the sample is left to ash for a duration of 16 hours. Once the ashing process is complete, the ash in the crucible dish is removed and placed in a desiccator to cool. After cooling, the ash is weighed, and the percentage of ash is calculated using a specific formula. Overall, the procedure involves weighing the crucible dish, adding and ashing a sample, and calculating the percentage of ash:

$$\text{Percentage ash} = \frac{\text{Total Weight of Ash}}{\text{Sample Weight}} \times 100$$

2.5.4. Crude Protein Determination

A sample was weighed into a 500 mL Kjeldahl flask, and two tablets of the catalyst mixture and 20 mL of concentrated sulphuric acid (H_2SO_4) were added. The flask was heated in a fume cupboard until clear. The mixture was cooled, and distilled water was added. 25 mL of 1% boric acid was added to a 250 mL conical flask, and the distillation apparatus was placed under the collection spigot. Ten mL of the digest was pipetted into the stopper portion of the condenser, and 20 mL of 40% sodium hydroxide solution was added. The solution was allowed to distil for 15 minutes, and the purple solution turned green. The distillate was titrated against 1.0 M Hydrochloric Acid (HCl) to a pink color point. A blank titration was performed using 20 mL of 1% boric acid. The percentage of crude protein was calculated as,

$$\text{Gram Nitrogen (\% g N) content} = \frac{0.014 \times (X-Y) \times \text{molarity of HCL} \times \text{dilution}}{\text{Sample Weight}} \times 100$$

$$\text{Percentage crude protein} = \%\ g\ N \times 6.25$$

Where, X = volume of titre for the test sample, and Y= volume of titre for blank.

2.5.5. Crude Fibre Determination

A sample of 200 mL is measured and placed in a 500 mL beaker. It is then boiled in 200 mL of 10% (v/v) H2SO4 for a duration of 30 minutes. After boiling, the suspension is filtered, and the remaining residue is washed with boiling water until it is no longer acidic. Next, the residue is boiled in 200 mL of 0.313M NaOH for another 30 minutes. It is then filtered and washed with ethanol and diethyl ether. The resulting residue is transferred into a pre-weighed crucible and placed in a hot air oven for 30 minutes to dry. Once dried, the residue is cooled in a desiccator and reweighed. To analyze the sample further, it is ashed in a furnace at 550°C, cooled, and weighed again. Finally, the amount of crude fiber in the sample is calculated as the acid-insoluble ash percentage.

$$\text{Fibre content (\%)} = \frac{\text{Weight of Crucible + Fibre -Weight of Crucible with Ash}}{\text{Sample Weight}} \times 100$$

2.5.6. Total Carbohydrate Determination

The carbohydrate content was measured by subtracting the sum of fibre, fat, ash, protein and fibre from 100.

$$\text{Carbohydrate} = 100 - (\text{Protein} + \text{Ash} + \text{Fibre} + \text{Fat})$$

2.6. Quantification of Mineral Elements

This method determines the contents of various elements in a sample using an Atomic Absorption Spectrophotometer. The elements analyzed include sodium, potassium, phosphorus, magnesium, calcium, iron, and zinc. To analyze the elements, the samples are first digested with a mixture of perchloric acid and nitric acid.

This digestion process involves measuring 10ml of each sample into a flask and adding perchloric acid, concentrated HNO3 and concentrated H2SO4. The mixture is then gently heated on a hot plate until dense white fumes appear. After cooling, distilled water is added to the solution, which is then filtered and transferred to a volumetric flask. The absorbance of the solution is measured using a triplicate atomic absorption spectroscopy.

The concentrations of potassium, calcium, iron, zinc, sodium, lead, and cadmium are determined using this instrument. The phosphorus content, on the other hand, is determined using the Vanado molybdate method with a spectrophotometer. In summary, the text outlines a procedure for analyzing the contents of potassium, calcium, iron, zinc, sodium, lead, cadmium, and phosphorus in a sample using an Atomic Absorption Spectrophotometer and the Vanado molybdate method. The samples are digested with a mixture of acids before analysis, and the absorbance of the solution is measured to determine the concentrations of the elements.

2.7. ***Antinutrients Quantification***

2.7.1. Phytate Content Determination

The filtrate was diluted with 0.03% NH4 SCN solution and 50 mL of distilled water to achieve the desired acidity. It was then titrated against a ferric chloride solution, which contained 0.005 mg of Fe2+ per FeCl3, to obtain the equivalent. The phytate content in mg/100 g was calculated.

$$\text{Iron Equivalent} = \text{Titre Value} \times 1.95$$

$$\therefore \text{Phytic Acid} = \text{Titre Value} \times 1.95 \times 1.19 \times 3.55 \text{ mg/phytic Acid} = 8.24$$

$$\therefore \%\ \text{Phytic Acid} = \frac{y \times 8.24}{1000} \times \frac{100}{\text{Weight of Sample}}$$

Where, y = Titre Value.

2.7.2. Tannin Content Determination

A sample (50mL) was poured into a bottle containing ten millilitres of each sample. The bottles were shaken for 2 hours at 30°C, then centrifuged and stored on ice. The supernatant was pipetted into a test tube, and standard tannic acid solutions were prepared from a stock of 0.5 mg/ml. Folin reagents were added to the test sample and standards, followed by 2.5 mL of 20% (w/v) Na2CO3. The solutions were vortexed and incubated for 40 minutes at room temperature. The absorbance was read at 725nm against the sample's reagent blank concentration. The optical density of the tannic acid was plotted, and the tannin content of the samples was obtained from the standard curve.

2.7.3. Oxalate Content Determination

To conduct the experiment, 10 millilitres of each sample were measured and placed in a 100 mL conical flask. Then, 75 mL of 3 M H2SO4 was added, and the solution was stirred intermittently with a magnetic stirrer for approximately one hour. Afterwards, the solution was filtered using Watman N0.1 filter paper and a 25 mL sample filtrate was collected. The filtrate was titrated hot (at a temperature of 80-90°C) against 0.1M KMnO4 solution until a faint color appeared that persisted for at least 30 seconds.

$$\text{Oxalate (mg/g)} = W_T \times 0.904$$

Where, W_T = Titre value

2.7.4. Total Phenolic Content Determination

The process involves adding 10 mL of a diluted sample to 2.5 mL of a 10% (v/v) Folin–Ciocalteu reagent. Then, 2 mL of a 7.5% (w/v) Na2CO3 solution is added to the mixture. The resulting solution is mixed well on a vortex

vibrator for 5 minutes and incubated in the dark at ambient temperature (29°C) for 1 hour. After incubation, the absorbance of the solution is measured at a wavelength of 765 nm. The concentration of the substance in the sample is determined using a calibration curve with gallic acid as the standard. The results are expressed as milligrams of gallic acid equivalents per 100 mL of the sample.

2.8. Statistical Analysis

The study used ANOVA to analyze numerical data, separated the means by using New Duncan's Multiple Range Tests in SPSS 16.0, and errors were calculated as standard errors.

3. Results

3.1. Physicochemical Characteristics of the Laboratory Prepared "Otika"

There was a gradual decrease in pH from steeping (0 hr) at 7.20 to 6.52 after milling. Then, there was a continuous decrease as the fermentation time progressed to 4.90 after 72 hr of fermentation (Figure 2). On the contrary, TTA increased along the production process. There was an increase in the TTA from steeping (0 hr) at the value of 0.04% to 0.1% after 72hr of fermentation.

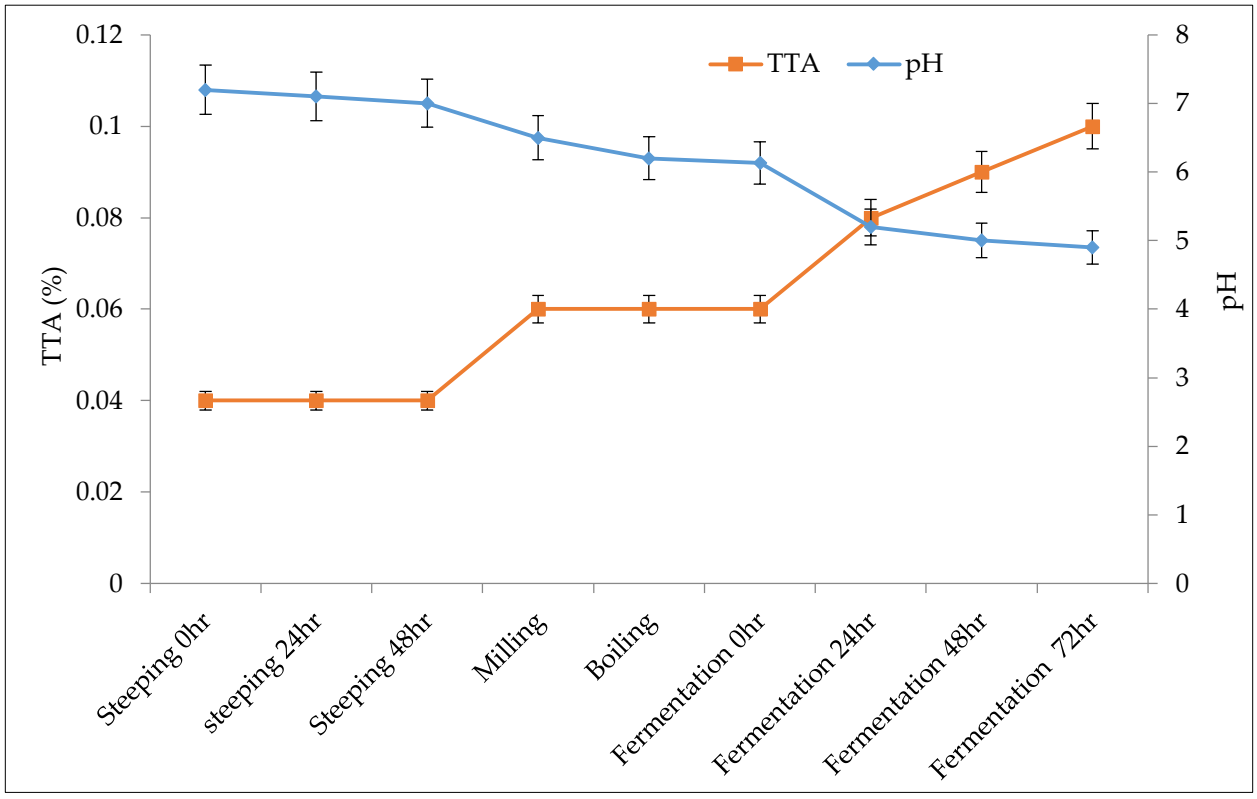

Fig. 2 pH and TTA during the production of "Otika"

The proximate composition of laboratory-prepared ''Otika'' with bacteria and yeast is shown in Table 1. The highest value with ash and moisture content of 1.15 ± 0.12%, 89.4 ± 0.66% having significant differences (P>0.05) was found with the sample containing W. cibaria while the least ash, moisture, and protein was found with the isolate S. cerevisiae. The highest protein content is found with L. plantarum with other isolates at 1.33 ± 0.05%. The carbohydrate content was found to be the highest with S. cerevisiae, 11.02 ± 0.02%, while the lowest was found with W. cibaria (8.24 ±0.6%). Fat and fibre contents were not detected in this study.

Table 1. Proximate composition of "Otika" produced from the laboratory with various isolates

Sample	Proximate Composition (%)					
	Ash	Moisture	Fat	Fibre	Crude Protein	Carbohydrates Content
A	0.67±0.12^{ab}	88.72±0.24^{ab}	0.00±0.00	0.00±0.00	1.02±0.11^{a}	9.59±0.36^{c}
B	1.15±0.12^{c}	89.46±0.66^{b}	0.00±0.00	0.00±0.00	1.15±0.05^{a}	8.24±0.61^{b}
C	0.98±0.01^{bc}	88.28±0.06^{a}	0.00±0.00	0.00±0.00	1.06±0.34^{a}	9.68±0.28^{c}
D	0.83±0.01^{bc}	88.19±0.07^{a}	0.00±0.00	0.00±0.00	1.33±0.05^{a}	9.65±0.02^{c}
E	0.32±0.24^{a}	88.03±0.01^{a}	0.00±0.00	0.00±0.00	0.88±0.00^{a}	11.02±0.02^{d}
F	0.36±0.08^{a}	93.99±0.08^{c}	0.00±0.00	0.00±0.00	1.19±0.03^{a}	4.45±0.70^{a}

Legend: A=Natural fermentation, B= W.cibaria, C= W.cibaria + L. plantarum+ S. cerevisiae, D= L. plantarum, E= S.cerevisiae, F= Control ("Otika" purchased from local manufacturer)

Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

The antinutrient composition of 'Otika' produced in the laboratory with specific bacteria and yeast is displayed in Figure 3, the lowest values of tannin and total phenol content (1.9± 0.0 mg/100g and 2.5 ± 0.00 mg/100g) were found with the culture of W. cibaria The phytate had its lowest value (3.2 ± 0.05 mg/100g) with the spontaneous fermentation of 'Otika'. In comparison, the least oxalate content (0.25 ±0.15 mg/100g) was found with the mixed isolate of W. cibaria, L. plantarum and S. cerevisiae. The highest values of all the antinutrients were found with the "Otika" containing S. cerevisiae except for phytate, the highest value (10.5 ± 0.11 mg/100g) was found with the mixed isolate of W. cibaria, L. plantarum and S. cerevisiae.

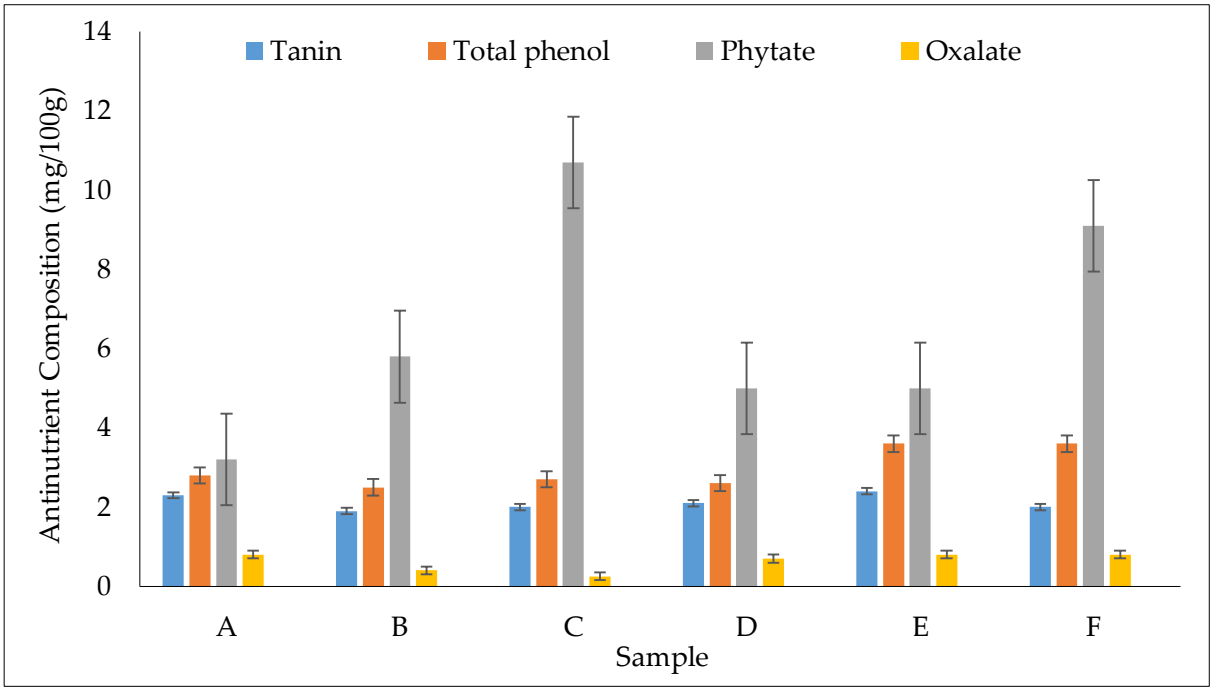

Fig. 3 Antinutrient composition of "Otika" produced in the laboratory with various isolates

A=Natural fermentation, B= W.cibaria, C= W.cibaria + L. plantarum+ S. cerevisiae, D= L. plantarum, E= S.cerevisiae, F= Control ("Otika" purchase from local manufacturer)

The mineral composition of ''Otika'' produced in the laboratory is shown in Figure 4 with various microorganisms. Potassium, magnesium, iron and zinc had their highest value with the value of 18.26 ± 0.02, 2.03 ±0.11, 0.42 ±0.14 and 2.02 ± 0.01 mg/100g, respectively, with control (the "Otika bought from a local producer) while the spontaneous fermentation, i.e. without isolates had the highest value (10.7 ± 0.03 mg/100g) with phosphorus alone. The highest value of calcium sodium and magnesium was found with "Otika" produced with W.cibaria at a value of 2.5± 0.0, 7.9 ±0.0, 2.03 ±0.11 mg/100g, respectively.

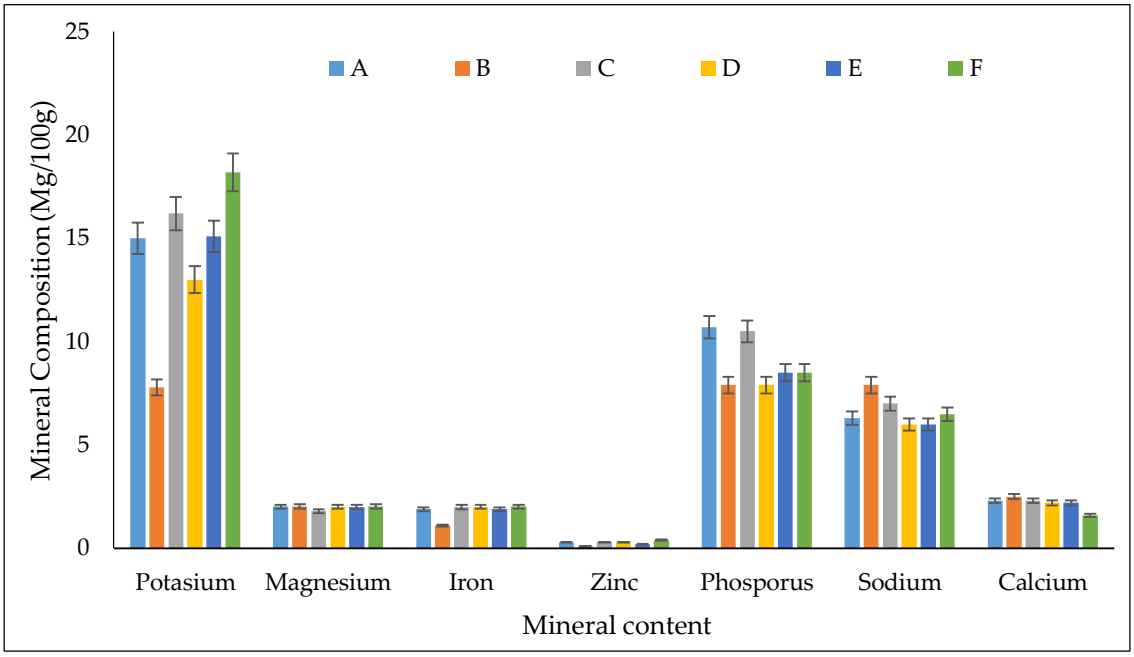

Fig. 4 Mineral composition of ''Otika'' produced in the laboratory with various isolates

A=Natural fermentation, B= W.cibaria, C= W.cibaria + L. plantarum+ S. cerevisiae, D= L. plantarum, E= S.cerevisiae F= Control ("Otika" purchase from local manufacturer)

4. Discussion

In this study, the pH of the steeped sorghum, which dropped a little on the second day, could be a result of the acid-producing microorganisms; the same was also found with the sorghum as fermentation progressed. The cause of the increase in acidity and reduction in pH during the fermentation of this cereal was likely due to the utilization of free sugars to organic acids by yeast and Lactobacillus bacteria. This research relates to the Akinleye [11] research, whose pearl millet fermentation pH was between 4.5 and 3.9. The low pH obtained during fermentation is important since bacteria, including pathogenic and spoilage microorganisms, do not survive in low pH environments, as this increases the shelf-life of the final products.

It was also observed that the total titratable acid had a slight increase with the period of fermentation. This could be attributed to the presence of lactic acid arising from the LAB during fermentation, and this result is similar to the report obtained by Adeshokan et al. [12], who said natural sorghum fermentation is mainly lactic acid produced by Lactobacillus sp. This could explain the apparent increase in lactic acid towards the end of fermentation.

The analysis of the proximate composition of beverages revealed the basic chemical and nutritional composition, including moisture, ash, crude fat, protein, crude fibre, and carbohydrate, which are crucial for assessing the food's nutritive quality. Moisture content is crucial for food maintenance and is a widely used

measurement for processing and shelf life. Ash composition is crucial for assessing mineral content in food samples, while protein is essential for body growth, repairs, and maintenance, making functional foods and nutraceuticals essential for supplementation. The addition of Lactobacillus plantarum in "Otika" increased protein content. This increase may be attributed to microbial growth during the fermentation process and the action of extracellular enzymes such as protease produced by the fermenting microorganism. The increase in microbial biomass may have also contributed to the increase in protein content. In comparison, the "Otika" without any addition of microorganisms did not show an increase in protein content. Some microbial strains have been found useful for protein enrichment of foods since no source of nitrogen was applied in this research; this could be due to the conversion of plant proteins or other nitrogenous compounds into microbial protein. Adebawo et al. [13] had earlier reported the increase in lysine content in the 'Ogi' produced by genetically engineered Lactobacillus plantarum. Mbata et al. [14] also reported that fermented cereal gruels have higher lysine, arginine, and methionine contents under laboratory-adapted conditions. The reduction carbohydrate content of "Otika" inoculated with starter cultures relative to the uninoculated beverage is probably due to the breakdown of some starch and sugar components by increased activity of alpha-amylase, which hydrolyses starch to simple sugar. It is a known fact that LAB increases food palatability and improves the quality of food by increasing the availability of proteins and vitamins [15, 16].

The antinutrients (total phenols, tannins, and phytic acid) are known to precipitate nutrients, and they are reduced during germination [17]. Germination (sprouting of the seeds) induces hydrolytic enzymes which might have indirectly resulted in contributing to the reduction in the antinutritional components. From this study, lower values of antinutrients were observed in 'Otika' produced with the use of W.cibaria compared to "Otika" produced with mixed starter cultures.

The findings from this study reveal that "Otika" brought from the local producer and W. cibaria had most of the higher mineral contents compared with the single starters. Mineral elements are important because they are essential for regulating and building up living cells and fighting depression. Calcium is essential for building the bones that make the human body balanced; it promotes a healthier cardiovascular system that helps maintain the volume of water necessary for life processes [18]. Magnesium helps keep the muscles relaxed and helps the formation of strong bones and teeth. Zinc is involved in the normal functioning of the immune system and is associated with protein metabolism [19]. It helps control blood pressure and the nerve transmitter. Iron is an important element that is necessary for the heamoglobin of the red blood cell and myoglobin in the muscle [20]. The increase in the mineral values of 'Otika' produced by the local vendor could be a result of the co-metabolism that existed between the microbes because a portion from the previous fermented brew, which serves as a starter, is usually initiated into the new batch. (Guessan et al. [21] stated that the coexistence between the microorganisms in gowe production from sorghum increases the nutritional properties. Also, according to the work of Lacaze et al. [22] The Strains of Weissella cibaria utilized as starter cultures for the production of wheat and sorghum sourdoughs synthesized EPS (0.08 to 0.8%). They increased the quality, shelf life, nutritional value and machinability of rye, wheat, and gluten-free breads.

5. Conclusion

The use of the W.cibaria strain as a single starter culture in the fermentation process of "Otika" helped to raise the nutritive value among other microorganisms that served as starter cultures. Summarily, the data obtained in this work will be useful for the producer of this beverage in the formulation of starters.

Conflicts of Interest

The writer affirms that she is impartial.

Authors' Contributions

OB conceptualized and planned the study and carried out the research. The draught, review and editing were written by OB. The article was read and approved by this same author.

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