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# Microorganisms Associated with the Fermentation of Gari Fortified with Sprouted Mung Beans Flour

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***Authors' contributions***

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

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## ABSTRACT

Gari' is consumed regularly by large populations of Nigeria, therefore, it is an excellent means of improving the diet of people through fortification with nutritionally rich foods hence, this study aim to produce 'Gari' by co-fermenting cassava mash and sprouted mung bean flour in different ratios in order to assess the effect on the nutritional composition as compared to a standard produced without sprouted mungbeans (SMF) flour fortification. In different ratios, composites of cassava mash (CM) and the sprouted mungbeans flour (SMF) were made (5% SMF + 95% CM, 10% SMF + 90% CM, 15% SMF + 85% CM; and 100% CM which serve as the control) and fermented using semi-solid state fermentation for four days. The Gari was then produced following the standard method of dewatering, sieving and roasting. Isolation of microorganism during the fermentation was carried out using standard microbiological techniques and identification was done using conventional and molecular techniques. The total bacterial (cfu/ml) of the fermented samples increased during the first day of fermentation, and reduced on subsequent days in all samples with the bacterial count ranging from 1.21 x$10^5$ CFU/g to 2.45 x$10^5$ CFU/g. The organisms isolated include *Lysinibacillus alkalisoli*, *Proteus mirabilis*, *Pediococcus acidilactici, Lactobacillus plantarum, Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Penicillium sclerotiorum, Diutina*

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*catenulate, Rhizopus stolonifer*, *Trichoderma viridae*, *Saccharomyces cerevisiae.* Most of the organisms isolated during the first and second day of fermentation such as *Proteus* spp*, Staphylococcus* spp*, Bacillus* spp*, Bacillus* spp*, Diutina* spp and *Trichoderma* spp, later disappeared toward the later days of the fermentation. Lactic acid bacteria, mold and yeast isolates such as *Lactobacillus plantarum, Pediococcus spp. Penicillium spp* and *Saccharomyces cerevisiae* dominate toward the later stage of the fermentation in all the fermented samples. Molecular identification of the bacterial isolates shows that *Lysinibacillus alkalisoli*, *Proteus mirabilis*, *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* were present in the samples. The outcome of this research showed that co-fermentation of cassava mash and sprouted mung bean flour eliminates pathogenic microorganisms and encourages the growth of beneficial microorganism in mung bean fortified Gari production.

*Keywords: Mung beans; gari; nutrition; dewatering; Nigeria.*

## 1. INTRODUCTION

In the microbiological sense, fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as moulds, yeasts, or bacteria. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (and in the case of food, more easily digestible) compounds and nutrients. For example, the enzyme protease breaks down huge protein molecules first into polypeptides, then into numerous amino acids, which are readily absorbed by the body. Fermentation also, according to Sandor [1], also creates new nutrients. As they go through their life cycles, microbial cultures create B vitamins, including folic acid, riboflavin, niacin, thiamine, and biotin. Ferments have been credited with creating vitamin $B_{12}$, otherwise absent from many plant-source foods.

Cassava (*Manihot esculenta Crantz*) is a staple of choice across cultures and social divides in Nigerian households. The majority of the tuber produced is consumed locally as traditional meals. It is the most important crop by production, and the second most important by consumption [2]. Africa produces over 54% of the world's cassava, with Nigeria taking the global lead with a production of about 54.8 million MT in 2014 [2].

Gari is one of the most popular foods in Nigeria, as well as in West Africa. It is a fermented, gritty, starchy food with slightly sour taste. Gari or cassava flakes is a rich source of carbohydrate, made from fermented, gelatinized fresh cassava tubers. This staple food is derived from cassava tubers through a processing method that includes soaking the tubers in water, grinding, fermenting and frying.

The problems with consistent consumption of unfortified, traditionally fermented gari and other cassava products stem from their poor nutritional value [3] and potential toxicity [4]. Gari is rich in calories but have very low protein, fat, and micronutrient contents. Diets made from low protein gari can predispose consumers to protein-energy malnutrition with compromised kidney functions [5]. Moreover, protein-energy malnutrition and micronutrient deficiencies constitute the most dreaded nutritional problems faced in developing countries [3].

In order to provide the body with all nurients it requires, foods are often combined or a particular food is fortified/nutrified with the nutrient(s) it lacks in other to make the nutrient(s) available for the body utilization.

The mung bean (*Vigna radiata* (L.) is a major edible legume seed in Asia (India, South East-Asia and East Asia) and is also eaten in Southern Europe and in the Southern USA. The mature seeds provide an invaluable source of digestible protein for humans in places where meat is lacking or where people are mostly vegetarian [6]. Mung beans are cooked fresh or dry. They can be eaten whole or made into flour, soups, porridge, snacks, bread, noodles and ice-cream. Split seeds can be transformed into dhal in the same way as black gram or lentils. Mung beans can be processed to make starch noodles (vermicelli, bean thread noodles, cellophane noodles) or soap. The sprouted seeds ("bean sprouts" in English, and incorrectly called "germes de soja" or "pousses de soja" in French) are relished raw or cooked throughout the world.

The immature pods and young leaves are eaten as a vegetable [7].

## 2. METHODS

### 2.1 Collection of Samples

Fresh and healthy cassava tubers (*Manihot esculenta*) used were purchased from Oja oba, Market, Ilesha while the Mung bean was bought from Ortese market in Gboko, Benue State.

### 2.2 Preparation of Samples

Extraneous particles like stones and metals were hand-picked from the beans. Broken kernels and wizened seeds were also removed. The seeds were soaked in clean water for eight (8) hours at room temperature. The soaked seeds were removed from the water and spread on trays which were then covered with moist towel. The trays were placed in a dark room for 3 days to allow sprouting of the seeds. After sprouting, the seed coats were removed and the seeds oven-dried and ground into flour to give the sprouted Mungbeans flour (SMF). The flour was stored in a polythene bag and sealed.

The cassava roots were peeled to remove the outer brown skin and the inner thick cream layer and washed using a plastic scourer and clean water to remove stains and dirt. The cleaned cassava tubers were then grated using a motorised grater with a stainless steel grating drum.

### 2.3 Preparation of Composites of Cassava Mash and Mungbeans Flour

In different ratios, composites of cassava mash (CM) and the sprouted mungbeans flour (SMF) were made. The mungbeans flour was worked into the cassava mash to produce homogenous composites as follows:

5% SMF (100 g SMF + 1900 g CM); 10% SMF (200 g SMF + 1800 g CM)

15% SMF (300 g MBF + 1700 g CM); 0% SMF (0 g SMF + 2000 g CM) (Control)

### 2.4 Fermentation of Samples

Each sample was produced in duplicates and put in separate polythene sacks, tied, and fermented. The samples were fermented using one of the methods described by (8). The bags containing the samples were put on a rack, to allow the milky water to drain freely from the bags and for the samples to ferment for 4 days. The bags were then removed from the rack and pressed with hydraulic press for a day to get rid of the rest of the milky water. The bags were removed when the water stopped dripping from the bags. The resulting wet cake was then sieved into smaller pieces known as grits, which were then roasted separately on a hot frying tray to form the final dry and crispy product. The frying tray was wiped clean after each sample was roasted to prevent the carrying of particles of one sample to another.

### 2.5 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe agar was used to isolate lactic acid bacteria. Techniques were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. De Man Rogosa sharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Akinrotoye [9].

### 2.6 Molecular Identification of Bacteria

#### 2.6.1 Isolate

Extraction of DNA using CTAB method was done according to (10), PCR analysis was run with a universal primer for fungi called 1TS1 and ITS4 and bacteria which was run with a universal primer called 16S rRNA. The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques.

### 2.7 Determination of pH and TTA

The pH of all fermenting samples was determined at 24 hours interval using a pocket size pH meter. A 1 g of the sample was dissolved in 10 ml of distilled water and filtered. The pH meter was calibrated with buffer solutions of pH 4, 7 and 9, this was followed by dipping the electrode of the pH meter into the sample solution and the observed pH was read and recorded in triplicates. The total titratable acidity of the fermenting samples was determined at 24

hours interval. A 2 g of macerated sample was weighed into a beaker. 20 ml of distilled water was added to it, it was mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added into it. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre value was read. Total titratable acidity was expressed as percent (%) lactic acid. The acidity was calculated as: TTA= Titre value × 9 mg/100. The pH and TTA of the samples were carried out according to the method described by [11].

## 3. RESULTS

### 3.1 Types of Microorganisms Isolated from Mungbeans Fortified and Unfortified Gari Samples during Fermentation

The organisms isolated from Mungbeans Fortified and Unfortified Gari samples during fermentation include *Lysinibacillus* spp, *Proteus* spp, *Pediococcus* spp, *Lactobacillus plantarum*, *Staphylococcus* spp, *Bacillus* spp and *Bacillus* spp for bacteria (Table 1) while the fungi isolate *Penicillium* spp, *Diutina* spp, *Rhizopus stolonifer*, *Trichoderma* spp and *Saccharomyces cerevisiae* (Table 2.)

### 3.1 Microbial Load of Microorganisms Isolated During Fermentation

The microbial load was observed throughout the four days of fermentation. The total bacterial (cfu/ml) of the fermented samples increased during the first day of fermentation followed by subsequent reduction in all the samples with the bacterial count ranging from 1.21 $x10^5$ CFU/g on the last day of fermentation in sample D to 2.45 $x10^5$ CFU/g on the first day of fermentation in sample C (Table 3). The yeast count (CFU/ml) reduces in count throughout the fermentation duration in all the samples ranging from 0.45 $x10^5$ SFU/g on the last day of fermentation in sample B to 2.16 $x10^5$ cfu/g on the first day of fermentation in sample D (Table 4). However, the total lactic acid bacteria increase in number throughout the fermentation duration in all the samples (Table 5).

### 3.2 Molecular Identification of Microbial Isolates

Molecular identification of the bacterial isolates are shown in Table 6. The sequence obtained was analyzed with the Basic Local Alignment Search Tool, BLAST, in National Centre for Biotechnology Information (NCBI) database. Based on the 16SrRNA sequences, the bacteria *Lysinibacillus* spp, *Proteus* spp, *Pediococcus* spp, *Lactobacillus plantarum*, *Staphylococcus* spp, *Bacillus* spp and *Bacillus* spp were confirmed to be *Lysinibacillus alkalisoli* , *Proteus mirabilis*, *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* while the fungi isolate *Penicillium* spp, *Diutina* spp, *Rhizopus stolonifer*, *Trichoderma* spp and *Saccharomyces cerevisiae* were confirmed to be *Penicillium sclerotiorum*, *Diutina catenulata*, *Rhizopus stolonifer*, *Trichoderma viridae* and *Saccharomyces cerevisiae.*

### 3.3 pH and Titratable Acidity of Fermented Broth Cultures

The pH of the fermented samples was found to decrease on daily basis with the highest pH on day 1 and the lowest on the last day of fermentation in all the samples (Table 7.). The titratable acidity (TTA) of the fermented broth cultures was found to increase on daily basis with the least TTA observed on day 1 and the highest on the last day of fermentation in all the samples (Table 8).

## 4. DISCUSSION

This study aimed to produce Gari by co-fermenting cassava mash and sprouted mungbeans flour in different ratios in order to assess the microorganisms associated with the fermentation process. Since the major constituents of cassava and mungbeans are proteins, fats and carbohydrates, the organisms responsible for its fermentation must be capable of utilizing these three constituents. Most of the organisms isolated during the fermentation are known to possess such characteristics. *Lactobacillus planetarium* and yeast isolate was predominant towards the latter stage of the fermentation in all the sample probably because of the reduced pH which favours their growth and the ability of *Lactobacillus plantarium* to produce lactic acid during fermentation might account for the initial isolation of some organism during the first and second day of fermentation in all the samples that later disappeared towards the end of the fermentation. This agrees with the observation of Enujiugha [12], who reported that *Lactobacillus* produces acid medium during fermentation to inhibit the growth of other microbes that cannot grow in acidic medium.

**Table 1. Morphological, biochemical characteristics and identification of bacterial isolate**

| Isolate No | Colony morphology | Gram's reaction | Catalase | Coagulase | Motility | Mannitol | Glucose | Fructose | Maltose | Lactose | Sucrose | Citrate | Indole | Spore forming | Methyl red test | Starch hydrolysis | Urease test | Probable identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cream, circular, opaque, flat, rough | + | + | NA | + | + | AG | AG | AG | AG | AG | + | - | + | - | + | - | *Bacillus* spp. |
| 2 | Cream, circular, raised and smooth | - | + | - | + | - | A | A | - | - | A | | - | NA | | + | | *Proteus* spp. |
| 3 | Circular, opaque, convex, cream, smooth colonies | + | - | - | - | | A | AG | AG | A | AG | - | - | - | + | - | + | *Pediococcus* spp. |
| 4 | Circular, opaque, convex, cream, smooth colonies | + | - | - | - | | A | AG | AG | A | AG | - | - | - | + | - | + | *Lactobacillus plantarum* |
| 5 | Cream, circular, smooth, entire | + | + | NA | - | - | AG | A | - | - | AG | - | - | - | - | - | | *Staphylococcus aureus* |
| 6 | Cream, circular, opaque, flat, rough | + | + | NA | + | + | AG | AG | AG | AG | AG | + | - | + | - | + | - | *Bacillus* spp. |
| 7 | Cream, circular, opaque, flat, rough | + | + | NA | + | + | AG | AG | AG | AG | AG | + | - | + | - | + | - | *Bacillus* spp. |

**Table 2. Cultural and morphological characteristics of fungi isolated**

| Isolate No | Cultural and morphological characteristics | Probable organism |
|---|---|---|
| **1** | Mycelium, sporangiophore and spores grow outside the Petri dish, Whitish growth and later become greyish-brown. Sporangiophores are tall, irregular, ovoid, solitary, smooth walled stolons opposite the branched rhizoids | *Rhizopus stolonifer* |
| **2** | Conidia one-celled,borne in single terminal cluster, branch hyaline conidiophores | *Trichoderma viride* |
| **3** | Dark colony colour, rough surface, no sclerotia, brown-green conidia with velvety surface. Conidiophores irregularly branched, consisting of short stipes. Conidia are cylindrical and smoth-walled | *Penicillum digitatum* |
| **4** | Colonies are with pseudo-hypae and absent of chlamydospore and germ tube. Urease negative, ferment glucose, sucrose and galactose but not lactose | *Candida tropicalis* |
| **5** | Creamy in colour, slightly smooth in chains, glabrous, pseudo-hyphae were formed with large globose to ellipsoidal cells with multilateral budding, colonies have psudohypae | *Saccharomyces cerevisiae* |
| | Creamy in colour, slightly smooth in chains, glabrous, pseudo-hyphae were formed with large globose to ellipsoidal cells with multilateral budding, colonies have psudohypae | *Saccharomyces cerevisiae* |

**Table 3. Bacterial load (x10$^5$ CFU/g) of mungbeans fortified and unfortified gari samples**

| Samples | 0 HOUR | 24 HOUR | 48 HOUR | 72 HOUR | 96 HOUR |
|---|---|---|---|---|---|
| Sample A | 2.10 | 2.25 | 2.00 | 1.61 | 1.50 |
| Sample B | 2.20 | 2.30 | 2.00 | 1.63 | 1.82 |
| Sample C | 2.35 | 2.45 | 1.62 | 1.72 | 1.51 |
| Sample D | 1.53 | 1.75 | 1.40 | 1.32 | 1.21 |

*Data are presented as Mean ± S.E (n=3). Values along the same colunm are not significantly different (P < 0.05*
*Keys: Sample A= 5% SMF (100 g SMF + 1900 g CM), Sample B= 10% SMF (200 g SMF + 1800 g CM), Sample C= 15% SMF (300 g MBF + 1700 g CM), Sample D=0%SMF (0 g SMF + 2000 g CM) (Control)*

**Table 4. Yeast load (x10$^5$ SFU/g) of Mungbeans Fortified and Unfortified gari samples**

| Samples | 0 HOUR | 24 HOUR | 48 HOUR | 72 HOUR | 96 HOUR |
|---|---|---|---|---|---|
| Sample A | 1.81±0.11$^b$ | 1.66±0.06$^b$ | 1.31±0.01$^b$ | 1.01±0.01$^a$ | 0.65±0.05$^a$ |
| Sample B | 1.61±0.11$^{ab}$ | 1.45±0.05$^{ab}$ | 1.11±0.01$^a$ | 0.79±0.09$^a$ | 0.45±0.05$^a$ |
| Sample C | 1.46±0.05$^a$ | 1.25±0.05$^a$ | 1.12±0.02$^a$ | 0.80±0.10$^a$ | 0.65±0.05$^a$ |
| Sample D | 2.16±0.06$^c$ | 1.96±0.06$^c$ | 1.86±0.06$^c$ | 1.07±0.03$^a$ | 0.95±0.05$^b$ |

*Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same colunm are not significantly different (P < 0.05);*
*Keys: Sample A= 5% SMF (100 g SMF + 1900 g CM), Sample B= 10% SMF (200 g SMF + 1800 g CM), Sample C= 15% SMF (300 g MBF + 1700 g CM), Sample D=0% SMF (0 g SMF + 2000 g CM) (Control)*

Molecular techniques are rapid, less laborious, more sensitive, specific and efficient compared to the conventional method [13]. This result revealed a difference in cultural identification of *Lactobacillus plantarum, Micrococcus roseus* and *Lactobacillus fermentum*. A similar observation was also reported by Akinyemi and Oyelakin [13], who reported differences in conventional method and molecular method of bacteria identification. However, the results of this study demonstrate clearly the interest and feasibility to introduce the 16S rRNA gene sequencing method in identification of bacteria, combination of conventional techniques and molecular approach will improve bacteriological investigation and authentication, allowing specific and efficient identification of microorganisms as against cultural method that is probable.

The microbial load was observed throughout the five days of fermentation. The increase in the total bacteria (CFU/ml) of the fermented samples observed during the first few days of fermentation showed that the microorganisms have adapted physiologically to the culture conditions and are now in the exponential phase. This agrees with the view of Yate and Smotzer (2007) who reported that in a batch culture, after

physiological adaptation of microorganisms to culture condition follows an exponential phase Subsequent reduction in the bacteria counts observed after the increase could be as a result of the accumulation of toxic wastes material, depletion of nutrient and overpopulation of the organism thereby resulting in their ultimate death (Koffler, 2016). This could also justify the reduction in the yeast count (CFU/ml) that was observed throughout the fermentation duration. However, the increase in the total lactic acid bacteria throughout the fermentation period could be associated to the reduce pH in the fermenting medium during the fermentation.

The decrease in pH value and increase in titratable acidity of fermented samples may be due to the production of organic acids such as the lactic acid in the samples. This is in agreement with the findings of Akinrotoye [9] who reported a decrease in the pH when checking for the effect of fermented palm wine on some diarrhoeagenic bacteria.

**Table 5. Lactic Acid Bacterial load (x10$^5$ CFU/g) of Mungbeans Fortified and Unfortified gari samples**

| Samples | 0 HOUR | 24 HOUR | 48 HOUR | 72 HOUR | 96 HOUR |
|---|---|---|---|---|---|
| Sample A | 1.13±0.02$^{c}$ | 1.36±0.05$^{b}$ | 1.56±0.06$^{bc}$ | 1.62±0.02$^{b}$ | 1.70±0.00$^{ab}$ |
| Sample B | 1.22±0.01$^{b}$ | 1.47±0.06$^{b}$ | 1.76±0.05$^{c}$ | 1.79±0.02$^{b}$ | 1.82±0.02$^{c}$ |
| Sample C | 1.24±0.02$^{b}$ | 1.55±0.06$^{b}$ | 1.57±0.05$^{b}$ | 1.66±0.05$^{b}$ | 1.74±0.04$^{b}$ |
| Sample D | 1.10±0.02$^{a}$ | 1.13±0.03$^{a}$ | 1.16±0.05$^{a}$ | 1.91±0.10$^{a}$ | 1.92±0.15$^{a}$ |

*Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same colunm are not significantly different (P < 0.05; Keys: Sample A= 5% SMF (100 g SMF + 1900 g CM), Sample B= 10% SMF (200 g SMF + 1800 g CM), Sample C= 15% SMF (300 g MBF + 1700 g CM), Sample D=0%SMF (0 g SMF + 2000 g CM) (Control)*

**Table 6. Molecular identification of isolated bacteria and yeast**

| Cultural and biochemical Identification | Gene sequence identification | Max Identity | Accession number |
|---|---|---|---|
| *Lysinibacillus* spp | *Lysinibacillus alkalisoli* | 100 | NR156042.1 |
| *Proteus mirabilis* | *Proteus mirabilis* | 99 | NR113344.1 |
| *Pediococcus* spp | *Pediococcus acidilactici* | 99 | NR042057 |
| *Lactobacillus* spp | *Lactobacillus plantarum* | 98 | NR104573.1 |
| *Staphylococcus* spp | *Staphylococcus aureus* | 97 | NR037007.2 |
| *Bacillus* spp | *Bacillus subtilis* | 98 | NR102783.2 |
| *Bacillus* spp | *Bacillus cereus* | 99 | NR074540.1 |
| *Penicillium sclerotiorum* | *Penicillium sclerotiorum* | 98 | MH484008.1 |
| *Diutina spp* | *Diutina catenulate* | 100 | MK394156.1 |
| *Rhizopus stolonifer* | *Rhizopus stolonifer* | 98 | AB025735.1 |
| *Trichoderma* spp | *Trichoderma viridae* | 99 | MK518057.1 |
| *Saccharomyces* spp | *Saccharomyces cerevisiae* | 99 | CP036473 |

**Table 7. pH Changes during fermentation of samples**

| Samples | 0 Hour | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
|---|---|---|---|---|---|
| Sample A | 5.05±0.15$^{a}$ | 4.80±0.20$^{b}$ | 4.30±0.20$^{a}$ | 3.10±0.10$^{a}$ | 3.10±0.10$^{a}$ |
| Sample B | 6.00±0.20$^{b}$ | 5.33±0.12$^{ab}$ | 4.55±0.15$^{a}$ | 3.60±0.20$^{ab}$ | 3.50±0.10$^{ab}$ |
| Sample C | 6.05±0.25$^{b}$ | 5.21±0.11$^{ab}$ | 4.35±0.25$^{a}$ | 3.80±0.10$^{b}$ | 3.75±0.15$^{b}$ |
| Sample D | 6.35±0.15$^{b}$ | 5.58±0.18$^{b}$ | 4.75±0.15$^{a}$ | 3.90±0.13$^{b}$ | 3.65±0.15$^{ab}$ |

*Data are presented as Mean ±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P < 0.05; Keys: Sample A= 5% SMF (100 g SMF + 1900 g CM), Sample B= 10% SMF (200 g SMF + 1800 g CM), Sample C= 15% SMF (300 g MBF + 1700 g CM), Sample D=0% SMF (0 g SMF + 2000 g CM) (Control)*

**Table 8. Titratable acidity changes during fermentation of samples**

| Samples | 0 Hour | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
|---|---|---|---|---|---|
| Sample A | 0.32±0.02$^{a}$ | 0.38±0.01$^{a}$ | 0.48±0.02$^{b}$ | 0.49±0.03$^{b}$ | 0.50±0.01$^{b}$ |
| Sample B | 0.33±0.02$^{a}$ | 0.34±0.01$^{a}$ | 0.35±0.01$^{a}$ | 0.41±0.01$^{a}$ | 0.48±0.01$^{ab}$ |
| Sample C | 0.41±0.01$^{b}$ | 0.55±0.02$^{b}$ | 0.61±0.01$^{c}$ | 0.63±0.01$^{c}$ | 0.64±0.01$^{c}$ |
| Sample D | 0.33±0.02$^{a}$ | 0.35±0.02$^{a}$ | 0.37±0.02$^{a}$ | 0.39±0.02$^{a}$ | 0.46±0.01$^{a}$ |

*Data are presented as Mean ±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P < 0.05; Keys: Sample A= 5% SMF (100 g SMF + 1900 g CM), Sample B= 10% SMF (200 g SMF + 1800 g CM), Sample C= 15% SMF (300 g MBF + 1700 g CM), Sample D = 0% SMF (0 g SMF + 2000 g CM) (Control)*

## 5. CONCLUSION

The outcome of this research showed that co-fermentation of cassava mash and sprouted mung bean flour eliminates pathogenic microorganisms such as *Proteus* spp*, Staphylococcus* spp*, Bacillus* spp*, Bacillus* spp*, Diutina* spp and *Trichoderma* spp and encourages the growth of beneficial microorganism such as *Lactobacillus plantarum, Pediococcus spp. Penicillium spp* and *Saccharomyces cerevisiae* in mung bean fortified Gari production.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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