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NUTRIENT COMPOSITIONS OF DIFFERENT GRAINS FOR USE IN THE FORMULATION OF BACTERIOLOGICAL MEDIA

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ABSTRACT

Some grains were analysed for their nutrient compositions for use in the formulation of bacteriological media in consideration of cheap and effective alternative to the conventional media. The media were respectively formulated using Acha (*Digitaria exilis*), Maize (*Zea mays*), Rice (*Oryza sativa*), Guinea corn (*Sorghum species*) and Millet (*Pennisetum glaucum*). Proximate analyses of the grains showed reasonable amounts of carbohydrates (55.0 to 73.9%), moisture contents (9.92 to 11.25%) and crude proteins (7.39 to 11.77%). Crude fibre and ash had the least % in the five grains used. Amongst the macro-elements, carbon is the most abundant followed by nitrogen. Using atomic absorption spectrophotometer (AAS), Zn, Co, Cu and Mo were not detectable in the grains, whereas Mn was present in minute quantities (≤ 0.015%). All the bacteria assessed grew on the formulated media. Statistical analysis of the data indicated homogeneity in growth rates of the test bacteria on most of the media formulated. However higher microbial counts observed on millet extract agar (MIEA) suggests that the medium could serve as an alternative growth medium for bacteria in place of the conventional nutrient agar (NA) with reduced cost.

Contribution/Originality: This study is one of the very few studies which have investigated nutrient compositions of different grains for use in the formulation of bacteriological media.

1. INTRODUCTION

Many microorganisms, including bacterial cells, are grown in the laboratory culture media. Cellular biosynthesis and energy generation are two most critical demands of most microorganisms attained by the metabolism of the nutrients in the growing environment of the organisms. The nutritional requirements of a bacterium such as *E. coli* correlates with the cells elemental composition consisting of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulphur (S), phosphorous (P), potassium (K), magnesium (Mg), iron (Fe), calcium (Ca), manganese (Mn) and traces including zinc (Zn), cobalt (Co), copper (Cu) and molybdenum (Mo) (Todar, 2012). These various elements are contained in the media where the organisms grow. Various studies on nutritional requirements for the growth of microorganisms have been identified to include sources of carbon, nitrogen, phosphorus, sulphur and metal ions including iron, protein and minerals (Arora and Arora, 2011). Bacteria utilise carbon for energy and growth.

For the design of a culture medium, determination of the nutrients needed is of paramount importance. The determination of the source of such components and the exact concentrations of each component must be taken into

account in the formulation of medium for microbiological work. Consequently, analysis of the nutrient composition of the media components is critical (Ogbonna, 2013).

The use of culture media dates back to the time of Louis Pasteur where simple broths were used, and Robert Koch who used potato pieces to grow bacteria. However, the need to get an easily assessable and cheap media cannot be overemphasised. Bacteriological media are used to isolate bacteria grow and identify bacteria. It is consequently used for the diagnosis of clinically important bacteria. Cultured bacteria are useful in obtaining antigens for serological work, genetic studies and many other biotechnological purposes. There are many types of microbiological culture media and the choice of which one to use is dependent on the purpose, affordability and cost. The reason for the varieties of culture media is not separated from the fact that microorganisms vary greatly in their nutritional requirements. The number, morphology, colour and pattern of growth of bacteria are largely determined by the type of media.

Some potential sources of components of industrial media include carbohydrate sources namely cassava, sweet potato, yams, cocoyam, millet, rice, sorghum, and Jerusalem artichoke. Others sources are of protein origin and include peanut meal, blood meal and fish meal (Okafor, 2007). There is a high potential for the production of cereal grains in Nigeria, giving the tropical climate and vegetation type and the favourable soil types. Nigerian is ranked high in the world production of these grains. The present work not only assessed the nutrient compositions of different grains for use in the formulation of bacteriological media but also evaluated the growth of some bacteria on the formulated media.

2. MATERIALS AND METHODS

Milled grains of Acha (*Digitaria exilis*), Guinea corn *(Sorghum bicolor*), Maize *(Zea mays)*, Rice (*Oryza sativa)* and Millet *(Pennisetum glaucum)* were purchased from a local grocery market in Lafia, Nasarawa State, Nigeria. Five grams each was separately decocted in 2 litres of distilled water by cooking for 30 min. Upon cooling, it was carefully filtered through a clean sheet of muslin cloth. Two hundred millilitres of the resulting extract of each grain was poured into 500 mL conical flasks solidified by adding 1.5% agar and sterilized by autoclaving at 121 ^{o}C for 15 min under pressure at 15 psi. The procedure adopted was that used by Ochei and Kolhatkar (2008) in the preparation of cornmeal extract agar. The pH of the medium was adjusted to 7.2 by the addition of sodium hydroxide.

2.1. Test Organisms

The test bacteria were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella* and *Proteus vulgaris*. They were obtained from Microbiology Laboratory at the National Veterinary Research Institute Vom, Plateau State, Nigeria and reinoculated into appropriate media for before further confirmation before use.

2.2. Determination of Bacterial Count.

This was carried out using standard microbiological techniques. A loopful of the organism contained in the agar slant was first serially diluted up to 10^{-6} folds and 0.1 mL of appropriate dilution was used to inoculate each of the plates.

2.3. Determination of Proximate Composition of the Grains

Proximate compositions of the samples were determined using standard methods as presented below. Analysis carried out where the moisture content, crude fibre, ash content, crude protein and total carbohydrate.

2.4. Determination of Moisture Content

All samples were separately crushed using the laboratory mill into fine powders. Forty grams (40 g) of each sample was weighed in a clean dish that has been weighed and recorded. The samples were transferred into a hot air oven at 105 °C for 24 h. After which the sample was removed and allowed to cool in desiccators for 1 hour and weighed. This was repeated until a constant weight was obtained. Percentage of moisture content was calculated as contained in equation 1.

$$\% \text{ moisture} = \frac{\text{loss in weight}}{\text{weight of sample before drying}} \times 100 \qquad \text{(Equation 1)}$$

2.5. Crude Fibre Determination

Five gram (5 g) each of the deflated samples was taken into 500 mL conical flasks, 200 mL of boiling (2.25%) sulphuric acid was added and boiled for 30 min, filtered and rinsed with distilled water. Sewage of the residue was transferred into crucibles after drain, dried at 105 °C, cooled in the desiccator and weighed. It was then placed in a muffle furnace at 300 °C for 30 minutes. After that, it was removed and kept in the desiccators to cool and weighed again.

2.6. Determination of Crude Protein

A 0.5 g each of the samples was weighed into 50 mL Kjeldahl flask and 5 mL concentrated sulphuric acid was added with a catalyst. The flask was treated gently then strongly until it digested completely leaving a clear (grey-white) digests. It was allowed to cool, filtered, washed and made up to 50 mL volume of the volumetric flask. The digest was distilled with 40% sodium hydroxide through 5 mL 2% boric acid solution. Three drops of mixed indicator were used and 40 mL of each sample was collected and back titrated using 0.01 M HCl. The % Nitrogen content was calculated according to the equation (equation 2)

$$\% \text{ Nitrogen} = \frac{(\text{mL standard acid} - \text{mL blank}) \times \text{N of acid} \times 1.4007}{\text{weight of sample in grams}} \qquad \text{(Equation 2)}$$

2.7. Determination of Total Carbohydrate

Carbohydrate content was determined using Anthrone reagent method. The samples and 5 mL of 2.5 M hydrochloric acid were kept on boiling water to hydrolyse for 3 hours. Sodium carbonate (solid) was used to neutralize the solution until there was no effervescence. The volume was made to 100 mL and centrifuged. Half millilitre (0.5 mL) and one millilitre (1 mL) aliquots were collected for analysis. A standard calibration curve was made with serial dilutions of the standard. The standards and samples were added 4 mL of Anthrone reagent and heated for 8 minutes. Samples standard and blank were read calorimetrically at 630 nm.

Carbohydrate was determined using the relationship (equation 2)

$$\frac{\textit{optical density test} \times \textit{Concentration of standard used}}{\textit{optical density standard} \times \textit{volume of the sample used}} \times 100 \qquad \text{(Equation 3)}$$

2.8. Determination of Nutrient Elements Contained in the Grains

2.8.1. Sample Preparation

Each of the grains was put in an oven, dried in about 96 °C and was grounded using porcelain mortar and pestle into a fine powder. Zero point two grams (0.2 g) of each sample was weighed into 50 mL volumetric flask and dissolved with 6 M HNO_3 and made to a volume of 50 mL. One millilitre (1 mL) was taken into 25 mL volumetric flask and made to volume (25% dilution). Each of this was read for Fe, Ca, Mn, Zn, Co, Cu, and Mo using a variant.

Each of the metal has its wavelength that is shown on Atomic Absorption Spectrophotometer (AAS). A standard was prepared for each of the metals and a graph was plotted and extrapolated.

2.9. Determination of Phosphorus Using Vanadomolybdate Method

A 0.2 g of each grounded powder was put into 100 mL beaker, 30 mL of mix acid (Nitric acid, sulphuric acid and perchloric) was added and heated to about 150 – 170 ^{o}C until the nitric acid escaped and digest reduced to a volume of about 10 mL when dense fumes were noticed coming out. Then the digest was transferred to 50 mL volumetric flask. A standard was prepared and read through a spectrophotometer (ammonium molybdate + ammonium metavanadate.

$$\frac{100\ ppm\ phosphorus\ standard}{comic/absorbance} \qquad \text{(Equation 4)}$$

$$\text{Calculate} \qquad \frac{PPM\ from\ graph\ x\ 50\ x\ 50\ x\ 100}{0.2\ x\ dilution}$$

2.10. Determination of Sulphur Using Barium Chloride Method

A standard of sulphur was prepared in the range of 1, 2, 3, 4, 5, 6. One millimetre (1 mL) of the sample was pipetted into 50 mL volumetric flask for each sample. One gram (1 g) of $BaCl_2$ and 1 mL of gelatine were added. The mixture was allowed to stay for 90 minutes for the colour to develop in a spectrophotometer. A graph was plotted with the standard and extrapolated.

2.11. Determination of Total Organic Carbon

Walkley-Black method of Determining Total Organic Carbon was employed. Five millilitres (5 mL) Potassium dichromate ($K_2C_{r2}O_7$) and 10 mL concentrated H_2SO_4 was added to 0.2 g of each sample. The solution was swirled and allowed to cool prior to adding water to halt the reaction. As a result, a correction factor of 1.33 was applied to the result to adjust the organic carbon recovery. Upon completion of the sample extraction, the quantity of organic carbon present in the sample was determined by manual titration. An indicator ortho-phenanthroline complex solution was added to the digest. The excess Cr_2O^{2-} was titrated with ferrous sulphate ($FeSO_4$). The colour change was green to reddish brown.

The titre value was used to calculate for g/dm^3 using the relation $C_1V_1 = C_2V_2$ and the result multiplied by correction factor 1.33 to obtain the total organic carbon.

2.12. Statistical Analysis

Statistical analysis (using SPSS) was carried out using analysis of variance (ANOVA).

3. RESULTS

3.1. Growth of *Staphylococcus aureus* on the Media

In day 1, there was no growth on all the media. However, the colony increased after 24 h to the fifth day of incubation where Nutrient Agar (NA) showed the highest no of colonies followed by MEA, MIEA, MZEA and REA having an equal number of colonies while AEA showed the least number (Fig. 1).

3.2. Growth of *Escherichia coli* on the Media

In day 1, there was no growth on all the media. However, in day 5, MEA and NA supported the growth showing the highest number of bacteria colonies followed by MIEA (Fig. 2).

3.3. Growth of *Salmonella typhi* on the Media

In day 1, there was no growth shown on all the media. In the fifth day of the experimental period, it was observed that NA, the control medium, showed the highest growth followed by MEA. MIEA was the formulated medium that had the highest number of colonies (Fig. 3).

3.4. Growth of *Klebsiella* species on the Media

The bacterium did not show growth on all the media on the first day. While the experiment remained, growth increased up to day 5, where MEA and NA showed equal and highest number of colonies. GEA showed the least growth (Fig. 4).

3.5. Growth of *Proteus vulgaris* on the Media

No growth of the bacterium on any of the media on day 1. At the end of the experimental period on day 5, MIEA showed the highest number of colonies followed by MZEA respectively (Fig. 5).

The comparison of the time course of bacterial growth on the formulated media showed that *S. aureus*, *S. typhi*, *Klebsiella* species, *P. vulgaris* showed equal growth on MZEA. *P. vulgaris* was highest on MIEA, AEA. *E. coli*, *S. typhi*, *Klebsiella* spp and *P. vulgaris* showed equal growth (Fig. 6).

3.6. Proximate Composition of Grains Used in the Formulation of the Media

Proximate composition of grains is shown in Table 1. Millet has the highest moisture content as compared to Acha, Guinea corn, Maize and rice. Maize has the highest crude fibre compared to millet, guinea corn and acha. Maize, millet and rice have equal amounts of ash while acha and guinea corn have lower values. Millet has the highest value of crude protein while guinea corn has the lowest protein content. Guinea corn has the highest carbohydrate level of 73.9 %. The range of values of moisture content, crude fibre and ash were 9.92 – 11.25, not detectable to 3.2, 1 ± 0.1 - 2 ± 0.1 respectively. Crude protein and total carbohydrate ranged from 7.39 – 11.77 and 46.38 – 73.9 respectively.

3.7. Percentage Nutrient Element Contained in the Grains

Millet has the highest percentage of carbon as compared to maize, guinea corn, rice and Acha (Table 4). However, these values were not significantly different. Amongst the macro- elements, carbon is the most predominant followed by nitrogen which is a component of amino acid. Most of the trace elements analysed namely Zn, Co, Cu, Mo were not detectable in the grains, whereas Mn was present in minute quantities in some of the samples. Calcium, a macronutrient was also not detectable in any of the grain samples.

4. DISCUSSION

When bacteria were inoculated on the culture media, there was no immediate increase in cell colonies. This could probably be because the cells were synthesizing new components which are in the lag period, a key phase prior to the start of cell division. Adenosine triphosphate (ATP), essential co-factors and ribosomes must be synthesized before growth can begin (Prescott *et al.*, 2012). In day 1, there was no growth on all the media. Subsequently, there were little variations in the growth of the *S. aureus* on the media although no definite format was followed in the progression of growth of the organism in the different media. However, the highest numbers of colonies formed were on NA followed by MEA and MiEA.

4.1. Escherichia coli

In day 1, there was no colony increase in any of the media. This could be because the bacterium has to synthesize new components. There was no clear-cut difference in the growth of *Escherichia coli* in the different

formulated media. In day 5, no significant difference in the growth of the bacteria on MEA and NA, but there are significant differences in the growth on the other media. The highest growth was recorded on MEA and NA. Both the malt extract agar (MEA) and nutrient agar (NA) are the standard media used as the control. The implication is that, even though the bacterium could grow on the formulated media, optimal growth was observed in the conventional media.

4.2. Salmonella typhi

In day 1, there was no growth on all the media. In days 4 and 5, there were lots of variations in the growth of the organism. There was a significant difference in growth on MEA and NA, NA and MzEA, MzEA and MiEA, MiEa and GEA, GEA and REA, as well as between REA and AEA. The highest colonies of the bacteria were recorded on NA (the standard medium) while MiEA recorded the highest number of colonies amongst the formulated media.

4.3. Proteus vulgaris

In day 1, there was no colony formed on any of the formulated media probably because the cells have to synthesize ATP. The organism demonstrated several growth differences on the different media during the experimental period. The growth of *P. vulgaris* on MiEA at the end of the experimental period was the highest amongst all the media. The percentage nutrient contained in millet extract agar (MiEA) is 53.54% carbon, 2.98% nitrogen, 0.77% sulphur, 0.38% magnesium, 0.04% iron, 0.015% manganese and 0.28 phosphorous. This could account for its better support of *Proteus vulgaris*.

4.4. Klebsiella

Growth rates of Klebsiella on MEA and NA were almost the same in day 5 of the experimental period. However, there was a significant difference in growth between NA and MzEA, MzEA and MiEA, MiEA and GEA, GEA and REA. Similarly, a significant difference in growth existed between REA and AEA. The highest growths were on MEA and NA however, MiEA best growth environment amongst the formulated media.

The bacterial colonies increased progressively with time. Bacteria such as *S. aureus*, *Clostridium*, *Klebsiella*, *E. coli*, *Salmonella and Streptococcus* can grow on ordinary media (Arora and Arora, 2011). Hence, the test bacteria used in this research did not show a significant difference in growth between the standard and formulated media.

At the end of the experimental period, MiEA demonstrated a most favourable environment for the growth of *P. vulgaris* while NA and MEA as standard media offered a most suitable environment for the growth of *S. aureus*, *S. typhi*, *E. coli* and *P. vulgaris*. However, MiEA stands out as the best media. GEA can be used as a selective medium for fungi while MiEA can be used as a selective medium for bacteria. According to Ochei and Kolhatkar (2008) an amino acid which is a component of protein was found to be a suitable growth factor for bacteria. Millet was shown to have a considerable high amount of crude protein of 11.77 g.

Carbohydrates dominated the proximate compositions of all the grains. Similarly, carbon was the predominant element. In the formulation of the media, additional water was added and this increased the moisture content and water activity (a_w) of the media. Millet has the highest moisture content as compared to others. High or low moisture contents of the grains could be attributed to the drying and storage conditions Acha, Guinea corn, maize and rice. The very low moisture content of rice and acha could be suggested that the two grains lose a considerable amount of water during storage resulting in a longer shelf life (Victor and James, 1991). Maize has the highest crude fibre compared to millet, guinea corn and acha. This could be as a result of the size of the grain. Maize, millet and rice have equal amounts of ash while acha and guinea corn have lower values.

5. CONCLUSIONS

All the bacteria assessed grew on the formulated media. Statistical analysis of the data indicated homogeneity in growth rates of the test bacteria on most of the media formulated. However higher microbial counts observed on millet extract agar (MIEA) suggests that the medium could serve as an alternative growth medium for bacteria in place of the conventional nutrient agar (NA) with reduced cost.

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Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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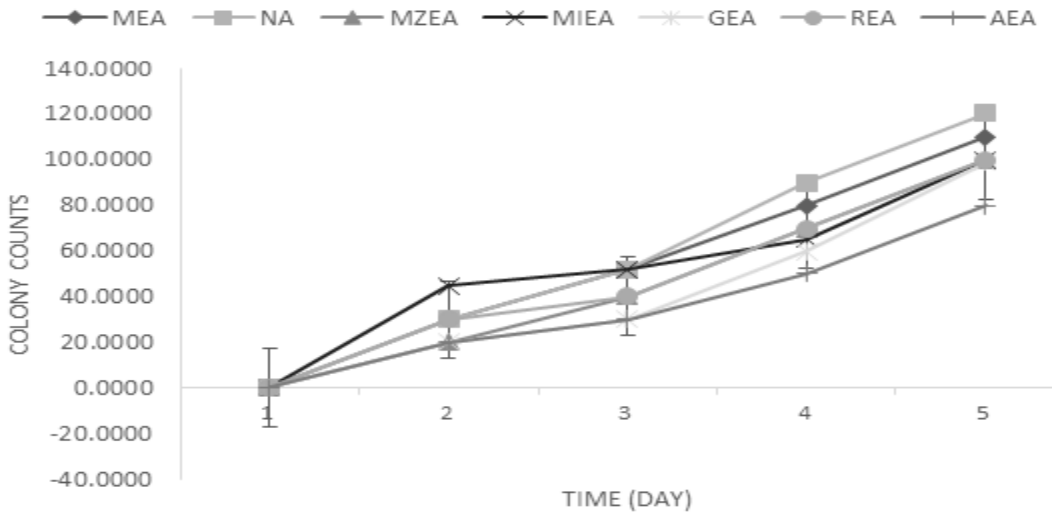

Fig-1. Time course of growth of S. aureus on grown on the formulated media.

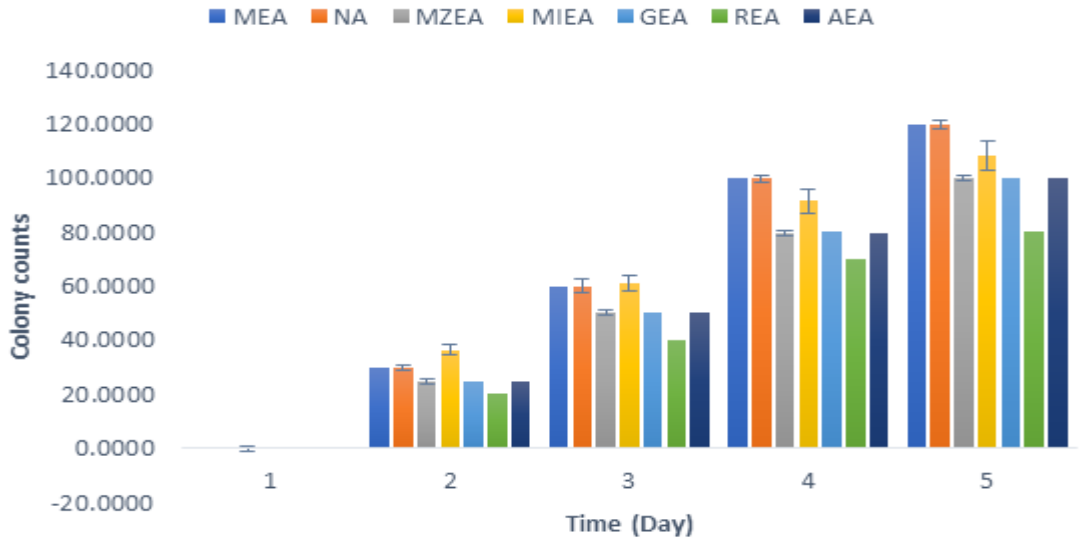

Fig-2. Growth of E. coli on all the media.

Source: Our present study being reported

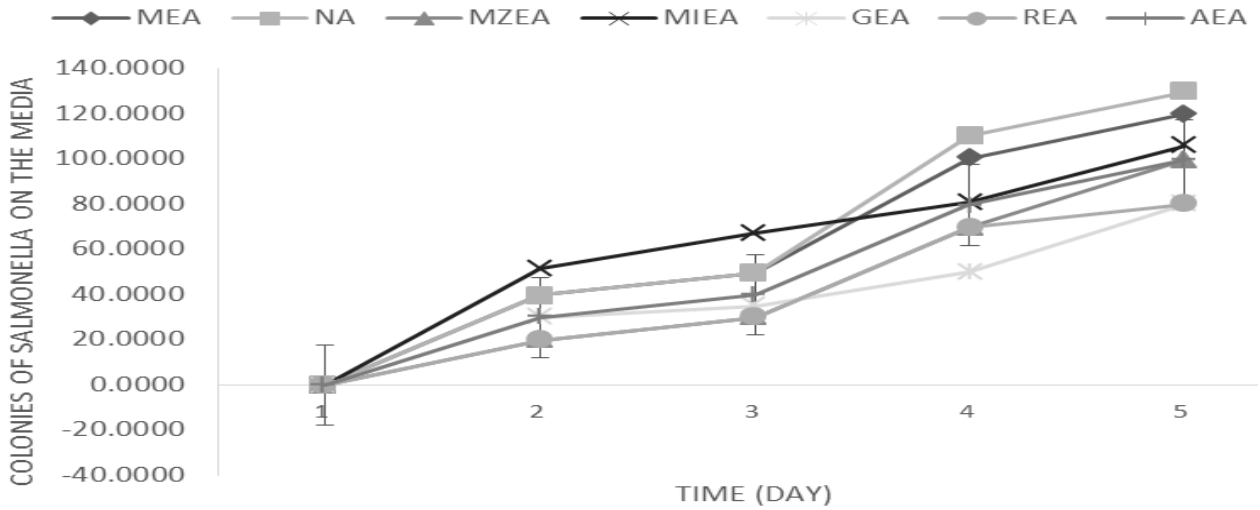

Fig-3. The growth of *S. typhi* on all the media.

Source: Our present study being reported

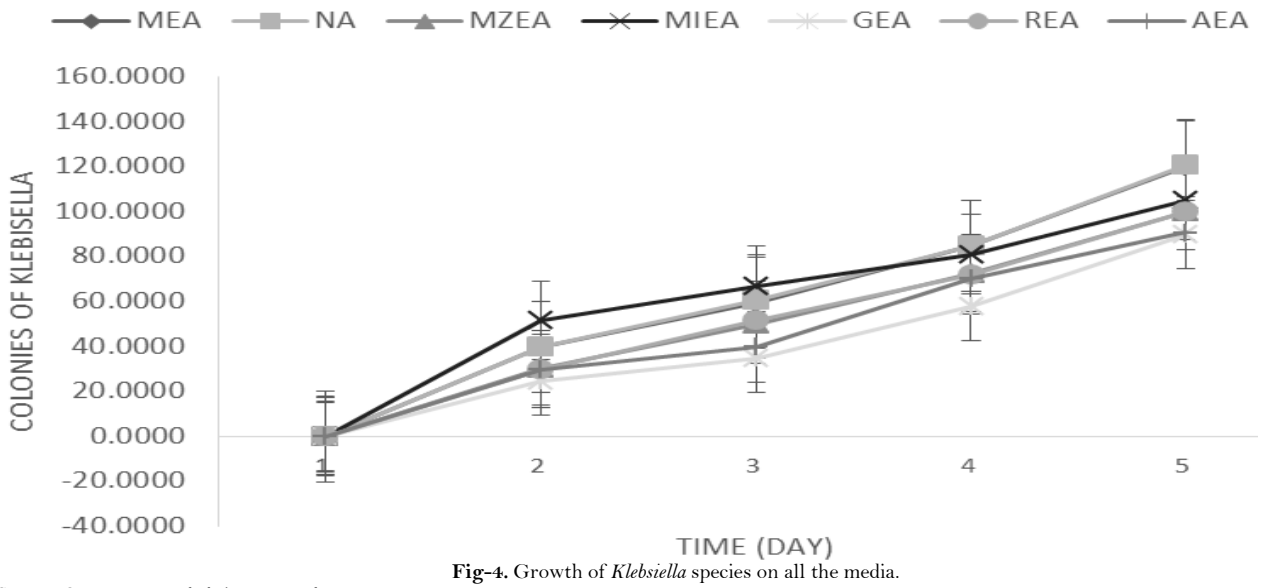

Fig-4. Growth of *Klebsiella* species on all the media.

Source: Our present study being reported

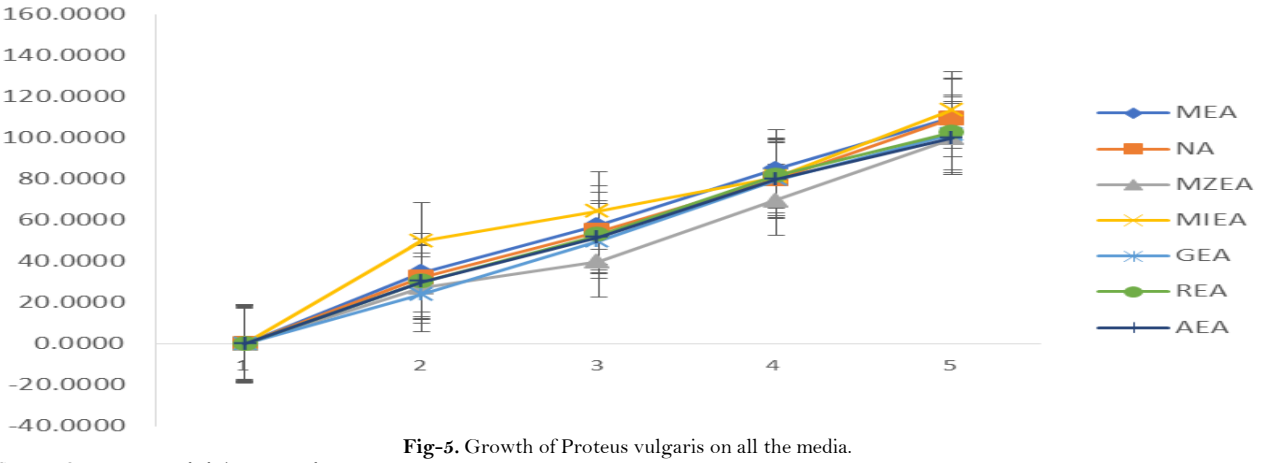

Fig-5. Growth of Proteus vulgaris on all the media.

Source: Our present study being reported

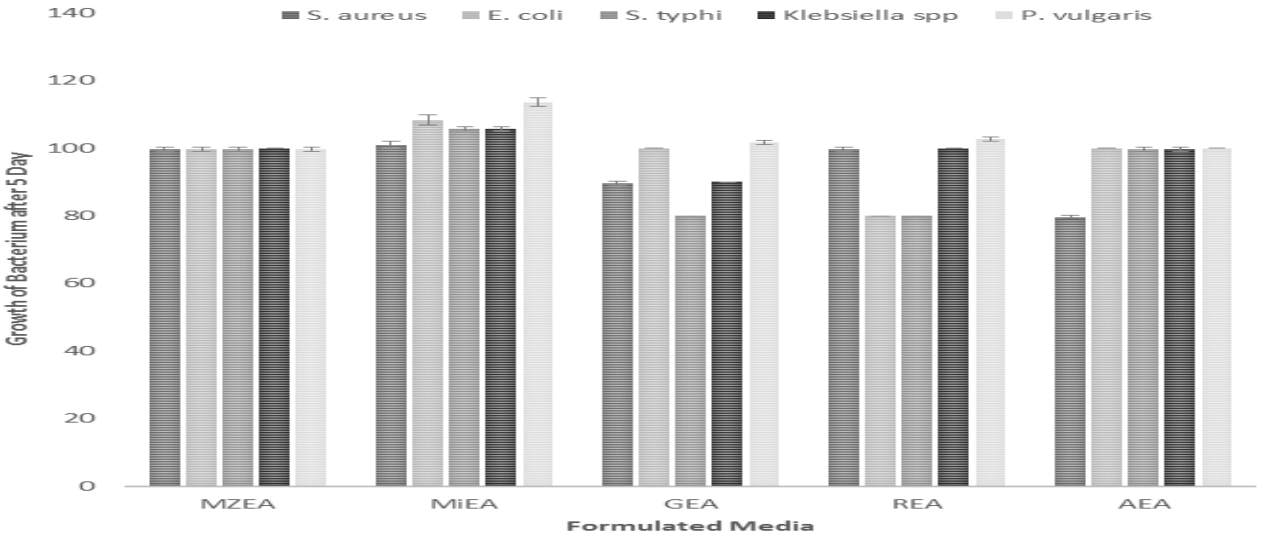

Fig-6. Growth of bacteria on the formulated media

Source: Our present study being reported

Table-1. Proximate compositions of the grains

Grain	Moisture (%)	Crude Fibre (%)	Ash Content (%)	Crude protein (%)	Total Carbohydrate Content mg/100 mL
Acha	10.28	0.8	1 ± 0.01	8.61	46.38
Guinea corn	10.73	1.4	1 ± 0.01	7.39	73.9
Maize	10.65	3.2	2 ± 0.01	10.04	56.52
Millet	11.25	2.0	2 ± 0.01	11.77	60.87
Rice	9.92	ND	2 ± 0.01	9.06	55.0

Source: our previous study (Adapted from Maikasua *et al.* (2018))

Table-2. Percentage (%) nutrient/element in grains.

Sample name	C	N	S	Mg	Fe	Ca	Mn	Zn	Co	Cu	P	Mo
Maize	44.85	7.00	0.74	0.26	0.24	ND	ND	ND	ND	ND	0.45	ND
Guinea corn	43.74	4.38	0.96	0.42	0.29	ND	0.005	ND	ND	ND	0.35	ND
Acha	38.55	3.67	0.70	0.24	0.18	ND	0.0006	ND	ND	ND	0.34	ND
Millet	53.45	2.98	0.77	0.38	0.04	ND	0.015	ND	ND	ND	0.28	ND
Rice	42.40	3.41	0.88	0.07	0.13	ND	0.014	ND	ND	ND	0.23	ND