



Production, Nutritional and Microbiological Evaluation of *Mahewu* a South African Traditional Fermented Porridge

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Authors' contributions

This work was carried out in collaboration between both authors. Author IFF designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OOS managed the analyses of the study, performed the statistical analysis and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the microorganisms associated with laboratory production of *Mahewu* and to evaluate its nutritional property.

Study Design: Completely randomized design (CRD) was used with fermentation time considered as treatments and levels of microorganisms as factors.

Place and Duration of Study: All works were carried out in the Department of Microbiology, Faculty of Science, University of Ibadan, from May 2013 – January 2015.

Methodology: The Microbiological evaluation was carried out using the culture dependent method. Physicochemical properties were studied using a pH meter and titratable acidity was determined using titrimetric method. Enzymatic assessment was carried out using Dinitrosalicylic acid method (DNSA) with the aid of a spectrophotometer. Nutritional analysis was determined using the Association of Analytical Chemist (AOAC, 2005) method and atomic absorption spectrophotometer, while sensory properties were carried by panel evaluation.

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Results: The study revealed that the microorganisms predominantly associated with the production of *mahewu* were yeast and lactic acid bacteria. The physicochemical study showed that an inverse relationship occurred between the pH and titratable acidity. The amylolytic activity was significantly higher ($P<0.05$) at the beginning of the fermentation process but declined towards the end. The moisture content increased significantly from 14.80% in the raw maize to 85.50% in *mahewu* while the protein, fat, ash, fibre and carbohydrate contents decreased significantly ($P<0.05$) from 11.00%, 4.83%, 1.55%, 1.10% and 66.72% respectively in raw maize to 9.21%, 2.02%, 1.03%, 0.83%, and 63.01% respectively in the produced *mahewu* sample. Similarly, the mineral contents analysis showed that sodium, potassium, calcium, iron, zinc and manganese contents decreased significantly ($P<0.05$) from 0.058 ± 0.01 mg/kg, 0.109 ± 0.03 mg/kg, 0.062 ± 0.02 mg/kg, 2.555 ± 0.01 mg/kg, 0.104 ± 0.03 mg/kg and 0.700 ± 0.08 mg/kg respectively in raw maize to 0.051 ± 0.00 mg/kg, 0.82 ± 0.01 mg/kg, 0.55 ± 0.01 mg/kg, 1.963 ± 0.06 mg/kg, 0.911 ± 0.09 mg/kg and 0.528 ± 0.01 mg/kg in the produced *mahewu* sample. The product was highly accepted by the consumers as indicated by the result of sensory evaluation.

Conclusion: The produced *Mahewu* was highly nutritious with good consumer's acceptability and the microorganism involved could serve as potential starter cultures.

Keywords: *Mahewu*; production; microbiological; nutritional evaluation; enzymatic assay; non-alcoholic; fermented porridge.

1. INTRODUCTION

Fermented foods are products obtained from the enzymatic modification of the substrate by the associated microorganisms to bring about desired biochemical changes. They form a considerable part of diet in many African countries and developing world. The art of fermentation can be used to extend the shelf-life of food substances and introducing new variety of foods into the diet of Africans and the oriental world [1,2]. It plays significant socio-economic role in the developing world. Examples of fermented food include fermented starchy roots, cereals, alcoholic beverages, vegetable proteins and animal protein [3,4]. They often possess pleasant flavour, aroma, texture, palatability and high nutritive values which make them to be highly acceptable to consumers [5]. The microorganisms associated with the fermentation of food imposed properties such as sensory characteristics, nutritional quality and high digestibility of fermented food constituents [6]. Traditionally fermented foods prepared from maize are well documented such as *ogi*, [7- 9] Blandino et al., *uji* and *kenkey* [7,10]. In some African communities they are used as weaning foods and in social functions such as marriage and naming ceremonies, they are served as inebriating drinks [11]. *Mahewu* a fermented non-alcoholic maize-based beverage is a popular drink taken in South Africa [12]. The spontaneous fermentation process during *mahewu* production is carried out by the natural flora of the malt at ambient temperature [13]. It is highly refreshing and consumed in Africa, some

Arabian Gulf countries schools, farms, mines by the adult and also used as weaning food [14]. It is known by various names in South Africa, in Zulu it is called *amahewu*, the Xhosas identify it as *amarehewu*, [15] the Swazis, know it as *emahewu* [5] the Pedis, call it '*metogo*' [16], while the Sothos, named it '*machleu*' [5]. *Mahewu* have been reported by several authors to exhibited bacteriostatic and bactericidal properties against enteric pathogens confirming its health benefits [17,18]. The wide consumption of this product by different age groups in various ethnic regions is well documented. Therefore the general objective of this present work is to produce *Mahewu* in the laboratory while the specific objectives are to investigate the microorganisms involved, physicochemical properties, nutritional quality, enzymatic activity and organoleptic attributes, during and after production.

2. MATERIALS AND METHODS

2.1 Sample Collection

Western white Maize grains (*Zea mays*) and wheat grains were purchased from Bodija central Market in Ibadan, Oyo State Nigeria and were brought into the laboratory in polyethylene bags for immediate use.

2.2 Laboratory Production of *Mahewu*

Mahewu was produced using the modified method of [20] as illustrated in Fig. 1. Eighty

grams of maize meal was soaked into 1000 ml of warm water in 2L Erlenmeyer flask (ratio 8:100 w/v) and then boiled for 15 minutes. It was cooled to room temperature and wheat flour (5% of the maize meal) was added as a source of inoculum and allowed to ferment for 36 hours at ambient temperature.

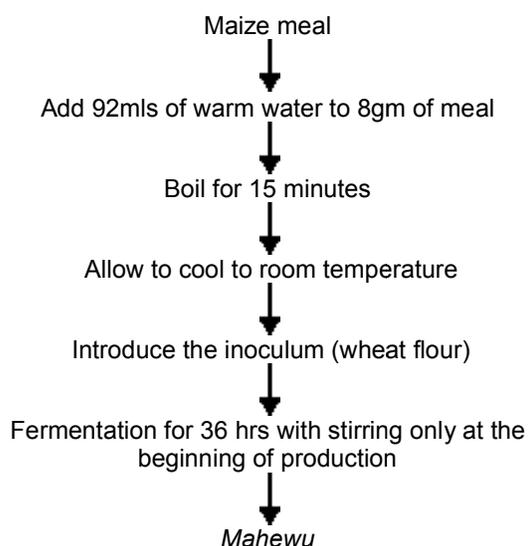


Fig. 1. Flow chart diagram for the traditional preparation of *Mahewu* [19]

2.3 Isolation of Microorganisms

Ninety ml was taken from the fermenting maize meal sample and diluted using the method of [20]. Dilutions 10^{-6} and 10^{-8} were differently plated on sterile petri dishes containing sterilized molten agar of de Mann Rogosa Sharpe agar (MRS) and Malt extract agar (MEA). The MRS plates were incubated anaerobically at 37°C for 24-48 h while the Malt extract agar plates were incubated aerobically at 30°C for 5 days. Pure cultures were stored on agar slants in McCartney bottles and kept in the refrigerator. The isolation procedure was limited two microorganisms which had been previously reported to dominate the natural fermentation of cereals.

2.4 Identification Procedure

Identification of the Lactic acid bacteria was carried out using API 50CH strips and 50CHL medium. (API system, Montalieu, Vericeu, France) while the yeast isolates were identified based on the method described by [21].

2.5 Physico-Chemical Changes during the Fermentation of Maize for *Mahewu* Production

2.5.1 pH measurement

Ten mls of the fermenting gruel was aseptically transferred into sterile bottles and pH was taken using a pH meter (Jenway model).

2.6 Total Titratable Acidity (TTA)

Total titratable acidity was evaluated according to the method of [22] by titrating 10ml of the filtrate against 0.1 M NaOH using phenolphthalein (3drops) as indicator. The acidity was calculated as % (w/v) lactic acid equivalent.

2.7 Enzyme Assay during Production of *Mahewu*

2.7.1 Enzyme extraction

Centrifugation of the sample was carried out 5000 rpm at 4°C for 30 minutes; the supernatant obtained was decanted and used for enzyme assay.

2.8 Amylase Assay

This was carried out based on the dinitrosalicylic acid (DNSA) method described by [23]. The amylolytic activity was measured using a spectrophotometer set at 550 nm. The amount of reducing sugar was calculated from a standard curve constructed from different glucose concentrations. One unit of the enzyme activity is equal to the amount of enzyme that releases reducing sugar corresponding to 1 mg of glucose per min

2.9 Nutritional Analysis

2.9.1 Moisture content determination

This was measured by weighing 2 g of sample into a pre-weighed moisture can and placed in an oven at 80°C for 24hours to dry to a constant weight [24]. The moisture content was calculated by subtracting the initial weight from the final weight.

2.10 Ash Content Determination

Five milliliters of the produced *Mahewu* sample were transferred into pre-weighed porcelain

crucible and weighed. The muffle furnace was set at 600°C for 6 hours and used to remove the all organic materials present in the sample placed in the crucible. The crucible was placed in a dessicator to cool and reweighed to determine the ash content [24].

2.11 Crude Fat Estimation

The fat content was determined by weighing two grams of dried sample into a flat bottom flask and extracted by adding an anhydrous diethyl ether. The solution obtained was boiled for 4h to evaporate the ether. The distilled fat was dried in an oven set at 80°C for 30 mins and weighed to obtain the fat content [24].

2.12 Crude Protein Determination

The crude protein content was quantified by digesting two grams of the sample with sulphuric acid and the reaction was catalysed by adding Kjeldahl tablets. Concentrated sodium hydroxide solution was added to make the solution alkaline. This was gradually distilled into a conical flask containing boric acid and methyl red indicator until the solution turned green. The solution was titrated against 0.01N hydrochloric acid and the appearance of a wine colorations indicated the end point. The quantity of crude protein was obtained by multiplying the % nitrogen by 6.25 [24].

2.13 Carbohydrate Content

This was calculated using the formular below:

$$\%CHO = 100 - (\%Moisture\ content + \%Protein + \%crude\ fiber + \%Fat + \%Ash\ content)$$

The above mentioned parameters were carried out in triplicates and expressed in percentage.

2.14 Determination of Mineral contents

The mineral analyzed were calcium, magnesium, potassium, sodium, iron and zinc. Determination was carried out in triplicates using atomic

absorption spectrophotometer (SP.191 Pye Unicam Spectrophotometer).

2.15 Sensory Evaluation of Mahewu

Mahewu sample was subjected to assessment by a 5 member panel; each panellist was instructed to taste the sample to indicate their degree of likeness on the questionnaire provided. The sample was evaluated for taste, aroma, appearance and overall acceptability. Each parameter was scored on 5 point hedonic scale ranging from dislike extremely (5) to like extremely (1).

2.16 Statistical Analysis

The data generated were subjected to ANOVA to determine significant difference between the means and this was expressed as \pm standard deviation (SD). The degree of freedom was put at $P \leq 0.05$. The SPSS version 17.0 was employed in the data analysis.

3. RESULTS

Species of lactic acid bacteria and yeasts isolated from fermenting maize meal during the production of *Mahewu* were identified as *Lactobacillus brevis*, *Lactobacillus casei*, *Lactococcus lactis* and *Lactobacillus plantarum* while the identities of the yeasts isolates were confirmed as *Saccharomyces cerevisiae* and *Saccharomyces pombe* (Table 1). *Saccharomyces cerevisiae* showed the highest occurrence of 70% followed by *L. brevis* with 54% and the least was recorded by *L. plantarum* 15%.

The result of Changes in pH and TTA of the fermenting maize meal during the production of *mahewu* is represented in Table 2. It was observed that the highest pH (6.75) was recorded at 0h which decreased to 3.30 at 36 hours with a corresponding TTA of 0.09% at 0h which increased to 1.35% at the end of the fermentation.

Table 1. Percentage occurrence of Microorganisms associated with fermentation of Mahewu

Lactic acid Bacteria		Yeast	
Species	% of occurrence	Species	% of occurrence
<i>L. brevis</i>	54	<i>S. cerevisiae</i>	70
<i>L. casei</i>	23	<i>S. pombe</i>	30
<i>L. plantarum</i>	15		
<i>L. lactis</i>	8		

Table 2. Changes in pH and TTA of the fermenting maize meal

Time	pH	TTA (%)
0	6.75±0.22a	0.09± 00.1a
12	4.74±0.25b	0.19±0.047b
24	3.78±0.18c	0.23±0.065c
36	3.30± 0.34d	1.35±0.11d

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

An amylase activity of 8.41±0.5 ug/ml/min was recorded at 12h which increased to 20.97±1.5

ug/ml/min and finally decreased to 15.10±1.5 ug/ml/min at 36h (Fig. 1).

The result of the proximate analysis is displayed in Fig. 2. It was observed that the moisture content increased from 14.80±0.01% in the raw maize to 85.50±0.02% in the produced *Mahewu* sample. Contrarily, the % protein, fat, ash, fiber and carbohydrate contents decreased significantly from 11.00±0.02, 4.83±0.04, 1.55±0.01, 1.10±0.03 and 66.72±0.02 in the raw maize respectively, to 9.21±0.05, 2.02±0.02, 1.03±0.03, 0.83±0.00 and 63.01±0.01 respectively in the produced *Mahewu* sample.

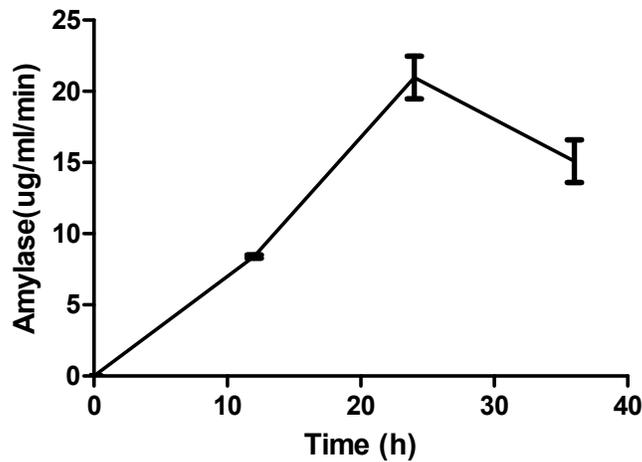


Fig. 1. Changes in Enzymes activity during *Mahewu* production

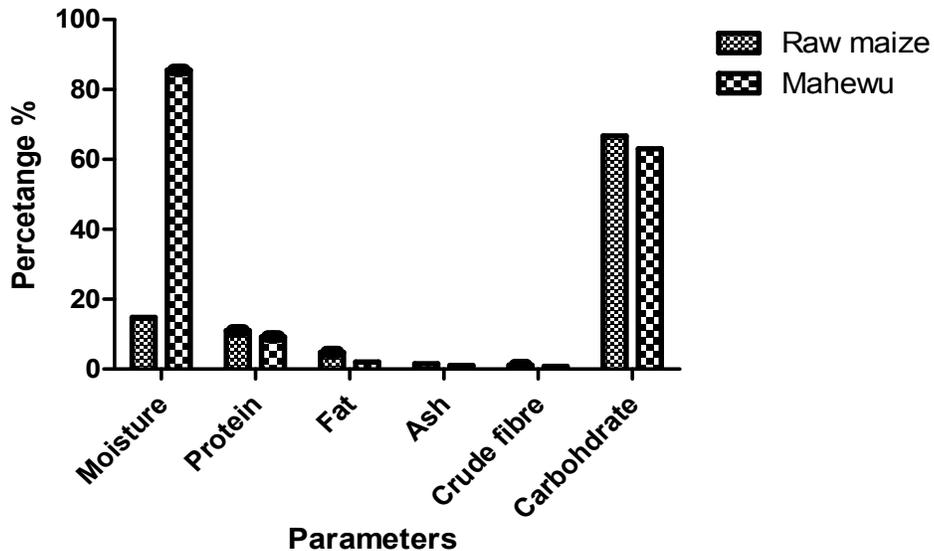


Fig. 2. Proximate analysis of "*mahewu*"

The sodium, potassium, calcium, iron, zinc and manganese contents decreased significantly from 0.058±0.01 mg/kg, 1.09±0.03 mg/kg, 0.062±0.055 mg/kg, 2.555±0.01 mg/kg, 1.04±0.03 mg/kg and 0.700±0.08 mg/kg respectively in raw maize to 0.051± 0.02 mg/kg, 0.82±0.01 mg/kg, 0.055±0.01 mg/kg, 1.963±0.06 mg/kg, 0.911±0.09 mg/kg and 0.528±0.01 mg/kg in the produced *Mahewu* sample (Fig. 3).

The taste attribute had the highest score of 3.95±0.77 followed by aroma and overall acceptability scoring 3.90±0.25 and 3.90±0.53 respectively while the appearance was scored 3.00±0.21 (Fig. 4).

4. DISCUSSION

The occurrence of LAB and yeast species in maize fermentation had earlier been documented

by [25]. Also Teniola and Odunfa [26] and [13] reported the isolation of *S.cerevisiae* from *kenkey*, *koko*, *ogi* and *munkoyo* production. However, Jespersen et al. [27] reported the high occurrence or the dominance of *Saccharomyces cerevisiae* in African indigenous fermented foods and beverages.

The fermentation of “*Mahewu*” in this study was characterized by a fall in pH and a corresponding rise in TTA. Agarry et al. [28] reported similar changes in spontaneous fermentation of maize and millet.

The observed decrease in pH during the fermentation process of *Mahewu* indicated that lactic acid and other organic acids were produced. This occurrence had been previously reported by several authors [29-32]. The production of organic acid was

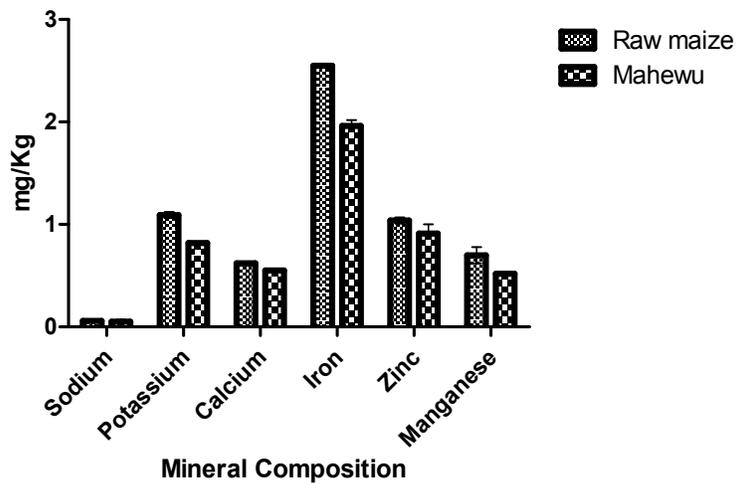


Fig. 3. Mineral Composition of “*mahewu*”

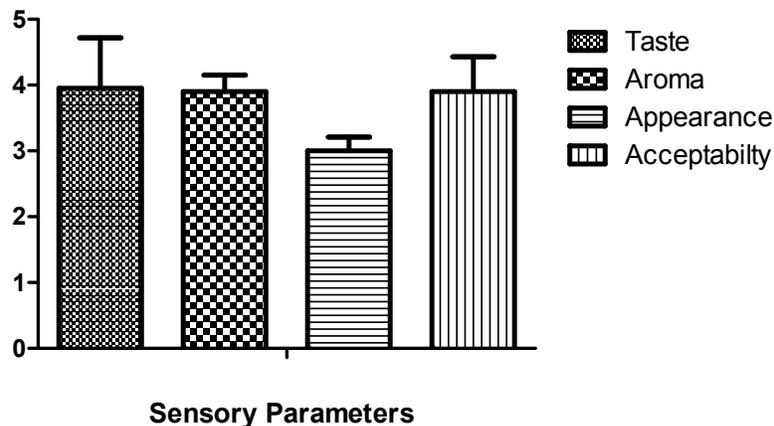


Fig. 4. Sensory evaluation of *mahewu*

probably due to microbial activities, degrading some of the carbohydrate content into organic acids, which led to the fall in pH [5,33,34]. The observed increase in the titratable acidity was previously reported by Annan [35,36] this occurrence might probably be caused by the effect of fermentation. The inverse proportional trend between pH and titratable acidity observed in this study was similar to the finding of [11]. It was equally observed that the variation in the two parameters (i.e. pH and TTA) was very rapid and significant; this signified a good rate of fermentation process [32]. In addition, Odunfa and Adeyele [29], reported that the occurrence of un-dissociated forms of organic acids at low pH could inhibit a broad spectrum of pathogens thus improving the microbiological stability of the food product [9,37]. According to Gadaga et al. [13], most pathogens are not able to survive at low pH, this is advantageous to the product in terms of storageability and shelf life. Significantly higher amylolytic activity was observed in the early stage of fermentation in this work, similar report had earlier been documented during *tempeh* and pearl millet fermentation [30,38]. This observation showed that the performances of α and β amylases enzymes were high probably due to high pH values [30] which favours enzymatic reactions. The observed reduction in the amylase activity toward the end of the fermentation period had earlier been reported [30,38] and reasons such as low pH and low substrate concentration recorded at the end of the fermentation period may be adduced for this occurrence [30]. Low pH i.e. increasing acidity tends not to support high enzymatic activity. In addition aerobic microorganisms such as *Saccharomyces cerevisiae* is associated with high amylolytic activity [39] and this microorganism was seen to be predominant in the fermentation of *Mahewu*.

The increased moisture content of the produced *Mahewu* emanated from the soaking exercise. High moisture content in food had been reported to reduce the period of storage, while low moisture content increased storage period because of low water activity [40,41] which does not favour microbial proliferation. The protein content in the produced *Mahewu* was noted to decrease significantly when compared to raw maize. Similar observations had been previously reported in studies conducted on fermented cereal-legume food mixture by action of bacteria and yeast [31,42]. According to the submissions of report of Sharma [43] protein content decreased during the fermentation process of

pearl millet with *L. acidophilus*. The observed reduction of protein content during fermentation could probably emanated from an increase in protein catabolism by the fermenting microflora which led to the generation of by-product (metabolic deamination) for example ammonia. Furthermore, Asiedu et al. [44], reported that low protein content observed in fermented food could be due to the effect of solid water ratio and fermentation technique. Ejigui et al. [31], revealed that reduction in protein content could not be linked to the metabolic activities of LAB which confer nutritional benefit on the food produced, but could be due largely to difference in cultivar of maize and the condition surrounding the fermentation process step, such as washing. However there are conflicting results on the protein content of maize during fermentation from different authors. Ejigui et al. [31], observed increase in the protein content during traditional fermentation of pearl millet. Reason such as decrease in other constituents of maize resulting from utilization of maize starch for metabolic activity could be responsible for the increase in protein content. Also noticed is the reduction in ash content of *Mahewu*. This observation is similar to the submissions of [44,45] during the fermentation of cassava for fufu production and Ejigui [31], during traditional fermentation of yellow maize. However the increased ash content reported by [46] was contrary to the submission of the authors mentioned above. Reasons such as the effect of fermentation that caused reduction in the level of anti-nutrient such phytate and oxalate could have led to increase in the bioavailability of mineral element, also the level of ash content, is a reflection of the total available minerals [46].

There was a significant ($P < 0.5$) decrease in fibre content of *Mahewu* when compared to raw maize. This finding is in agreement with the report of [47]. The reduction of fibre content is an indication of low digestibility of nutrients and net energy production. It was also observed from this study that the fat content decreased significantly in the *Mahewu* sample produced. Reduction in fat content was observed by [32,48] during the production of *Doku*. The utilization of oxidised lipids to generate energy for growth and cellular activities by the fermenting microorganisms might have led to decrease in fat content [21, 49].

The starch content of the *Mahewu* produced was significantly lower than the value obtained in raw maize. This recorded observation was in

conformity with the reports of [30,50]. In addition the indigenous microflora associated with the natural fermentation of maize has been reported to be amylolytic thereby causing high hydrolysis of starch to simple molecules [50]. Reports by Chavan and Kadam [14] and [19] revealed that generally, fermentation of cereals leads to decrease in the level of carbohydrates.

The observed decrease in the mineral contents of the produced *mahewu* in this study is in agreement with the submissions of [30,51]. Reason such as soaking process which the maize seeds were subjected to during production of *Mahewu* might be responsible for this loss. *Ebirien et al.* [51] and [52], reported that there was increased leaching of minerals from the germ and endosperm of cereals during soaking despite their low level of occurrence in cereals. Mineral elements are important because they are essential for regulating and building the living cells and aid in fighting depression.

From the result of the sensory evaluation carried out on the produced *Mahewu*, it could be inferred that it well acceptable to the consumers in terms of appearance, taste, aroma, overall acceptability. The high consumers acceptability observed could be due to the activities of the fermenting microorganisms which have been reported to synthesis some compounds such as diacetyl which improves flavour enhancement and some enzymes which brings about bioavailability and digestibility of the nutrients present in food substance [39].

Vogel et al. [53], reported that Spontaneous fermentation has been used for the production of fermented foods based on the microflora present in the raw material and the quality of end-product was dependent on the types and number of microorganisms present in the raw material.

5. CONCLUSION

The produced *Mahewu* was highly nutritious with good consumer's acceptability and the microorganism involved could serve as potential starter cultures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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