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The bacteriological evaluation of cereal-based pap enriched with soybean in Abakaliki, Ebonyi State.

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ABSTRACT

The evaluation of bacteriological qualities of cereal-based pap enriched with soybean in Abakaliki, Ebonyi State was investigated. Varieties of maize, sorghum and soybean seeds randomly purchased from the international market were subjected to steeping at ambient temperature. Bacteriological qualities of cereal steeped liquor and akamu sample were analyzed by standard microbiological procedures. Soybean seeds were prepared by washing, soaking, dehulling and parboiling at 100 ^{0}C for 20 min before being wet-milled to slurry. Ground samples of cereal and soybean were mixed together in different ratios and fermented to yield the enriched akamu. The result showed that the least bacterial load was recorded by unenriched white sorghum with a bacteria load of 9.60 x 10^3cfu/ml after 24 h fermentation. The 4:1 enriched white maize had the highest load of 2.20 x 10^4cfu/mlafter 24 h fermentation, this was followed by unenriched white sorghum with a load of 2.11 x 10^4cfu/ml.The least distributed organism occurred in white sorghum with *S. aureus*, *E. coli,P. aeruginosa* and *K. pneumoniae* all having a frequency of 1±0.092.

Keywords: Bacteria, cereal, pap and soybeans

INTRODUCTION

Cereals fulfil an important role in the diet as a source of starch and dietary fibre that make up to 70 to 77 % of the whole grain [1] while proteins account for only 6 to 15 % [2]. As whole grain, cereals can potentially contribute to vitamins [3] and minerals intake [4,5,6]. One of the popular indigenous cereal-based fermented foods in Nigeria is *akamu* or *ogi*, a kind of pap, which is a fermented cereal porridge made from maize (*Zea mays*), sorghum (*Sorghum vulgare*) or millet (*Pennisetum typoideum*) [7,8,9,10,11]. Ogi can be simply described as a kind of diet that does not require chewing. The cereal-based ogi is very smooth in texture and has a sour taste reminiscent of that of yoghurt. Typically, Ogi has a distinct aroma and fine texture. The colour of the Ogi is mainly depending on the type of feedstock used for the processing. It could either be consumed as porridge (pap) or as a gel-like product (*agidi*) in some West African countries [12,13,14,15]. It is an essential weaning food for infants as well as a dietary staple for adults in West Africa [16,17,18,19,20]. Generally, traditional cereal foods play a vital role in the diet of the people of tropical Africa particularly in cereal producing regions [21,22,23,24]. However, cereal proteins are low in several essential amino acids potentially causing protein malnutrition. Ogi or Akamu has been reported as being poor in protein and essential nutrients because during processing, the protein are lost in the steeping and wash water. Similarly, Ogi has been implicated in the cause of protein-energy malnutrition in children during the weaning period [25,26]. [27], revealed that the protein contents of ogi was too low even to support the growth of rats in a feeding trial with 50 suckling rats at birth. The pups became malnourished and most of them died, indicating the inadequacy of ogi as a weaning food. In terms of production quantity, sorghum is the fifth most important cereal crop in the world after rice, wheat, maize and barley, and the most grown cereal in Sub-Saharan Africa, after maize [28]. It remains one

of the most versatile cereal crops on the continent, serving as a staple and main meal for millions of people [29]. It is an important source of calories, variety of nutrients and beneficial food components [30]. With the increasing world population, decrease in water supply and the effects of climate change, this drought resistant food crop is vital for human utilization and will be an important crop for the future. Ogi production, despite many reported research works is still on a small scale. In this production, domestic equipment are often used [31]. Soaking and sedimentation have been widely reported as two important fermentation stages involved in the traditional method of processing ogi [32,33,34]. Soaking is often carried out at room temperature [35]. The soaking period is expected to reduce the hardness of the maize grain while the sedimentation period gives the required tartness often desired by some consumers [36]. The soaked grains are then washed, wet milled, sieved and allowed to sediment for 24–48 h. The ogi slurry may then be processed into varieties of products for infants, children and adult's meal [8]. Maize (*Zea mays* subsp. mays), also known as corn (North American and Australian English), is a cereal grain first domesticated by indigenous peoples in southern Mexico about 10,000 years ago [14]. The leafy stalk of the plant produces polleninflorescencesand separate ovuliferous inflorescences called ears that yield kernels or seeds, which are fruits [16]. Maize has become a staple food in many parts of the world, with the total production of maizesurpassing thatof wheat or rice . In addition to being consumed directly by humans (often in the form of masa), maize is also used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup. The six major types of maize are dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn. Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels.While field corn varieties are used for animal feed, various corn-based human food uses (including grinding into cornmeal or masa, pressing into corn oil, and fermentation and distillation into alcoholic beverages like bourbon whiskey), and as chemical feedstocks. Maize is also used in making ethanol and other biofuels [15]. Maize is widely cultivated throughout the world, and a greater weight of maize is produced each year than any other grain [16]. In 2014, total world production was 1.04 billion tonnes. Maizeand cornmeal (g round dried maize) constitute a staple food in many regions of the world. Maize is used to produce cornstarch, a common ingredient in home cooking and many industrialized food products. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, a sweetener; and also fermented and distilled to produce grain alcohol. Grain alcohol from maize is traditionally the source of Bourbon whiskey. Corn flour is used to make cornbread and other baked products [9].

Aim of the Study

This study is aimed at bacteriological evaluation of cereal-based paps enriched with soybean in Abakaliki, Ebonyi State.

MATERIALS AND METHODS

Study Area

The study area of this research was Abakaliki Metropolis of Ebonyi State, Nigeria. Ebonyi State is located in the south-eastern part of Nigeria which lies approximately within longitude 7 ^{0}30 ^{1} and 7 ^{0}E and, latitude 5 ^{0}40 ^{1} and 6 ^{0}45 ^{1} N. It has a population of 149,683, and a land mass of about 5,935 square kilometers. Ebonyi State is bounded to the north by Benue State, to the south by Abia State and to the east by Enugu State and west by Cross River State

respectively. Abakaliki, the State capital, has tropical climate with an average relative humidity of 75 % and may reach 80 % during rainy season. The vegetation characteristics are predominantly rainforest with atmospheric temperature of 30 ^{0}C. Two seasons are distinguishable in Ebonyi State: a dry season (November to March) and a wet season (April and October). Abakaliki residents utilizes various cereal-based food products for weaning purposes and general nutritional purposes.

Sample Collection

Exactly 2 kg each of white and yellow maize, white and red sorghum grains and 1 kg of soybean seed were randomly bought from traders at the Abakaliki international market Ebonyi State, Nigeria (i.e. the 5 samples were bought from 5 different shops). The samples were collected in polythene bags from the market and transported to the Applied Microbiology Laboratory of Ebonyi State University, Abakaliki for analysis. The cereal grains were authenticated by a taxonomist in the Department of Crop science, Faculty of Agriculture and Resource Management as *Zea mays, Sorghum bicolor* and *Glycine max* respectively.

Preparation of Cereal-Based Akamu

Preparation of ogi was done according to the methods of [16]. 500g of each cereal grains were washed in distilled water to remove dust particles. The washed grains were steep in 1 Litre autoclaved distilled water for 48 h in covered cleaned plastic containers. Water was decanted and the fermenting grains were re-washed twice to reduce fermenting odour and then wet-milled with a blender. Wet milling of the different cereals grains was followed by wet-sieving to remove bran, hulls and germs. The waste was restrained on the muslin cloth and later dried as poultry feed, while the filtrate was fermented for another 24 h to yield ogi which is either white, cream or chocolate coloured depending on the pigment of the cereal grain.

Media Preparation

All media were aseptically prepared according to the manufacturer's specifications. The media includes: Peptone water, Nutrient agar, MacConkey agar and Plate count agar.

Preparation of Liquid Media (0.1 Peptone Water)

This was prepared by dissolving 10g of commercial peptone powder and 5g of sodium chloride in one litre of distilled water. It was sterilized in laboratory autoclave at 121°c for 15 minutes in conical flask.

Preparation of Nutrient Agar

Exactly 2.8 g of nutrient agar powder was dissolved in 100ml of distilled water contained in a sterile conical flask was plugged with non-absorbent cotton wool. The neck of the conical flask was tied firmly with a masking tape. It was mixed by shaking, boiled so as to dissolve completely and autoclaved at 121°C for 15 min at 15 psi. It was allowed to cool at 45°c before it was dispensed aseptically in 20ml volume petri dishes. The medium was allowed to gel on the petri dishes.

Preparation of MacConkey Agar

Forty-eight grams (48g) of MacConkey agar powder was weighed and dissolved in 1 litre of distilled water – swirled to mix and sterilized by autoclaving for 15 min at 121°c, cooled to 47°C, mixed well and 20ml each was poured aseptically into sterile petri-dish.

Preparation of Plate Count Agar

Exactly 1.8 grams of plate count agar powder was dissolved in 100 ml of distilled water contained in a sterile beaker. It was shaken to enhance homogeneity after which 5ml was dispensed into sterile test tubes and covered with absorbent cotton wool. The neck of the conical flask was tied firmly with a masking tape. It was sterilized by autoclaving at 121°C for

15 minutes at 15 psi and allowed to cool to 45°c before use.

Isolation and Enumeration of viable Bacteria Load

The bacterial load of the steeped grain liquor was enumerated using pour plate method. 10 fold serial dilutions of the steeped grain liquor were made for preliminary investigation to confirm the dilution factor that recorded more discreet colonies. After which 1 ml of 10^{-5} dilution was pipetted into duplicate sterile Petri dishes and 15 ml of molten plate count agar at 45 ^{0}C was aseptically added. The medium and the inoculum contained in the Petri dishes were immediately mixed by shaken to ensure a homogenous mixture. The Petri dishes were allowed to solidify and incubated at 37 ^{0}C for 24 h for the enumeration of the bacterial load. On the other hand, the bacterial load of each prepared akamusamples involved in making 10-fold serial dilutions of 1g akamu samples. To reduce the bacterial count, 10^{-5} dilutions was aseptically inoculated in duplicates on plate count agar Petri dishes and incubated at 37 ^{0}C for 24 h for the enumeration of bacterial load [16]. Then the mixed cultures of the isolated bacteria were severally subcultured on nutrient agar, tryptone soy agar and MacConkey agar plates and further incubated at 37 ^{0}C for 24 h to obtain pure bacteria cultures [19].

Characterization and Identification of Isolates

The identification of isolates was carried out in three phases; cultural characterization,morphological characterization and biochemical characterization.

Cultural characterization

Grouping of pure cultures of bacteria randomly picked from inoculated plates was carried out on the basis of their colonial characteristics such as colony elevation, color, size, shape, surface, edge, and pigmentation. They were aseptically inoculated in duplicates on Mannitol Salt agar, cetrimide agar, Bushnell-Hans agar and MacConkey agar plates and incubated at 37°C for 24 h for bacterial growth.

Morphological characterization

This was done by examining the isolates microscopically for cellular morphology. Day- old cultures of the bacterial isolates was gram-stained, and their color (purple or pink), shape (cocci or rods), and arrangement (singles, pairs, chains, or clusters) were observed and recorded.

Gram staining

Using a sterile loop, a light suspension of organism in sterile distilled water was smeared on a clean microscope slide. The film was air-dried and heat-fixed by passing the slide twice through a gas flame. The slide was allowed to cool. The slide was placed on a staining rack, flooded with crystal violet solution, and left for 30 s before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 30 s before washing off with running tap water. To decolorize, acetone alcohol was ran over the film and washed off immediately with running tap water. The film was flooded with safranin solution and left for 1 minutes before washing off with running tap water. The film on the slide was allowed to air- dry. A drop of immersion oil was placed on the film, and it was examined under the microscope using the × 100 oil immersion lens. Dark purple indicated Gram- positive reaction and pink indicated Gram-negative reaction. The shapes and arrangement of the cells were recorded.

Motility test (hanging drop method)

Two drops of wet suspension of the test organism was placed on a grease-free coverslip and quickly inverted over a depression grease-free glass slide supported by a ring of petroleum jelly. It was examined under the

microscope with 10x and 40x objective lenses. Visible movement of the organism indicates motility. Absent of this movement indicates no motility.

Biochemical characterization

Conventional biochemical tests were carried out on the bacterial isolates for further identification such as catalase test, oxidase test, Indole test, methyl red test, Voges–Proskauer test, citrate utilization, urease test, nitrate reduction, starch hydrolysis, casein hydrolysis, spore test, and sugar fermentationtest.

Catalase test (slide method)

Catalase is an enzyme produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen (H_2O_2) for their protection. Anaerobes generally lack this enzyme, and yeasts are catalase positive. This test was carried out to distinguish *Staphylococci* and *Bacillus* spp. which are catalase positive and to identify the Enterobacteriaceae family. A drop of 3 % hydrogen peroxide (H_2O_2) was placed on a clean glass microscopic slide. Using a sterile inoculating loop, a small amount of organism was picked from a well- isolated 24 h colony and gently rubbed into the H_2O_2. Positive reactions was evident by immediate bubble formation.

Oxidase test

This test was used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. It was used to identify Candida spp. which are yeasts and are oxidase positive. A piece of filter paper in a Petri dish was moistened with 2drops of Kovac's oxidase reagent (1 % tetramethylphenylenediamine hydrochloride). Using a wire loop, a colony of the test organism was transferred to the filter paper and rubbed on the moistened area. Purple coloration within 30 s indicated the production of cytochrome c oxidase.

Indole test

This test was carried out to determine the ability of bacteria to break down tryptophan to indole by the enzyme tryptophanase. The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and incubated at 37 °C for 48 h. Indole was tested for by adding 0.5 ml (5 drops) of Kovac's reagent (isoamyl alcohol; para-dimethyl aminobenzaldehyde; concentrated hydrochloric acid) and shaken gently. A red color in the surface layer within 10 min indicated a positive reaction while a yellow color indicated a negative reaction.

Methyl red test

This test was carried out to identify enteric bacteria based on their pattern of glucose metabolism (mixed acid fermenters are positive to this test). The bacterium was inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37 °C for 48 h. The pH of the medium was tested by the addition of five drops of methyl red reagent. The tube was gently rolled between the palms to disperse the methyl red reagent. Development of red color was taken as positive and yellowasnegative.

Voges–Proskauer test

This test was carried out to detect acetoin in enteric broth culture with the aim of differentiating the enteric bacteria. The bacterium was inoculated into glucose phosphate broth and incubated for 48 h. Alpha- naphthol solution (0.6 ml of 5 %) in ethanol was added to the broth and shaken. The tube was allowed to stand for 15 min. Cherry red color was taken as positive while no color change indicated negative.

Citrate utilization test

This test was carried out to differentiate the enteric bacteria. Bacterial colonies from fresh (24h) plates was picked up with wire loop,

inoculated onto a slope of Simmons citrate agar and incubated overnight at 37 °C. A change of medium from green to blue indicated a positive reaction, that is, the organism has the ability to utilize citrate as sole source of carbon andenergy.

Sugar fermentation test

The test was used to assess the sugar fermentation abilities of bacteria and yeasts isolated. Ten percent (10 %) solutions of some test sugars (carbohydrates) such as glucose, fructose, lactose, galactose, mannitol, sucrose, raffinose, rhamnose, and melibiose was prepared and sterilized at 115 °C for 10 min so as not to denature the sugars. To 90 ml sterile peptone water, 10 ml of the sterile 10 % sugar solution was added with 2 ml 0.01 % phenol red indicator. The sugar solution (5 ml) was transferred aseptically into sterile test tubes with inverted Durham tubes to check for gas production. The tubes were incubated overnight to check for sterility, and then, the tubes were inoculated with pure culture of the test organisms and incubated at 37 °C for 4 days. Yellow coloration indicated acid production while gas production was indicated by displacement of the medium in the Durham tube.

Nutritional Improvement Process

Exactly 500g of each cereal grains was washed with distilled water to remove dust particles. The grains were then steeped in distilled water for 48 h after which they were wet-milled using electric blender and sieved with muslin cloth. Similarly, 500g of soybean seeds were washed with distillled water and dehulled. The soybean seeds were then subjected to 20 min boiling, after which they were cooled, wet milled and sieved. Each of the cereal grains slurry and soybean slurry was then combined in different proportions shown in thenext page and fermented to yield the enriched akamu.

500g of each cereal grains = traditionallyfermented to produce unenriched akamu

400g of each cereal grain + 100g soybean slurry to produce enriched akamu

300g of each cereal grain + 200g soybean slurry to produce enriched akamu

250g of each cereal grain + 250g soybean slurry to produce enriched akamu

Statistical Analysis

The raw data obtained in the course of the study was presented as mean ± standard deviation in tables and bar charts while relevant data was interpreted using simple descriptive statistics such as minimum, maximum, and one way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Excel 2013 software. $P < 0.05$ was considered to be statisticallysignificant.

RESULTS

Bacterial Load of Maize and Sorghum steepedliqour at Zero Fermentation

Table 1 shows the bacterial load of maize and sorghum steeped liquor for production of akamu at zero fermentation. It revealed that the 3:2 enriched white maize had the highest bacterial load of 1.130 x 10^4cfu/ml at zero fermentation, this was followed by unenriched yellow maize with a count of 1.120 x 10^4cfu/ml. The least count was recorded by unenriched white sorghum with a load of 0.960 x 10^4cfu/ml.

Table 1: Bacterial Load of Maize and Sorghum steepedliqouratZero Day Fermentation.

S/No	Sample Code	Growth (CFU/ml)	SD
1	WM_5	1.080×10^4	0.945
2	YM_5	1.120×10^4	1.673
3	WS_5	0.960×10^4	-1.240
4	RS_5	1.020×10^4	-0.148
5	WM_4	0.990×10^4	-0.694
6	YM_4	1.100×10^4	1.309
7	WS_4	1.010×10^4	-0.330
8	RS_4	1.000×10^4	-0.512
9	WM_3	1.130×10^4	1.855
10	YM_3	1.010×10^4	-0.330
11	WS_3	0.980×10^4	-0.876
12	RS_3	0.970×10^4	-1.058
13	WM_2	1.060×10^4	0.580
14	YM_2	0.980×10^4	-0.876
15	WS_2	1.050×10^4	0.398
16	RS_2	0.990×10^4	-0.694
Average Mean = 1.028, SD = ± 0.055			

KEY: WM_5 = White maize at 500g (unenriched), YM_5 = Yellow maize at 500g, WS_5 = White sorghum at 500g, RS_5 = Red sorghum at 500g, WM_4 = White maize at 400g (enriched), YM_4 = Yellow maize at 400g, WS_4 = White sorghum at 400g, RS_4 = Red sorghum at 400g, WM_3 = White maize at 300g (enriched), YM_3 = Yellow maize at 300g, WS_3 = White sorghum at 300g, RS_3 = Red sorghum at 300g, WM_2 = White maize at 240g (enriched), YM_2 = Yellow maize at 250g, WS_2 = White sorghum at 250g, RS_2 = Red sorghum at 250g. SD = Standard Deviation. Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence ($P < 0.05$).

Bacterial Load of Maize and Sorghum steepedliquor at 24 h Fermentation

The bacterial load of maize and sorghum steeped liquor for production of akamu at 24 h fermentation is shown on Table 2. It revealed that the 4:1 enriched white maize had the highest bacterial growth of 2.20 x 10^4cfu/mlafter 24 h fermentation, this was followed by unenriched white sorghum with a load of 2.11 x 10^4cfu/ml. The least load was recorded by unenriched white maize with a load of 1.880 x 10^4cfu/ml.

Table 2: Bacterial Load of Maize and Sorghum steepedliqourat 24 h Fermentation

S/No	Sample Code	Growth (CFU/ml)	SD
1	WM_5	1.880×10^4	-1.036
2	YM_5	2.010×10^4	0.217
3	WS_5	2.110×10^4	1.181
4	RS_5	2.100×10^4	1.084
5	WM_4	2.200×10^4	2.048
6	YM_4	1.920×10^4	-0.651
7	WS_4	1.890×10^4	-0.940
8	RS_4	2.020×10^4	0.313
9	WM_3	1.780×10^4	-1.999
10	YM_3	1.930×10^4	-0.554
11	WS_3	2.050×10^4	0.602
12	RS_3	2.010×10^4	0.217
13	WM_2	2.000×10^4	0.121
14	YM_2	1.990×10^4	0.024
15	WS_2	1.890×10^4	-0.940
16	RS_2	2.020×10^4	0.313
		Average mean = 1.98; SD =± 0.104	

KEY: WM_5 = White maize at 500g (unenriched), YM_5 = Yellow maize at 500g, WS_5 = White sorghum at 500g, RS_5 = Red sorghum at 500g, WM_4 = White maize at 400g (enriched), YM_4 = Yellow maize at 400g, WS_4 = White sorghum at 400g, RS_4 = Red sorghum at 400g, WM_3 = White maize at 300g (enriched), YM_3 = Yellow maize at 300g, WS_3 = White sorghum at 300g, RS_3 = Red sorghum at 300g, WM_2 = White maize at 240g (enriched), YM_2 = Yellow maize at 250g, WS_2 = White sorghum at 250g, RS_2 = Red sorghum at 250g, SD = Standard deviation. Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence ($P < 0.05$).

Bacterial Load of Maize and Sorghum steepedliquor at 48 h Fermentation

The bacterial load of maize and sorghum steeped liquor for production of akamuat 48 h fermentation is shown on Table 3 cfu/ml. It revealed that the 4:1 ratio of enriched white maize had the highest bacterial load of 2.28 x 10^4cfu/ml at 48 h fermentation, followed by 3:2 ratio of enriched white maize with a load of 2.21 x 10^4cfu/ml.The least load was recorded by unenriched white maize with a load of 1.96 x 10^4cfu/ml.

Table 3: Bacterial Load of Maize and Sorghum steepedliqourat

S/No	Sample Code	Growth (CFU/ml)	Standard Deviation
1	WM_5	1.960 x 10^4	-2.138
2	YM_5	1.990 x 10^4	-1.773
3	WS_5	2.170 x 10^4	0.418
4	RS_5	2.200 x 10^4	0.784
5	WM_4	2.280 x 10^4	1.758
6	YM_4	2.150 x 10^4	0.175
7	WS_4	2.080 x 10^4	-0.677
8	RS_4	2.100 x 10^4	-0.434
9	WM_3	2.210 x 10^4	0.906
10	YM_3	2.200 x 10^4	0.784
11	WS_3	2.170 x 10^4	0.419
12	RS_3	2.190 x 10^4	0.662
13	WM_2	2.120 x 10^4	-0.190
14	YM_2	2.110 x 10^4	-0.312
15	WS_2	2.080 x 10^4	-0.677
16	RS_2	2.160 x 10^4	0.297
		Average mean = 2.135; SD = ± 0.082	

KEY: WM_5 = White maize at 500g (unenriched), YM_5 = Yellow maize at 500g, WS_5 = White sorghum at 500g, RS_5 = Red sorghum at 500g, WM_4 = White maize at 400g (enriched), YM_4 = Yellow maize at 400g, WS_4 = White sorghum at 400g, RS_4 = Red sorghum at 400g, WM_3 = White maize at 300g (enriched), YM_3 = Yellow maize at 300g, WS_3 = White sorghum at 300g, RS_3 = Red sorghum at 300g, WM_2 = White maize at 240g (enriched), YM_2 = Yellow maize at 250g, WS_2 = White sorghum at 250g, RS_2 = Red sorghum at 250g. SD = Standard Deviation.Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence ($P < 0.05$).

Bacterial Load of unenriched and enriched processed Ogi

Table 4 shows the bacterial load of enrichedakamu. It revealed that the enriched white sorghum had the highest bacterial load of 1.96 x 10^4cfu/ml, this was followed by enriched yellow maize with a load of 1.71 x 10^4cfu/ml. The least load was recorded by 2.5:2.5 enriched white maize with a load of 0.36 x 10^4cfu/ml.

Table 4: Bacterial Load of enriched processed akamu

S/No	Sample Code	Growth (CFU/ml)	Standard Deviation
1	WM_5	1.60 x 10^4	1.284
2	YM_5	1.71 x 10^4	1.496
3	WS_5	1.96 x 10^4	1.977
4	RS_5	1.68 x 10^4	1.438
5	WM_4	0.49 x 10^4	-0.855
6	YM_4	0.44 x 10^4	-0.951
7	WS_4	1.08 x 10^4	0.282
8	RS_4	0.90 x 10^4	-0.065
9	WM_3	0.48 x 10^4	-0.874
10	YM_3	0.52 x 10^4	-0.797
11	WS_3	0.60 x 10^4	-0.643
12	RS_3	0.76 x 10^4	-0.335
13	WM_2	0.36 x 10^4	-1.106
14	YM_2	0.72 x 10^4	-0.412
15	WS_2	0.80 x 10^4	-0.258
16	RS_2	0.84 x 10^4	-0.181
		Average mean = 0.934; SD = ± 0.519	

KEY:WM_5 = White maize at 500g (unenriched), YM_5 = Yellow maize at 500g, WS_5 = White sorghum at 500g, RS_5 = Red sorghum at 500g, WM_4 = White maize at 400g (enriched), YM_4 = Yellow maize at 400g, WS_4 = White sorghum at 400g, RS_4 = Red sorghum at 400g, WM_3 = White maize at 300g (enriched), YM_3 = Yellow maize at 300g, WS_3 = White sorghum at 300g, RS_3 = Red sorghum at 300g, WM_2 = White maize at 240g (enriched), YM_2 = Yellow maize at 250g, WS_2 = White sorghum at 250g, RS_2 = Red sorghum at 250g. SD = Standard Deviation.Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence ($P < 0.05$).

Morphology and Biochemical characteristic of bacteria species from fermented maize and sorghum-based akamu

Morphological characteristic shows that all probable bacteria species were rod shape except for *S. aureus* which is cocci shaped. They showed positive Biochemical characteristic in the fermentation of galactose and glucose. *Klebsiella* species, *Pseudomonas aeruginosa* and *Lactobacillus* species were negative (-) to indole testwhile production of Acid/Gas (A/G) was observed against *Escherichia coli* as shown in Table 5.

Table 5: Morphology and Biochemical characteristic of bacteria species from fermented maize and sorghum-based akamu
KEYS: A/A- Acid/Alkaline, A-Acid,A/G-Acid/Gas, VP-Voges-Proskauer, TSI-Triple Sugar Iron, (+)- positive, (-)- negative

Gram staining	Shape	Colour	TSI	Oxidase	Motility	Catalase	VP	Methyl red	Indole	Citrate	Glucose	Lactose	Galactose	Arabinose	Sorbitol	Xylose	Fructose	Probable Bacteria
-	Rod	Black	A	-	+	-	-	+	-	-	+	-	+	-	+	+	+	*Lactobacillus* species
-	Rod	Green	A	+	+	+	-	-	-	+	+	-	+	-	-	+	+	*Pseudomonas aeruginosa*
-	Rod	mucoid Whitish-blue	A	-	-	+	+	-	-	+	+	+	+	+	+	+	-	*Klebsiella pnuemoniae*
-	Rod	Pink	A/G	-	+	+	-	+	+	-	+	+	+	+	+	-	-	*Escherichia coli*
+	Coccus	Golden Yellow	A/A	-	-	+	+	+	-	+	+	+	+	-	+	-	+	*Staphylococcus aureus*

Distribution of Bacteria isolates in steepedcerealliqour at zero-hour fermentation

The distribution of bacterial isolates in steepedcerealliqour at zero-hour fermentation were shown on Figures 1 to 12. Figure 9 revealed that *E coli* from white maize and Red sorghum was the most distributed with frequency of 5±0.052 among cereals of 500g weight, while the least was *Lactobacillus* speciewith a frequency of 1±0.017. At 400g measured weight, *E. coli* in yellow maize and white maize was the most distributed with a frequency of 3±0.128, while the least were *Lactobacillus* specie, *S. aureus*, and *P. aeruginosa* from acrossall the cereal grains. Figure 1 shows the distribution of bacteria in steepedcereal grain liquor for 300g weighed enriched cereal. It revealed that *P. aeruginosa* and *S. aureus* from white maize and yellow maize respectively were the highest distributed, with a frequency of 3±0.008. The least distributed was *E. coli* from yellow maize, white sorghum and red sorghum with a frequency of 1±0.022. The distribution of bacteria isolates in steeped grain liquor for 250g weight of enriched cereal is shown on figure 1. It revealed that *E. coli* in white sorghum was the highest distributed organism with a frequency of 4±0.021. While the least distributed organisms were *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* from white maize, white sorghum and red sorghum with a frequency of 1±0.061 respectively (Figure 1).

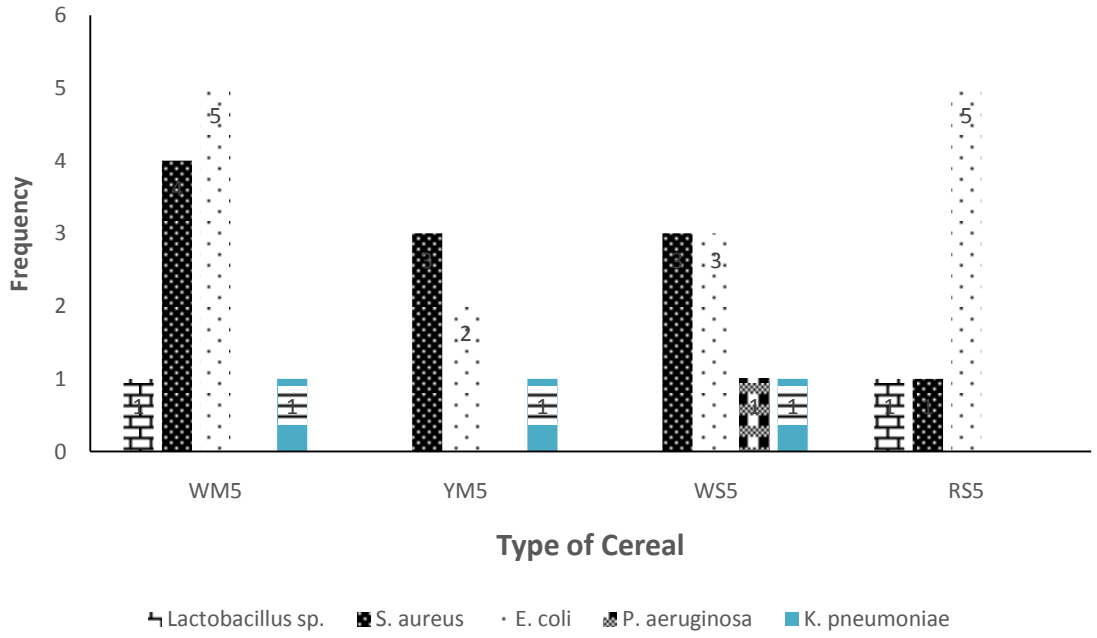

Figure 1: Distribution of bacterial isolates in steepedliqour at zero fermentation for 500g weighed un-enriched cereal

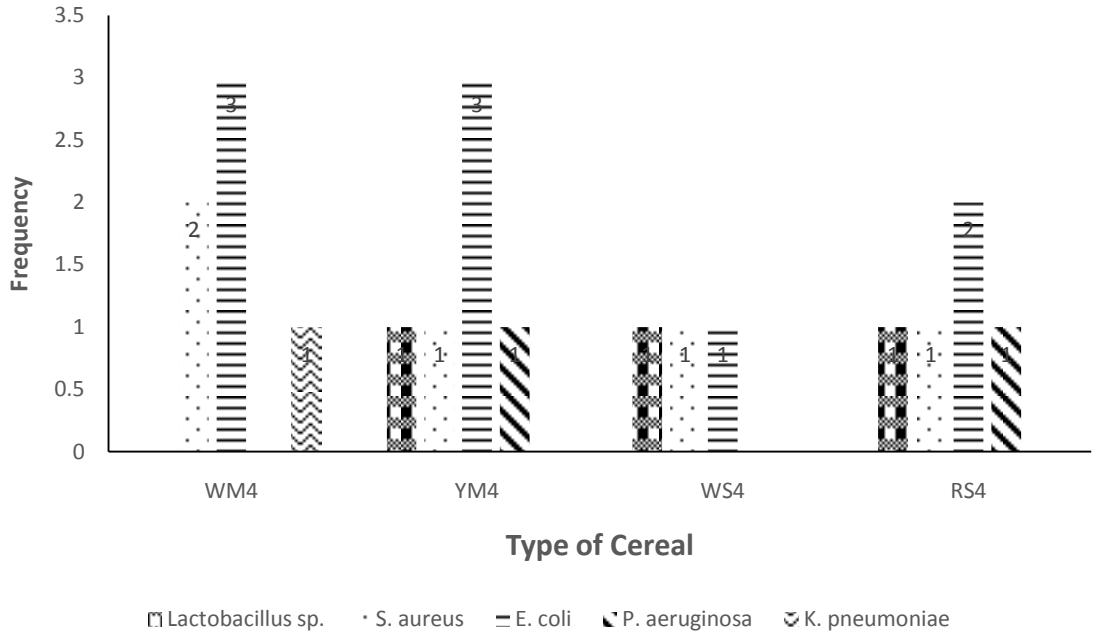

Figure 2: Distribution of bacterial isolates in steeped cerealliqour at zero fermentation for 400g weighed enriched cereal

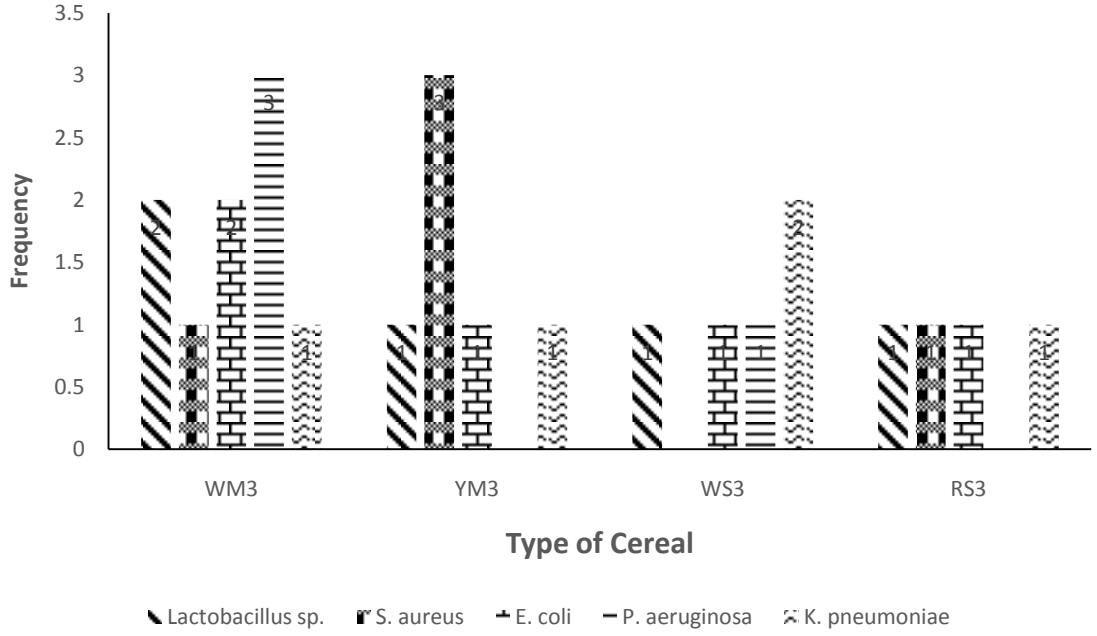

Figure 3: Distribution of bacterial isolates in steeped cerealliqour at zero fermentation for 300g weighed enriched cereal

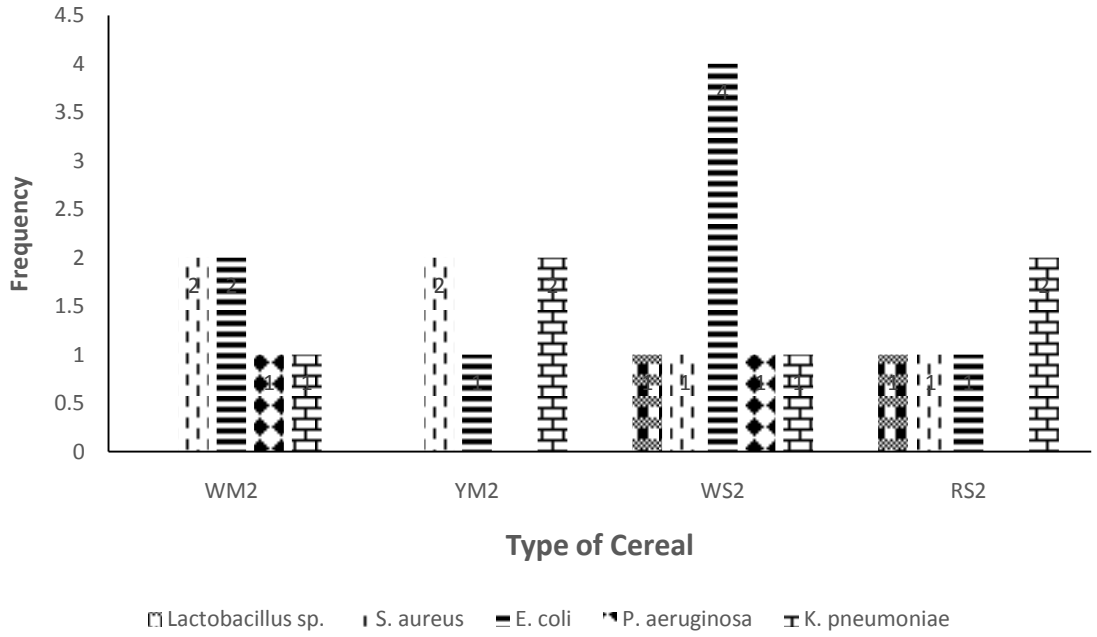

Figure 4: Distribution of bacterial isolates in steeped cerealliqour at zero fermentation for 250g weighed enriched cereal

Distribution of Bacterial isolates in steepedcerealliqour at 24 hour fermentation.

Figure above shows that *E. coli* in white maize and red sorghum were the most distributed with frequency of 5±0.001 respectively. This was followed by *P. aeruginosa* in white sorghum with a frequency of 4±0.023. The least distributed organism were*Lactobacillus* specie, *K. pneumoniae*, and *P. aeruginosa* in yellow maize, white maize, and red sorghum with a frequency of 1±0.046 respectively. The distribution of bacterial isolates in steeped cerealliqour for 400g enriched cereal at 24 h fermentation is shown on figure 4. It revealed that *E. coli* in white maize and yellow maize were the most distributed with a frequency of 3±0.004, followed by *E. coli* and *S. aureus* in red sorghum and white sorghum respectively with a frequency of 2±0.018. The least were *K. pneumoniae* in white maize, *S. aureus* and *P. aeruginosa* in yellow maize, *Lactobacillus* specie, *E. coli*, and *S. aureus* in white sorghum and red sorghum, all with a frequency of 1±0.022. Figure 5 shows the distribution of bacteria for 300g steeped cerealliquor of enriched cereal. It revealed that *E. coli* in red sorghum was the most distributed, while *S. aureus* and *Lactobacillus* specie in white maize, white sorghum and red sorghum were least distributed. At 250g weighed cereal,the distribution of bacteria isolates revealed that *E. coli* in red sorghum was the most distributed with a frequency of 3±0.128, while *S. aureus* and *Lactobacillus* specie in white maize, white sorghum and red sorghum were least distributedwith frequencies of 1±0.051 (Figure 6).

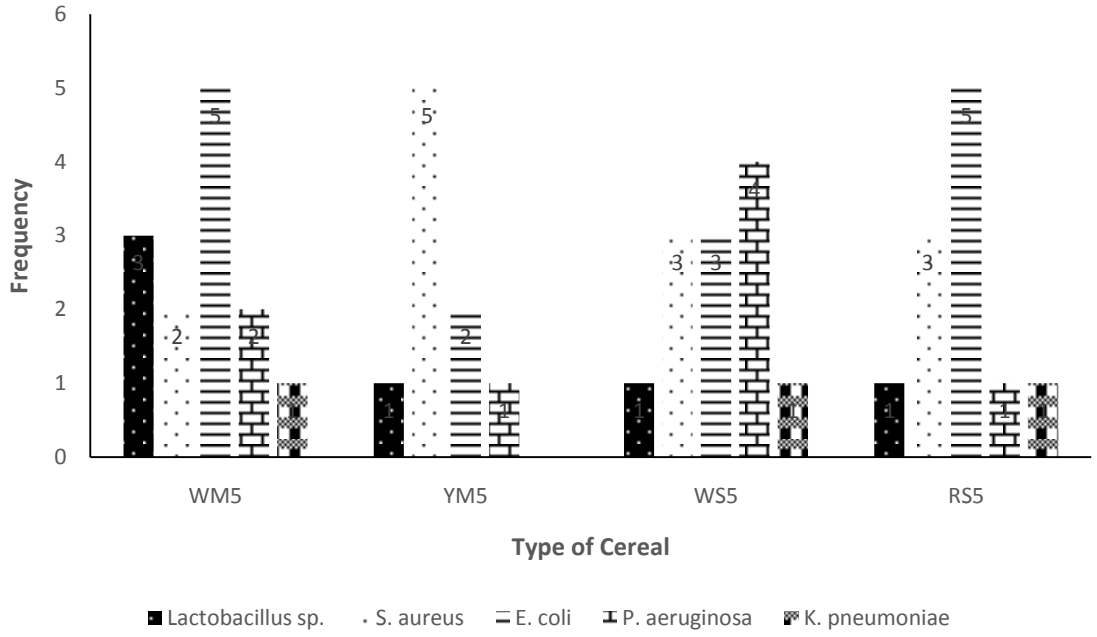

Figure 5: Distribution of bacterial isolates in steepedcereal liquor at 24 h fermentation for 500g weighed un-enriched cereal

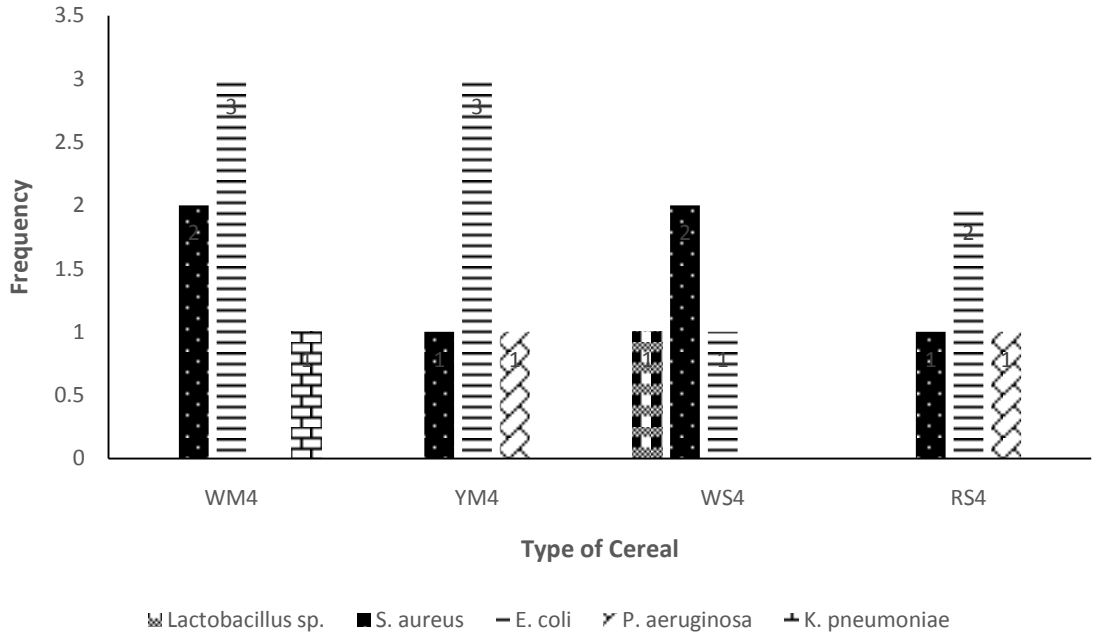

Figure 6: Distribution of bacterial isolates in steeped cerealliqour at 24 h fermentation for 400g weighed enriched cereal

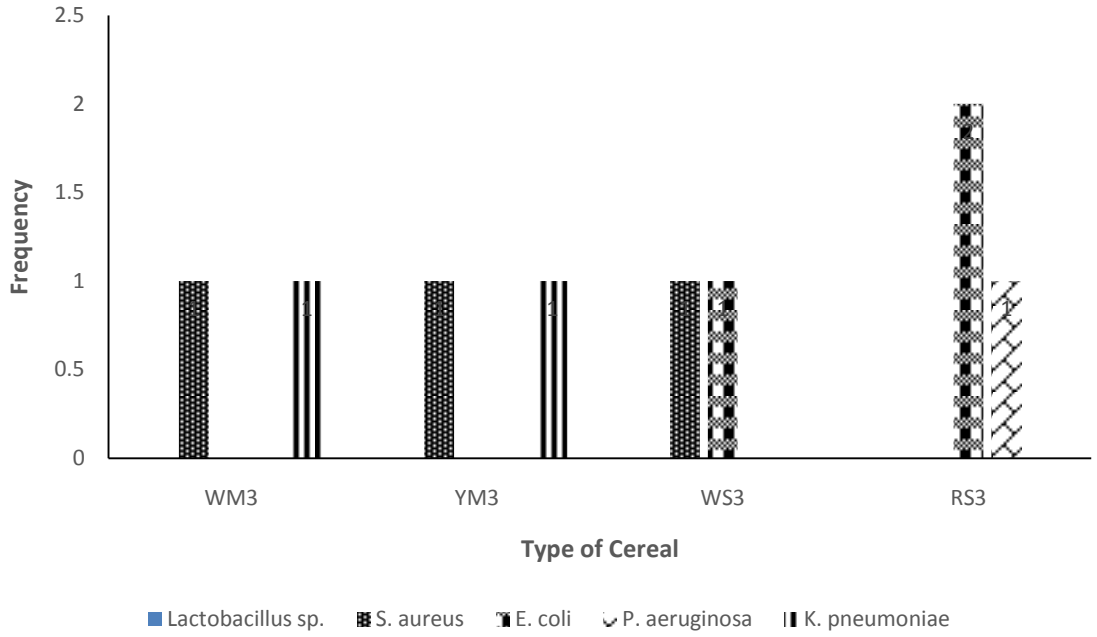

Figure 7: Distribution of bacterial isolates in steeped cerealliqour at 24 h fermentation for 300g weighed enriched cereal

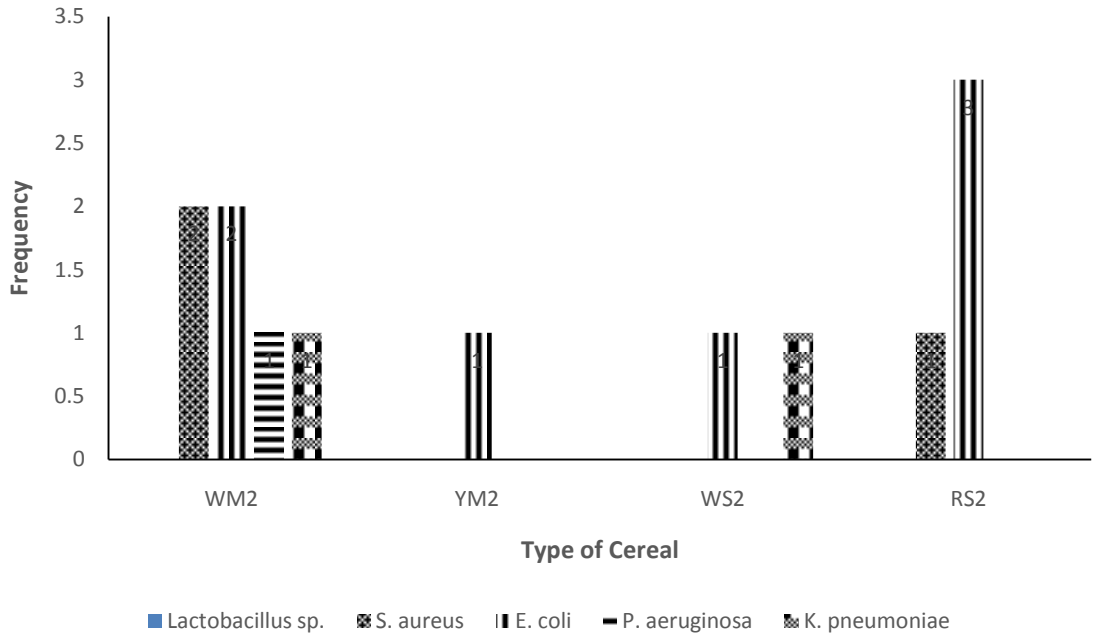

Figure 8: Distribution of bacterial isolates in steeped cerealliqour at 24 h fermentation for 250g weighed enriched cereal

Distribution of Bacterial isolates in steeped cerealliqour at 48 h fermentation

The distribution of bacterial isolates in steeped cereal liquor at 48 h fermentation are shown on Figure 8 to 12. Figure 17 shows the distribution of bacterial isolates in steeped cerealliqour for 500g weighed unenriched cereal. It revealed that *E. coli* in white maize (4±0.006) was

significantly (P<0.05) the most distributed, this was followed by *Lactobacillus* specie in yellow maize with a frequency of 3±0.028. The least distributed organism occurred in white sorghum with *S. aureus*, *E. coli,P. aeruginosa* and *K. pneumoniae* all having a frequency of 1±0.092. For 400g enriched cereal. Figure 18 revealed that *S. aureus* in yellow maize and white sorghum was the most distributed organisms with a frequency of 2±0.341 each. Meanwhile, red sorghum had the least distribution of organism. The distribution of bacteria in steeped cerealliquor of cereals weighing 300g was shown on figure 19. It revealed that *Lactobacillus* specie was the most distributed organism with a frequency of 2±0.089. The other isolated organism had a frequency distribution of 1±0.033 across all types of cereal.Figure 20 shows the distribution of bacteria isolates in steeped liquor of cereal weighing 250g. It revealed that S*. aureus, E. coli*, and *Lactobacillus* species were the most distributed organisms with a frequency of 2±0.190. No organism was isolated from red sorghum steep liqour at 48 h fermentation.

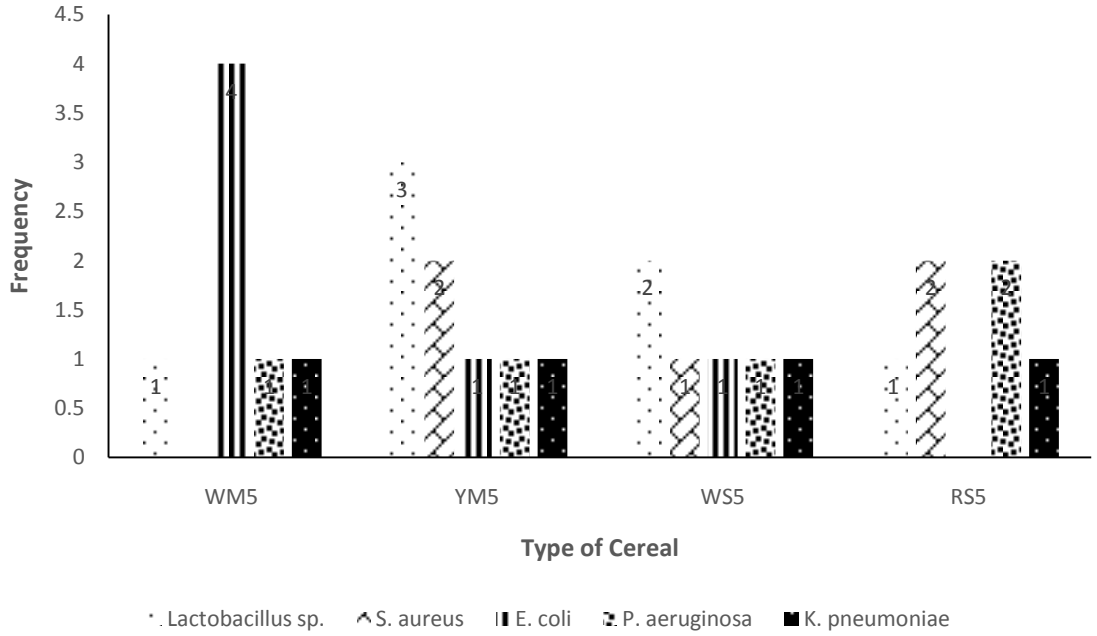

Figure 9: Distribution of bacterial isolates in steeped liqour at 48 h fermentation for 500g weighed un-enriched cereal

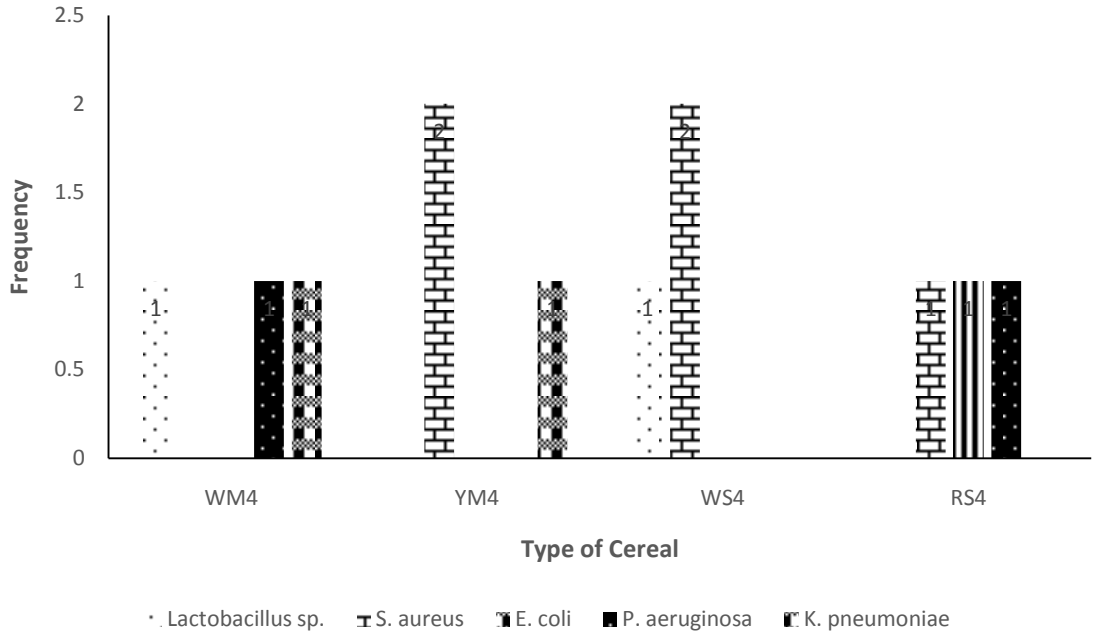

Figure 10: Distribution of bacterial isolates in steeped liqour at 48 h fermentation for 400g weighed enriched cereal

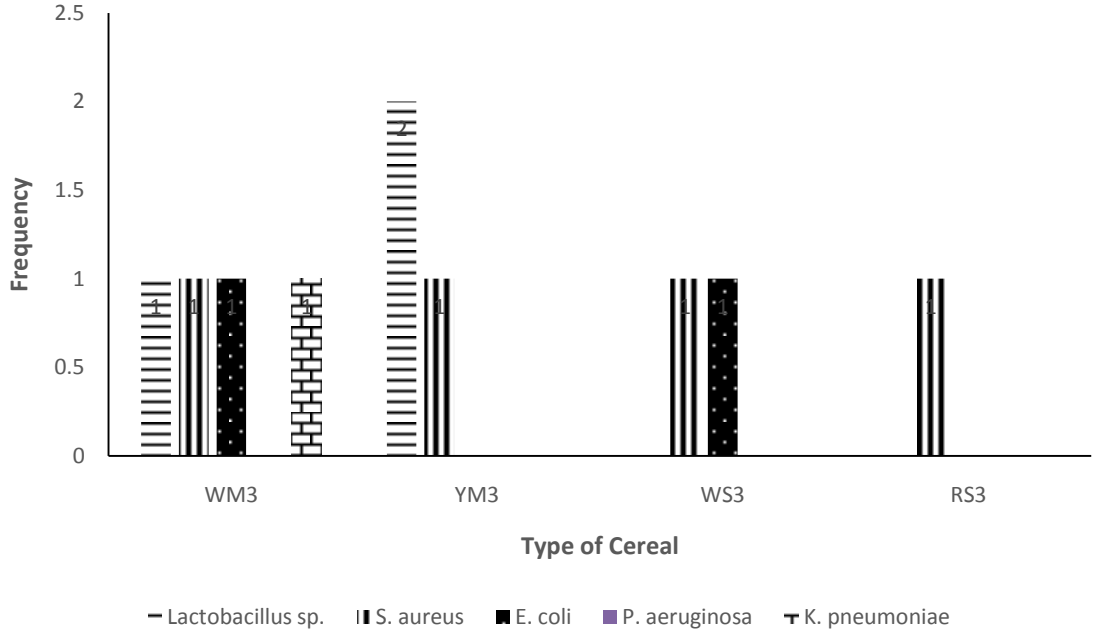

Figure 11: Distribution of bacterial isolates in steeped liquor at 48 h fermentation for 300g weighed enriched cereal

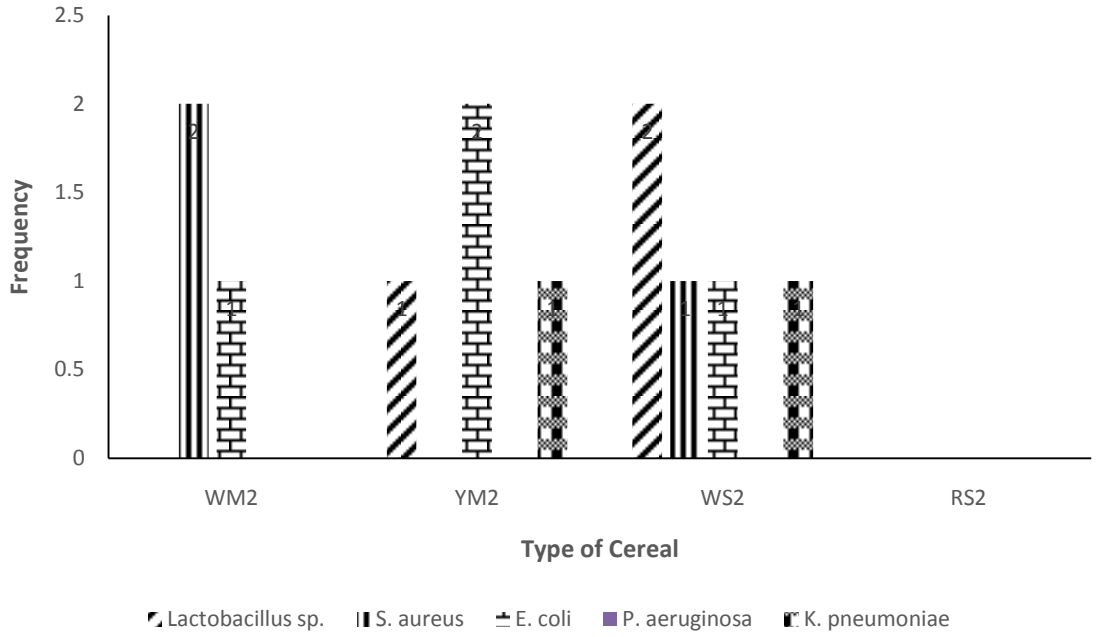

Figure 12: Distribution of bacterial isolates in steepedliqour at 48 h fermentation for 250g weighed enriched cereal

DISCUSSION

Enumeration of the bacterial load/counts of the steeped grain liquor

The result of this study revealed that the increase in bacteria load with time of fermentation must have been due to the microbial build up as the water had not been changed. This finding was in agreement with the report of [15] who reported that over a period of time, the bacterial load in a steeped cereal sample was on the increase. The increase in the lactic acid bacteria count with fermentation as observed in all the fermentation setups could be due to an increase in the acidity and the anaerobic condition of the fermenting medium, favoring the growth of only facultative anaerobes, and/or aciduric organisms. It may also have been as a result of the inhibitory effect of antimicrobial products from the lactic acid bacteria on the growth of unwanted harmful or spoilage organisms [9]. This is in agreement with the report of [17] whom reported a systematic increase of LAB buildup with respect to time. Toxic substances such as hydrogen peroxide, diacetyl, carbon dioxide (CO_2), organic acid, and bacteriocins have been shown to be released by lactic acid bacteria into the fermenting medium during food fermentation [17,19]. These substances are toxic to pathogenic organisms that may be present in the fermenting substrates.

Characterization and distribution of bacteria from unenriched and enriched cereal-based

The bacteria isolated from the purchased cereal samples were *Lactobacillus* species, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.However, the presence of pathogenic organisms (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiellapneumoniae*) observed in the cereal samples bought in the open markets could be attributed directly to poor distribution chain of the cereals, storage methods, poor sanitary conditions of the markets and unhygienic state of handlers. This finding is in line with the reports of who studied the microbial quality of ogi prepared from cereals like maize sold in Bauchi markets, Nigeria. They reported the isolation of *Klebsiella*, *Staphylococci*, *Lactobacillus* and *E. coli*

bacteria. A significant reduction in the growth of *E. coli* and *Klebsiella* specie towards the end of fermentation has been reported by [20], and this must have been as a result of build up of secondary metabolites. Also,the presence of *S. aureus* in the medium at the beginning of fermentation was high. *S. aureus* is ubiquitous, and as a normal flora of the skin and nasal cavity of man, it might have been unhygienically introduced during washing of grains and other activities which led to its introduction as contaminant. The higher prevalence of rod-shaped LAB in this study corroborated a previous study by [25]. Also, [28] reported that *L. plantarum* and *L.lactis* strains were also isolated at a high frequency of 24.6 and 26.3%, respectively during fermentation of akamu. Previous studies have revealed that *L. plantarum* is a highly versatile species capable of colonizing different environments, such as fermented cereals, legumes, fruits and meat, as well as being an inhabitant of the human and animal gut-intestinal tract [22]. Its presence in akamu only confirms its adaptive characteristics to a broad range of environments *E coli* from white maize and Red sorghum was the most distributed with frequency of 5±0.052 among cereals of 500g weight, while the least was *Lactobacillus* speciewith a frequency of 1±0.017. Previous reports by [8,11,15,18] have also isolated *E. coli* in the fermentation of maize for ogi production, and they have been reported to show exponential activities. At 400g measured weight, *E. coli* in yellow maize and white maize were the most distributed with a frequency of 3±0.128, while the least were *Lactobacillus* species, *S. aureus*, and *P. aeruginosa* from acrossall the cereal grains. For 300g weighed enriched cereal. It revealed that *P. aeruginosa* and *S. aureus* from white maize and yellow maize respectively were the highest distributed, with a frequency of 3±0.008. The least distributed was *E. coli* from yellow maize, white sorghum and red sorghum with a frequency of 1±0.022.

CONCLUSION

There was an increase in bacteria load with respect to fermentation time from 1.130 x 10^4 at zero fermentation to 2.28 x 10^4 after 48 h of fermentation. This must have been due to the microbial build up as the water had not been changed. The increase in the lactic acid bacteria count with fermentation as observed in all the fermentation setups could be due to an increase in the acidity and the anaerobic condition of the fermenting medium, favoring the growth of only facultative anaerobes, and/or aciduric organisms. It may also have been as a result of the inhibitory effect of antimicrobial products from the lactic acid bacteria on the growth of unwanted harmful or spoilage organisms.Morphological characteristic identified the organisms as *Pseudomonas aeruginosa, Klebsiella pnuemoniae*, *Escherichia coli*, *Lactobacillus* species and *Staphylococcus aureus.* With *E coli* from white maize and Red sorghum being the most distributed with frequency of 5±0.052 among cereals.

REFERENCES

1. Abioye, V.F. and Aka, M.O. (2015). Proximate Composition and Sensory Properties of Moringa Fortified Maize-*Ogi*. *Journal of Nutrition and Food Sciences*, **12:** 5-10
2. Achi, A. O (2005) Traditional fermented protein condiments in Nigeria. *African Journal of Biotechnology,***4** (13): 1612-1621
3. Achi, O. K. and Ukwuru, M (2015). Cereal-Based Fermented Foods of Africa as Functional Foods. *Int. J. Microbiol. Appl.* **2** (4): 71–83.
4. Agarry, O., Nkama, O. I. and Akoma, O. (2010) Production of Kunun-zaki (A Nigerian fermented cereal beverage) using starter culture.

*International Research Journal of Microbiology,***1** (2): 018-025

5. Agbor-Egbe, T. and Lape, M.I. (2006) The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. *Journal of Food Composition and Analysis,* **19**: 354-363.
6. Bankole SA, Adebanjo A (2003b) Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology,* **2**: 254-263.
7. Bankole, S.A. and Adebanjo, A. (2003). Aflatoxin contamination of dried yam chips marketed in Nigeria.Tropical Science **43:** 3-4.
8. Bankole, M. O. Consumer's Knowledge, Attitude, Usage and Storage Pattern of Ogi–A Fermented Cereal Gruel in South West, Nigeria. *Food Public Health*. 2015, 5(3), 77–83.
9. Cisse, M., Megnanou, R., Kra, K.A.S., Soro, R.Y. and Niamke, S. (2013). Physicochemical, biochemical and nutritive properties of QPM and regular maize flours grown in Côte d'Ivoire. *International Journal of Research in BioSciences,* **2**: 26-32.
10. Coreym M. (2009). *Ph.D. Thesis.* University of Georgia; Athens, GA, USA: 2009. Developing functional food products through novel processing, ingredient, and shelf stability evaluation.
11. Correia, I., Nunes, A., Guedes, S., Barros, A.S. and Delgadillo, I. (2010). Screening of lactic acid bacteria potentially useful for sorghum fermentation. *Journal of Cereal. Science,* **52**: 9–15.
12. Curiel J.A., Rodríguez H., Acebrón I., Mancheño J.M., de las Rivas B., Muñoz R. (2009). Production and physicochemical propertiesofrecombinant *Lacto bacillus plantarum* tannase. *J. Agric. Food Chemistry,* **57**: 6224–6230
13. Dandessa C. (2019). Review on Ethiopian traditional fermented foods, its microbial ecology and nutritional value. *Int. J. Curr. Res. Acad. Rev,* **7**: 13–27.
14. Etuk E.B., Okuedo N.J., Esonu B.O. and Udedibie, A.B.I. (2012). Antinutritional factors in sorghum: Chemistry, mode of action and effects on livestock and poultry. *Online J. Anim. Feed Res,.***2**: 113–119.
15. Evers, A. D. (2012). Ash determination - a useful standard or a flash in the pan; 2001. Retrieved on October 20,2012
16. Eze, V.C., Eleke, O.I. and Omeh, Y.S. (2011). Microbiological and nutritional qualities of *burukutu* sold in mammy market Abakpa, Enugu State, Nigeria. *Am. J. Food Nutrition,* **1**:141–146.
17. Ezedinma, F.O.C. (1964).The soyabean in Nigerian. *Proceeding of the Agriculture society of Nigeria.* pp13-16. Ibadan. October 1964.
18. Food and Agriculture Organization (2000) United Nations Food and Agricultural Organization, Evaluation of Certain Mycotoxins in Food: Fifty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva.
19. Food and Agriculture Organization of the United Nations (FAO). (2005). The cassava transformation in Africa. Rome: P 32
20. Franz C.M.A.P., Holzapfel W.H. Examples of lactic-fermented foods of the African continent. In: Vinderola G., Ouwehand A., Salminen S., von Wright A., editors. *Lactic Acid Bacteria: Microbiological and Functional Aspects.* CRC Press; Boca Raton, FL, USA: 2019. pp. 235–254

21. Ijabadeniyi, A.O. (2007) Microbiological safety of gari, lafun and ogiri in Akure metropolis, Nigeria. *African Journal of Biotechnology,***6** (22): 2633-2635.
22. Ijabadeniyi, A. and Adebolu, T. (2005). The Effect of Processing Methods on the Nutritional Properties of Ogi Produced from Three Maize Varieties. *J. Food Agric. Environ*, **3**: 108–109.
23. Ijabadeniyi, A. O. (2007). Micro organisms associated with ogi traditionally produced from three varieties of Maize. *Research Journal of Microbiology,* **2** (3): 247–253.
24. Iken, J. E., Amusa, N. A. and Obatolu, V. O. (2002). Nutrient composition and weight evaluation of some newly developed maize varieties in Nigeria. *Journal of Food Technology in Africa,***7**: 27-29.
25. Iken, J. and Amusa, N. (2010). Consumer Acceptability of Seventeen Popcorn Maize (Zea Mays L.) Varieties in Nigeria. *Afr. J. Agric. Res*, **5**(5): 405–407.
26. Proteins Advisory Group (1975). Guideline on protein-rich mixtures for use as supplemental foods. The pag compendium vol. E. Worldmark Press Ltd., John Wiley and Sons, New York, 63.
27. Pulver, E.L.; Kueneman, E.A. & Ranga-Rao, V. (1985). Identification of promiscuous nodulating soybean efficient in N_2 fixation. *Crop Science* 25:1065-1070
28. Shayo N.B., Kamala A., Gidamis A.B. and Nnko, S.A. (2000). Aspects of manufacture, composition and safety of *orubisi*: A traditional alcoholic beverage in the north-western region of Tanzania. *Int. J. Food Sci. Nutr.* **51**: 395–402.
29. Shekhar, H., Uddin, H., Zakir H. and Kabir, Y. (2016). *Exploring the Nutrition and Health Benefits of Functional Foods*. IGI Global. p. 223
30. Shu, X.O.; Zheng, Y.; Cai, H.; Gu, K.; Chen, Z.; Zheng, W. & Lu W. (2009). Soy food intake and breast cancer survival. *The Journal of the American Medical Association.* 302(22):2437-2443
31. Shurtleff, W. & Aoyagi, A. (2009). *History of soybeans and soyfoods in Africa (1857-2009).* SoyInfo Center. ISBN: 978-19289114-25-9. Lafayette, CA.
32. Simango C. and Rukure G. (1991).Survival of *Campylobacter jejuni* and pathogenic *Escherichia coli* in *mahewu*, a fermented cereal gruel. *Trans. R. Soc. Trop. Med. Hyg.* **85**: 399–400
33. Van der Aa Kühle A., Jespersen L., Glover R.L., Diawara B. and Jakobsen, M. (2001). Identificationandcharacterizatio nof *Saccharomycescerevisiae* st rains isolated from West African sorghum beer. *Yeast.* **18**: 1069–1079.
34. Yousif N.M.K., Dawyndt P., Abriouel H., Wijaya A., Schillinger U., Vancanneyt M., Swings J., Dirar H.A., Holzapfel W.H. and Franz, C.M.A.P. (2005). Molecular characterization, technological properties and safety aspects of enterococci from 'Hussuwa', an African fermented sorghum product. *J. Appl. Microbiol.* **98**: 216–228.
35. Yousif N.M.K., Huch M., Schuster T., Cho G.S., Dirar H.A., Holzapfel W.H. and Franz, C.M.A.P. (2010). Diversity of lactic acid bacteria from P*Hussuwa*, a traditional African fermented sorghum food. *Food Microbiol.* **6**: 757–768.
36. Zhai Q., Guo Y., Tang X., Tian F., Zhao J. and Zhang, H. (2019). Removal of cadmium from rice by *Lactobacillus*

plantarum fermentation. *Food Cont.* **96**: 357–364.