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PRODUCING *BACILLUS* FROM THE RIPE BANANAS, JACKFRUIT SEEDS AND SWEET POTATO TUBERS BY SOLID FERMENTATION AND ITS EFFECT ON THE PRODUCTS' BIOCHEMICAL PROPERTIES

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Background: Fermentation is defined as a biochemical process that produces energy using microorganisms from various raw material substrates. It is an ancient form of bio-preservation of food and improvement of its nutrient content. In addition to enriching the nutritional components of plant materials and preserving food, fermentation promotes the growth of beneficial microbes that play a role in human and animal health, especially in the digestive tract, commonly known as probiotics. **Objectives:** The current study investigated the fermentation of ripe banana, jackfruit seeds and sweet potato tubers to produce *Bacillus subtilis* and also examined the effect of fermentation on the biochemical properties of the plant materials used in the current study. The mentioned plant materials are the most cultivated crops in many parts of Uganda and generate a lot of food waste. **Methods:** The study was carried out at the soil laboratory of Mountains of the Moon University in Fort Portal tourism western Uganda. The solid fermentation of ripe bananas, jackfruit seeds and sweet potato tubers in this study was a natural process hence no inoculation of microbes onto the sample was done. Solid fermentation involved the putting the solid substrates into fermentation vessel with limited moisture. In every 3 days, temperatures, relative humidity and pH were monitored in the fermentation vessels to establish the conditions in which microbes are growing. Gram staining was done on the sub cultured microbial colonies to view the shapes of the cells and endospore structures of the *Bacillus species*. There were 2 biochemical tests performed on sub cultured colonies i.e. catalase test and starch hydrolysis to confirm the presence of *Bacillus* in the fermented samples of ripe banana, jackfruit seeds and sweet potato tubers. For catalase tests: the microbial colonies from nutrient agar on petri-dishes of ripe banana, jackfruit seeds and sweet potato tubers cultures were transferred and smeared on microscope slides. For starch hydrolysis tests, the sub cultured microbial colonies from ripe banana, jackfruit seeds, sweet potato and mix petri-dish were transferred to starch agar. **Results:** There was decrease in carbon-nitrogen ratio due to fermentation effect except the ripe banana which experienced an increase in carbon content from 2.44 carbon: 2.8 nitrogen to 3:12 carbon: 1.7 nitrogen. In additional to that, ripe banana produced highest population of microbes with colony forming units of $1.6 \cdot 10^4$ on day $3.4 \cdot 10^4$ on day 6, $1.05 \cdot 10^5$ and $1.08 \cdot 10^5$ on day 12. **Conclusion:** All the fermented samples ripe banana, jackfruit seeds, sweet potato tubers and mixed samples contained *Bacillus* since their microbial colonies displayed rod shaped cells and gram positive and they tested catalase. Fermentation improves the biochemical properties of ripe bananas as evidenced by increase of crude proteins and fats and carbon. For samples jackfruit seeds, fermentation negatively affect the biochemical properties since they lost carbon, crude proteins and fat.

Keywords: fermentation; *Bacillus*; biochemical properties; microbial production; beneficial microbe.

INTRODUCTION

In the study by Attri & Goel (2023) fermentation is defined as a biochemical process which produces energy by use of microorganisms from different raw substrates. It is the ancient form of bio-preservation of food stuffs and improves their nutrient content. There are four major types of fermentation such as: (i) alcohol fermentation is the process in which ethanol is produced by fermentation by yeasts which are the predominant organisms (e.g., wines and beers); (ii) lactic acid fermentation is mainly work of lactic acid bacteria (LAB) and occurs chiefly in cereals and milk products; (iii) acetic acid fermentation is work of *Acetobacter* species. *Acetobacter aceti* converts alcohol to acetic acid in the presence of excess oxygen; (iv) alkali fermentation takes place during the fermentation of either fresh poultry eggs, fish, seeds, or any protein rich raw materials and this type of fermentation is popularly used as condiments (Kaur et al., 2019).

In addition to enhancing the nutritional components of plant materials and preserving foods, fermentation promotes the growth of beneficial microbes which play a role in human health as well as animal health, especially in digestive tract, commonly known as probiotics (Soemarie et al., 2021). During that process, several genera are produced and they are used as probiotics, including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Pediococcus*, and several yeasts. Current research focuses on the production of *Bacillus* species.

Bacillus subtilis is a gram positive bacterium in the genus of *Bacillus* with in the family of *Bacillaceae* (Su et al., 2020; Nayak, 2021). It is renowned for its versatility and potential applications in agriculture productions such as:

– it is probiotic in aquaculture as enhancing feed utilization, immune system, stress response, antioxidant defines, water quality, reproductive health, and disease resistance (Olmos & Paniagua-Michel, 2014);

– it improves on soil health, promoting plant growth and biological control for plant pathogens in additional to conferring biotic and abiotic stress tolerance to plants by induced systemic resistance (ISR), biofilm formation and lipopeptide production (Mahapatra et al., 2022);

– it is used as a cell factory for microbial production of chemicals, enzymes, and antimicrobial materials for industry, agriculture, and medicine (Su et al., 2020).

Based on these facts, *Bacillus subtilis* plays a positive role of in agriculture and other aspects of sustainable development. This is why this study explored the fermentation of ripe bananas, jackfruit seeds and sweet potato tubers to produce this bacterium as well as studying the effect of fermentation on the biochemical

properties of the plant materials used in the experiment. The plant materials mentioned are most cultivated crops in many parts of Uganda and a lot of food wastes are generated from them.

MATERIALS AND METHODS

Experimental set up

The study was carried out at the soil laboratory of Mountains of the Moon University in Fort Portal tourism western Uganda. The experiment aimed at producing beneficial microorganism *Bacillus* by solid fermentation of ripe banana, jackfruit seeds, sweet potato tubers and their mixtures (mixed sample). Thus the 4 treatments and each treatment was replicated 3 times in complete randomized block design (Figure 1).

Figure 1. Solid fermentation of the studied products in plastic dishes (fermentation vessels) for 12 days

Preparation and fermentation of ripe bananas, jackfruit seeds and sweet potato tubers

Cook-able variety of banana locally known as Kitika because the ripen banana are always regarded as "food wastes" so they are thrown or fed to livestock. Ripe bananas were obtained from own plantation. After harvesting a mature bunch and ripening process was induced. By keeping bananas in the nylon for 2 weeks. This was followed by washing the fruits and chopping them into small pieces by use of the common knife. These pieces are easy to pack into fermentation vessels. After slicing, these pieces were sterilized by cooking them for 30 min so as to eliminate plant pathogens cause rotting hence disrupting the process of fermentation. Lastly the 500 g of cooked substrates were put into plastic dishes which were used as fermentation vessels (Figure 1) to carry out solid fermentation process which take place in 12 days.

Jackfruit seeds were obtained from the local market Kabundaire Fort portal city Uganda. Seeds were washed to remove the soil particles and chopped by use of mess like knife so as to create particles for microbes to grow and do fermentation. After chopping them, like ripe banana were sterilized by cooking them for 30 min. Then the 500 g of cooked substrates put into fermentation vessels (plastic dishes) (Figure 1).

Sweet potato tubers were also obtained the local market Kabundaire Fort portal city Uganda. Tubers were washed to remove residual soil particles and chopped by common knife like ripe bananas. This followed by sterilization mentioned in ripe banana and jackfruit seed preparation and putting the 500 g of cooked substrates into fermentation vessels (Figure 1).

The experiment consisted of mixed sample. Whereby 150 g of each cooked material of sample previously put into fermentation vessel and shaken for 1 min for thorough mixing. After putting them into the fermentation vessels were left on table at room temperature with counterpart vessels to allow solid fermentation to take place in period of 12 days as adopted from (Nicomrat & Chamutpong, 2016).

The solid fermentation of ripe bananas, jackfruit seeds and sweet potato tubers in this study was a natural process hence no inoculation of microbes onto the sample was done. Solid fermentation involved the putting the solid substrates into fermentation vessel with limited moisture.

Data collection and analysis

Monitoring temperatures, relative humidity and pH in fermentation vessels

In every 3 days, temperatures, relative humidity and pH were monitored in the fermentation vessels to establish the conditions in which microbes are growing. This was done by using by hand held VWR international pH and hygrometer to measure pH, relative humidity and temperatures inside the vessel. Hygrometer has sensor which was dipped into the substrate in vessel then temperatures and relative humidity were read and recorded (Figure 2). After measuring, sensor was cleaned by hand sanitizer to avoid transferring microbes from one vessel to another.

For pH, the 1 g of fermented substrates were dissolved in 50 cm^3 of ionized water, then probe of VWR international pH meter was dip in the sample solution and pH value was read and recorded (Figure 3).

Figure 2. Monitoring relative humidity and temperatures inside fermentation vessels

Figure 3. Measuring pH from the fermentation vessels

Nutrient agar culture, identifying and counting colonies

To establish the time when microbes start colonizing the substrates of ripe banana, jackfruit sweet potato and mixed samples in fermentation vessels. In every 3 days, the nutrient agar which is universal agar, was used to culture microbes from fermented substrates. The process begins with obtaining 1 g of sample substrates from each fermentation vessel and dissolve it in 9 mL of sterile water by shaking with vortex for 30 s. After that, 1000 µL of this solution was diluted with 20 mL of sterile water for easy counting colonies. This diluted solution was spread on nutrient agar on petri-dishes.

Putting the nutrient agar into use, the 7 g of nutrient agar powder was melted in 250 mL of rain water using autoclave at temperature of 121 °C and pressure of 15 kg/cm^2 for 15 min. Then cooled the solution for 30 min and poured into the petri-dishes. After solidifying inside the petri-dishes, the agar was inoculated with 1000 µL of diluted solutions from the fermented substrates of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples. This was followed by putting the petri-dishes in the incubator at temperature of 37 °C for 24 h. Then physical counting microbial colonies and measuring their diameters to determine genera of microbes as suggested by Fladerer et al., 2025. Then the colony forming unit (CFU)

was calculated as number of colonies X dilution factor/volume plated. Data analysis was quantitative where by CFU analysed by Stata software edition 13th to perform the Kruskal – Wallis test by since number of observations were few (less than 10 sample size) $n_{-1} = 5$ and un equal variance. To proceed for further tests, colonies were sub cultured into nutrient agar to have pure cultures. The Kruskal–Wallis test is aimed at testing the hypothesis that several samples are from the same population. This test is a multisample generalization of the two-sample Wilcoxon (Mann–Whitney) rank-sum test.

Gram staining

Gram staining was done on the sub cultured microbial colonies to view the shapes of the cells and endospore structures of the *Bacillus* species. This procedure began with transferring the colony masses from nutrient agar and smeared on microscope slide in form circular at diameter of 1 cm. This was followed by gently heating slide for 1 min to fix the colony on slide. Gram crystal violet was applied for 30 s, then washed off with running water. After that lugol's iodine was applied on stain then decolourized by clinic safe sanitizer. They were then dried on a hot plate for 2 min to remove water and immersion oil to ensure clear images under a Kruss microscope. The resulting images were taken at 40x magnification. The data were qualitative and involved observation of the cells under a Kruss microscope, with image interpretation based on cell shape. Endospore counts were performed on a computer linked to the microscope and analysis was performed by Kruskal – Wallis (Stata software edition 13th).

Biochemical test reactions

There were 2 biochemical tests performed on sub cultured colonies i.e. catalase test and starch hydrolysis to confirm the presence of *Bacillus* in the fermented samples of ripe banana, jackfruit seeds and sweet potato tubers (Awais et al., 2007). In fact *Bacillus* produce catalase enzyme which split hydrogen peroxide and digest the starch agar.

For catalase tests: the microbial colonies from nutrient agar on petri-dishes of ripe banana, jackfruit seeds and sweet potato tubers cultures were transferred and smeared on microscope slides. Then fixing the colonies by gently warming and 1 drop of hydrogen peroxide was poured on colonial stain. The bubble like reactions on slides (Figure 4) and coded as "–" no reaction, "+" less intense, "++" more intense and "+++" most intensive. Since it qualitative data so their analyses were based on intensity of their reactions.

Figure 4. Catalase test on microbial colony of the samples

For starch hydrolysis tests, the sub cultured microbial colonies from ripe banana, jackfruit seeds, sweet potato and mix petri-dish were transferred to starch agar. The procedure began with melting the 15 g of starch agar in 250 mL of water using autoclave at 121 °C and pressure of 15 kg/cm^2 for 15 min. This was followed by cooling by naturally for 20 min and filling it in the petri-dishes. Then waiting it to solidify in petri-dishes, and putting them upside in the Gen lab incubator to drain excess evaporation moisture. Inoculation was done after 12 h and returning the petri-dishes in Gen lab incubator where they stayed for 24 h at temperature of 37 °C. Lastly iodine was spread over the inoculated agar and observing colour from blue black to brown (Figure 5). This would indicate the digestion of starch by microbes. This data was also coded as "+" less intense, "++" more intense, "+++" most intensive and "–" not able to digest starch.

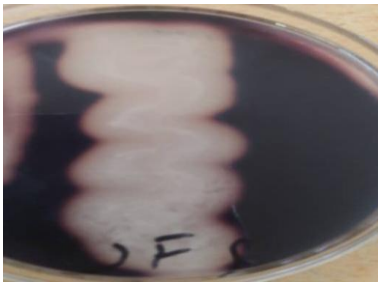

Figure 5. Starch hydrolysis

Crude protein analysis

The method Kjeldahl (AOAC International, 1995) Method which involved digestion, distillation and titration. A sample of 0.3 g of dry substrates of either dry ripe banana or other dry samples of jackfruit seed and sweet potato tubers. They are put in the test tube followed by adding 3 Kjeldahl tablets containing copper sulphate and potassium sulphate, 15 mL concentrated sulfuric acid and mixing the sample. Then digestion process is carried out whereby samples were digested in a heating block at 400 °C for 75 min or until the contents become clear green solution is formed. After 20 min of cooling the colour changed to blue then samples are diluted with 75 mL deionized water.

Distillation: 25 mL of boric acid with bromocresol with methylred indicator is added is the flask on platform. The 60 mL 35% NaOH is pumped into glass tube and distillation took 5 min. Ammonia distillate drops into boric acid in flask.

Titration: flask is put on magnetic stirrer 0.1 N1 hydrochloric is slowly added to the distillate when the colour changes to slight pinkish which marks the completion of titration. The moles of hydrochloric acid equals to the moles of nitrogen in the sample. The mL of 0.1 hydrochloric acid (HCL) is noted and nitrogen is calculated as:

$$\frac{\text{ml HCLsample} - \text{ml HCL blank} \cdot \text{Conc HCl} \cdot 14.01 \cdot 100}{1000 \cdot \text{weight of sample}}, \quad (1)$$

where ml HCLsample is volume of HCl for titration; ml HCL blank is volume of HCl used for blank titration; Conc HCl is concentration; 14.01 is equivalent weight of nitrogen; weight of sample is the actual weight of the sample in grams.

The crude protein will be then attained by multiplying the % nitrogen by a factor (6.25 which is conversion factor assumes that proteins contain approximately 16% nitrogen. By Stata software edition 13th was perform Wilcoxon signed rank test since number of observations were few $n_{-1} = 5$ and unequal variance therefore not fulfilling the conditions of paired test to find out significant effect on crude proteins on the samples.

Crude fat

The Soxhlet extraction method (AOAC International, 1995) was used determine to amount of Crude fat content of the substrate. This involved the following procedures: thimble preparation, sample preparation, fat extraction, taking the final weight and calculation.

Thimble preparation: this involved taking a square of the filter paper and wrap it like cup and seal it at the bottom. Then weighing it on digital scale and note the weight.

Sample preparation involved grinding the sample and loading 5 g into thimble and note sample weight. Then covering the

sample with cotton wool and close the openings of the filter paper. Taking the cellulose thimble and label the sample and put it inside cellulose thimble. Taking a clean and dried flat bottom flask and placing on the balance machine.

Fat extraction involved setting up the Soxhlet extraction unit and placing the sample in it. Then adding sufficient amount of n-Hexane. Then running the water through the condenser of Soxhlet extractor. This was followed by turning on the power and run the Soxhlet extractor. After 6 h of extraction bringing a glass bottle and placing the funnel on the bottle; taking out the thimble and collecting the wastage n-Hexane from the flask by condensation; rotate the flask to evaporate the excess n-Hexane from it. And extracted fat was seen inside the flask.

Taking the final weight includes: placing the flask inside the oven to remove moisture and n-Hexane at 110 °C for 30 min. Then taking out the dried flask and placing and cooling in desiccator. After cooling, taking the weight of a flask with fat. The calculation of crude in percentage in sample as:

$$\text{Crude fat } \% = \frac{W2 - W1}{Ws} \cdot 100, \quad (2)$$

where W2 is weight of flask with fat; W1 is weight of flask alone; Ws is weight of the sample.

By Stata software edition 13th was perform Wilcoxon signed rank test since number of observations were few $n_{-1} = 5$ and unequal variance therefore not fulfilling the conditions of paired test to find out significant effect of fermentation on crude fat.

Determining Carbon-to-Nitrogen (C/N) ratio of fermented and non-fermented samples

The procedure involved two methods namely loss on ignition and Kjeldahl method to determine of C/N ratio of fermented and non-fermented samples of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples.

Loss of Ignition involved weighing the samples and heating them in oven at 550 °C for 2 h. The differences in weight before and after ignition indicate amount of organic matter content. Final weight of sample after ignition was divided by its initial weight before ignition then multiplied by 100 to get organic carbon in percentage.

Amount of nitrogen in the fermented and non-fermented samples was determined by Kjeldahl method. Following the procedures of crude protein analysis. By Stata software edition 13th was perform Wilcoxon signed rank test since number of observations were few $n_{-1} = 5$ and unequal variance therefore not fulfilling the conditions of paired test to find out significant effect of fermentation on C/N of samples.

RESULTS

Microbial growth in the fermented samples

On day 3: the highest microbial growth was observed in ripe banana with $1.6 \cdot 10^4$ colony forming units (CFUs) and lowest colony forming units in jackfruit seeds $1.05 \cdot 10^2$ CFU (Figure 5). There were significant differences among fermented samples in microbial colony production with $p < 0.05$ (Kruskal – Wallis test) as different letters in Figure 6.

On day 6: the highest production of microbial colonies was in ripe banana vessel with $4.6 \cdot 10^4$ CFU and lowest production in jackfruit seed $3.7 \cdot 10^4$ CFU (Figure 5). There were significant differences among the fermented samples in microbial colony production with $p < 0.05$ as different letters in Figure 6.

On day 9: the highest microbial growth was in ripe banana $1.05 \cdot 10^5$ CFU and the lowest microbial production in sweet potato tubers $1.3 \cdot 10^4$ CFU (Figure 5). There were significant differences among the fermented samples in microbial colony production with $p < 0.05$ as different letters in Figure 6.

Finally on day 12: the highest microbial growth was in ripe banana $1.08 \cdot 10^5$ CFU and lowest microbial growth in sweet potato tubers $1.4 \cdot 10^4$ CFU (Figure 5). There were significant differences among the fermented samples in microbial colony production with $p < 0.05$ as different letters in Figure 6.

Nature of microbial colonies

The ripe banana sample was dominated by *Bacillus* colonies which are circular flat and opaque with 20 microbial colonies and 8 small convex smooth and glistening colonies (Table 1). Jackfruit seed sample consisted mainly by small convex smooth and glistening colonies with 80 microbial colonies and 4 circular flat and opaque colonies. The sweet potato tuber sample were also dominated by Small convex smooth and glistening colonies with 35 microbial colonies and 2 circular flat and opaque colonies. The mixed sample consisted of 50 small convex smooth and glistening colonies and 3 circular flat and opaque (Table 1).

Endospores

The most endospores were observed in the mixed sample with 110 endospores and the least was observed in sweet potato tubers sample 14 endospores (Figure 7). There were significant differences in the number of endospores among the samples with $p < 0.05$ (Kruskal – Wallis test) as indicated by different letters in Figure 7.

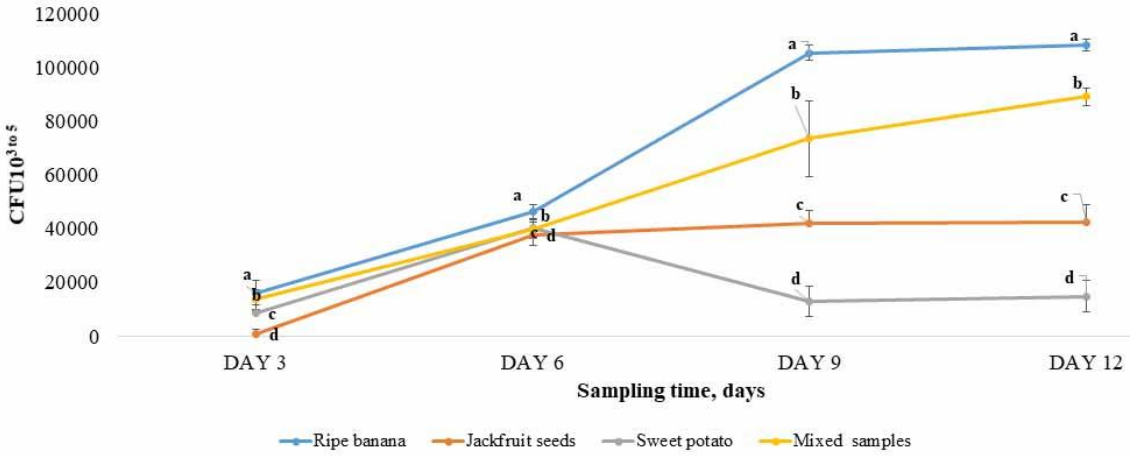

Figure 6. Microbial growth in the fermented samples of ripe bananas, jackfruit seeds, sweet potato tubers and mixed samples in CFU

Table 1. Nature of microbial colonies from fermented samples of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples

Nature of microbial colonies on nutrient agar	Ripe banana	Jackfruit seeds	Sweet potato	Mixed samples	Remarks
Circular, flat and opaque white colonies whose diameter 2 – 4 mm irregular or ragged margin	20.55 ± 3.5 microbial colonies	4.5 ± 3.5 microbial colonies	2.5 ± 2.1 microbial colonies	3.5 ± 2.3 microbial colonies	These colonies associated with *Bacilus* subtilis (Pandav et al., 2021)
Small convex smooth and glistening colonies 2 – 5 mm	8.51 ± 5.4 microbial colonies	80 ± 8.51 microbial colonies	35 ± 8.51 microbial colonies	50.7 ± 30.5 microbial colonies	These colonies are associated with *Lactobacillus* (Meng et al., 2021), *E. coli* (Hossain et al., 2021)
Black 1 mm colony – unidentified	30.46 ± 15.4 microbial colonies	0.0 ± 0.00 microbial colonies	0.0 ± 0.00 microbial colonies	0.0 ± 0.00 microbial colonies	Several species of bacteria *Corynebacterium species* (Shukla et al., 2003) *Clavibacter lycopersici* (Osdaghi et al., 2023)

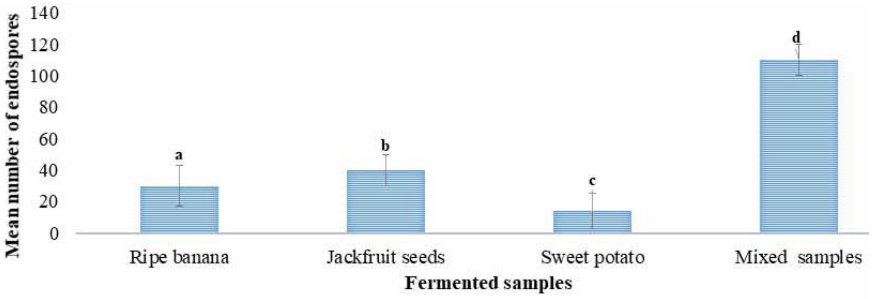

Figure 7. Mean number of endospores in samples ripe banana, jackfruit seeds, sweet potato tubers and mixed samples

Fermentation conditions

Far as fermentation temperature is concerned, on day 3 the highest was observed in ripe banana vessel 27.9 °C and lowest in sweet potato tuber vessel 27.6 °C (Figure 8). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ (Kruskal – Wallis test) as indicated by same letters in Figure 8.

On day 6: the highest temperature in jackfruit seed vessel 25.5 °C and lowest in the mixed sample 25.1 °C (Figure 7). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by same letters in Figure 8.

On day 9: the highest temperature was observed in sweet potato tuber and mixed samples with 27.5 °C and the lowest one in jackfruit seeds 26.6 °C (Figure 7). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by same letters in Figure 8.

Day 12: the highest temperature was in ripe banana and jackfruit seed vessels 26.6 °C and lowest temperature in mixed sample 26.4 °C (Figure 7). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$.

With relative humidity the highest on day 3 was observed in sweet potato tuber vessel 52.6% and lowest in 51.7% in ripe banana and mixed samples (Figure 9). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by the same letters in Figure 9.

On day 6: the highest relative humidity in mixed samples 60.7% and lowest in jackfruit seeds 59.3% (Figure 9). There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by the same letters in Figure 9.

On day 9 the highest relative humidity in jackfruit seed 59.5% and lowest in sweet potato tubers 56%. There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by the same letters in Figure 9.

On day 12 the highest relative humidity 61% in the mixed sample and lowest in jackfruit seeds 58.5% There were no significant differences in fermentation temperatures among the samples with $p > 0.05$ as indicated by the same letters in Figure 9.

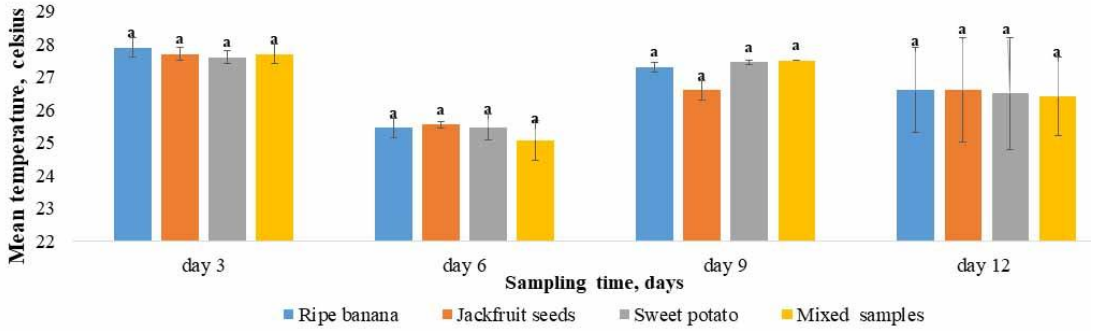

Figure 8. Mean temperatures in the fermentation vessels of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples. Error bars standing for standard deviation and same letters for no significant differences among samples

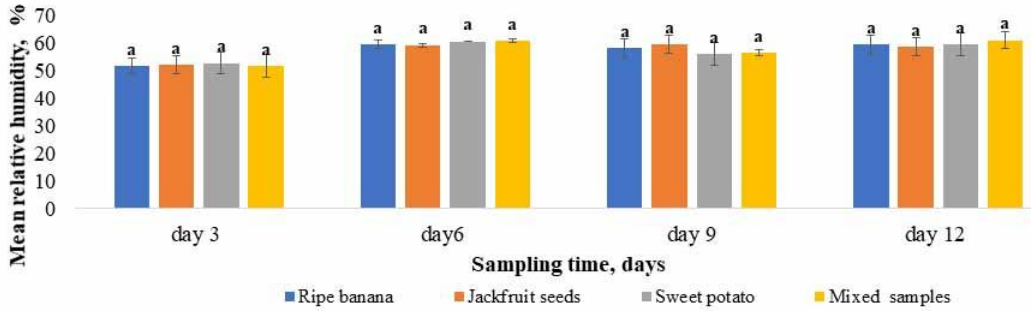

Figure 9. The mean relative humidity in fermentation vessels of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples

As far as pH is concerned, all samples experienced decline in pH during the fermentation process. For instance, pH of ripe banana decreased from 5.2 on day 3 to 3.6 on day 12. While jackfruit seeds' pH decreased from 4.5 on day 3 to 3.4 on day 12 and sweet potato tubers pH decreased from 4.4 in day 3 to 2.7 in day 12 (Figure 10). The mixed sample pH was 4.6 on day 3 to 3.3 on day 12. There were no significant difference in pH among the samples with $p > 0.05$ as indicated by the same letters in Figure 10.

Biochemical reactions

As the catalase test is concerned, colonies of all the sample reacted positively to hydrogen peroxide and jackfruit seed colonies was more reactive as compared to other samples (Table 2). While with starch hydrolysis, jackfruit seed and ripe banana colonies tested positive by digesting starch. Sweet potato tuber and mixed sample colonies tested negative so not able to digest starch.

Gram staining of colonies from the fermented samples

Colonies of ripe banana displayed rod shaped and purple cells. There were 3 chains of cells observed (Figure 11) and many brown endospores. While jackfruit seed colonies displayed purple rod shaped cell but there were no cell chains displayed (Figure 12). Whereas Sweet potato tuber cells are rod shaped but shorter than ones of ripe banana and jackfruit seeds, no chain and few endospores (Figure 13). The mixed sample are rod shaped cells are also short like sweet potato cells (Figure 14).

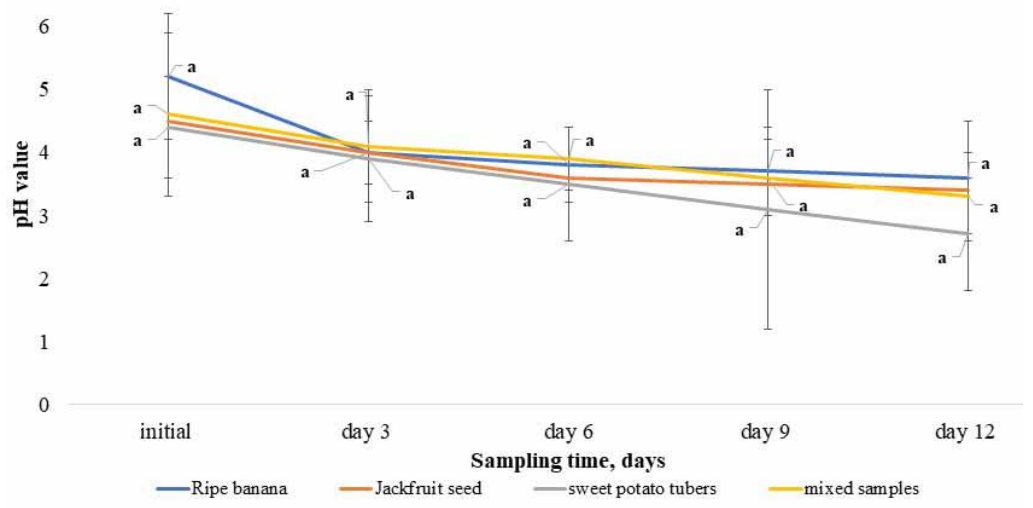

Figure 10. Change in pH in fermented samples of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples

Table 2. Biochemical tests of colonies from the fermented samples

Biochemical reactions	Ripe banana	Jackfruit seeds	Sweet potato tubers	Mixed sample
Catalase test	+	++	+	+
Starch hydrolysis	+	+++	-	-

Crude proteins

There was increase crude protein content in all samples after fermentation except the jackfruit seeds whose crude protein decrease from 5.3 to 4.8%. Before fermentation, the highest amount of crude proteins was in jackfruit seeds with 5.3% and lowest in mixed sample 3.3% (Figure 15). After fermentation ripe banana had highest amount of crude proteins with 5.4% and lowest amount in jackfruit seed with 4.8%. There were no significant differences in crude proteins among sample before and after fermentation (Wilcoxon signed rank test).

Crude fat

There was slight increase of crude fat in ripe banana from 3.4% before fermentation to 4.21% after fermentation as indicated in Figure 16. Whereas in jackfruit seeds there insignificant increase of crude fat from 4.51 to 4.58% due to fermentation effect. Other samples i.e. sweet potato tubers and mixed sample lost their crude fat during fermentation from 4.49 to 4.22% and 4.41 to 4.1422% respectively. There were no significant differences in crude fat before and after fermentation among the samples (Wilcoxon signed rank test).

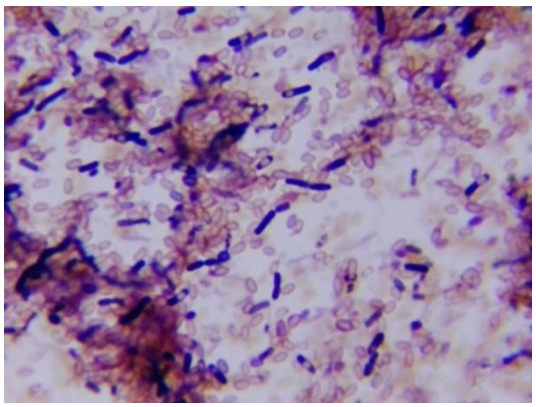

Figure 11. Ripe banana cells are rod shaped and purple with a lot of endospores after gram staining

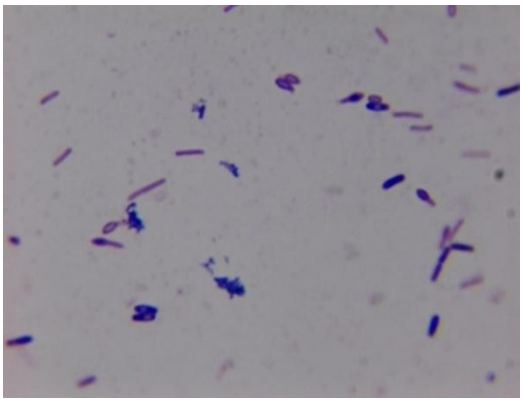

Figure 12. Jackfruit seed cells are rod shaped and purple with endospores

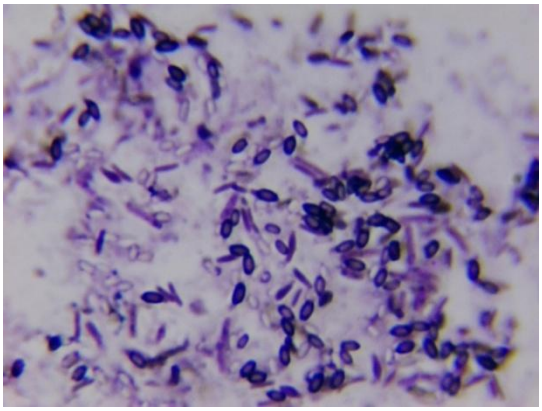

Figure 13. Sweet potato cells are rod shaped and purple but shorter than ripe banana and jackfruit seed cells

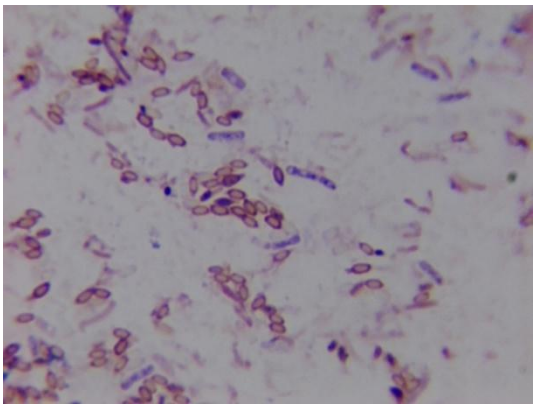

Figure 14. Cells of mixed sample are rod shaped cells short and a lot of endospores and no cell chains seen

Carbon-Nitrogen ratio

There was decrease in carbon-nitrogen ratio due to fermentation effect except the ripe banana which experienced an increase in carbon content from 2.44 carbon : 2.8 nitrogen to 3.12 carbon : 1.7 nitrogen. Before fermentation it is sweet potato tubers with highest C/N ratio of 6.09 : 0.78 which decreased to 5.51 : 1.25 after fermentation. There were significant differences in C/N ratio among the samples (Wilcoxon signed rank test) (Table 3).

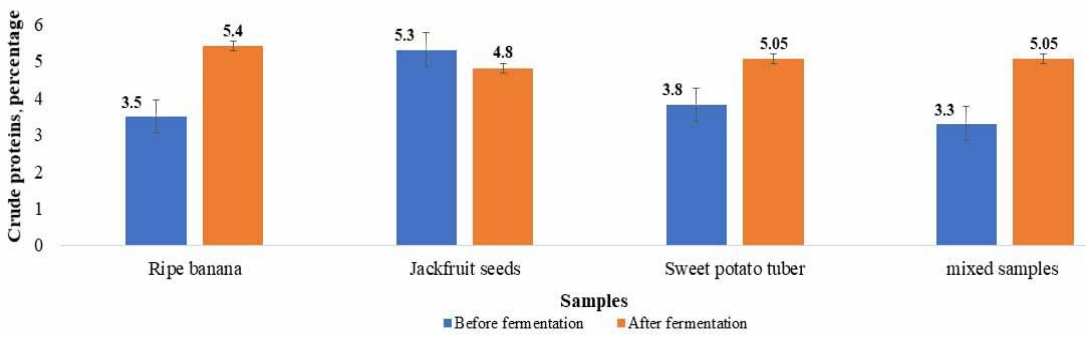

Figure 15. Amount of crude protein in fermented and non-fermented samples of ripe banana, jackfruit seeds, and sweet potato tubers and mixed samples

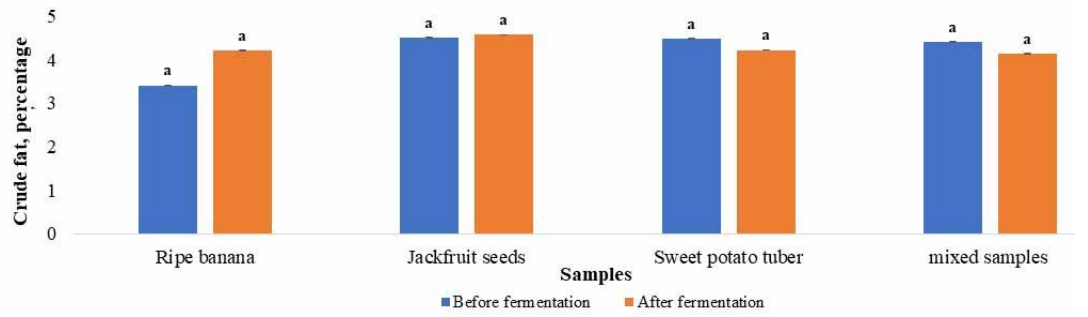

Figure 16. Mean crude fat before and after fermentation of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples

Table 3. Changes in Carbon-Nitrogen ratio of ripe banana, jackfruit seeds, sweet potato tubers and mixed samples during fermentation

Samples	Before fermentation		After fermentation	
	Carbon	Nitrogen	Carbon	Nitrogen
Ripe banana	2.44 ± 0.59^{a}	2.81 ± 0.99^{a}	3.12 ± 1.91^{a}	1.71 ± 0.51^{a}
Jackfruit seeds	4.41 ± 1.50^{ab}	0.24 ± 0.01^{a}	2.87 ± 0.78^{a}	1.23 ± 0.61^{a}
Sweet potato tubers	6.09 ± 2.45^{b}	0.78 ± 0.5^{a}	5.51 ± 1.35^{b}	1.25 ± 0.81^{a}
Mixed samples	4.49 ± 1.59^{ab}	1.03 ± 0.51^{a}	3.11 ± 0.99^{ab}	0.78 ± 0.49^{a}

DISCUSSIONS

Microbial production from the fermented samples

Ripe banana produced highest population of microbes with colony forming units of $1.6 \cdot 10^4$ on day 3, $3.4 \cdot 10^4$ on day 6, $1.05 \cdot 10^5$ and $1.08 \cdot 10^5$ on day 9 and 12 respectively. This was followed by mixed sample with colony forming units of $1.3 \cdot 10^4$ on day 3, $3.9 \cdot 10^4$ on day 6 and $8.9 \cdot 10^4$ on day 12 (Figure 6). The performance of these samples could be attributed to moisture, nutrient and pH level (Hamad, 2012). The ripe banana sample, the pH ranged from 5.2 on initial day 1 to 3.6 on day 12 while for mixed sample, pH ranged from 4.5 to 3.3 on day 12. This pH is below the suitable pH for growing Bacillus which is 6.5 to 7.5 (Gauvry et al., 2021) and 7 – 8 (Basamma Hadimani & Shripad Kulkarni, 2017). However there are some studies which report the growth of Bacillus subtilis surviving in acidic environments (Liu et al., 2024; Yadav et al., 2011).

Banerjee et al. (2019) explains how extrinsic factors such as temperatures, relative humidity and atmospheric gases influence the microbial growth on products. In this study, the Fermented samples were kept in plastic vessels (Figure 1) at room temperature. There were no significant differences in temperatures inside the fermentation vessels (Figure 8). The mean temperatures ranged from 25.4 to 27.7 °C in all the fermented from day 3 to day 12 so temperature may not influence microbial growth significantly among the fermented samples. With relative humidity inside the fermentation vessels increased with time for instance in ripe bananas 51.6% on day 3 to 59.5% on day 12 (Figure 9). Therefore it increased by 8 units which was a bit lower than in the mixed sample where it increased by 10 units. The lowest rate was in jackfruit seed vessels where it increased by 6 units from day 3 to 12. While sweet potato tuber vessel relative humidity increased by 7 units. This can be attributed to the production of water through metabolic processes like respiration release water vapor (Sargantanis et al., 1993). Mixed sample had the highest relative humidity on day 12 with 61% but its microbial growth was lower than that of ripe bananas with 59.5%. The fact the two samples produced the most microbial growth $1.6 \cdot 10^4$ CFU in ripe banana and $8.9 \cdot 10^4$ CFU on day 12. Relative humidity may have interacted with the amount of simple sugars in the samples since ripe banana experienced highest microbial growth as compared to the rest of the samples in the study. According to Timmermans et al. (2022) explains how simple sugars are primary source of energy for fermenting microbes so this explains high microbial growth in ripe banana and mixed samples. A medium sized ripe banana contains 14 – 15 g of fructose, glucose and sucrose (Phillips et al., 2021) whereas 1 kg of jackfruit seeds contain 749 g total carbohydrates (Ravindran, 1996). However, majority of them are form of resistant and low digestibility starch (Li, et al., 2022) so this was low microbial growth in jackfruit seed sample. Because microbes find it difficult to digest it. For the case of sweet potato tubers contain sucrose, glucose, and fructose, ranging from 4.10 – 10.82 g/100 g basing on the different cultivars (Adu-Kwarteng et al., 2014). Maybe the sweet potato tubers used in the study contained less sugars so they produced the lowest microbial growth $1.4 \cdot 10^4$ on day 12. Mixing the samples improved the microbial production of jackfruit seeds, sweet potato tubers but not that of ripe banana.

Microbial species presence in the fermented samples

All the fermented samples i.e. ripe banana, jackfruit seeds, sweet potato and mixed sample displayed the characteristics such as purple rod shaped cells as indicated in Figures 11 – 15. This would imply that these microbes are gram positive bacteria (Badar et al., 2022). In additional to that, endospores were observed so there is probability of samples containing microbes belonging to the phylum *Bacillota* which includes *Bacillus* and *Clostridium*. Since these microbes use endospores to survive the harsh environment (Laue et al., 2018). To find out which of the two species of bacteria is actually present in the samples, the biochemical tests were carried out i.e. the catalase test and starch hydrolysis. As far as starch hydrolysis test is concerned, samples of jackfruit seeds and ripe banana produced colonies on nutrient agar which were digest starch. This is a characteristics of *Bacillius*, *Clostridium*, *Aspergillus species* (Angelin & Kavitha, 2022). With the catalase test, all the fermented samples produced colonies on nutrient agar which tested positive hence ruling out the presence of *Clostridium* species. Since they are catalase negative and Bacillus species are catalase positive (Reiner, 2010). This confirms the presence of *Bacillus* species in the samples of ripe banana, jackfruit seeds, sweet potato tubers and mixed sample.

Effect of the fermentation on biochemical properties of the samples

Fermentation improves the biochemical properties of ripe banana since the sample experienced increase in crude fat and proteins as well as carbon increase. This is attributed to the high microbial population in the sample and microbes are capable of synthesizing proteins, lipids and other carbon-rich compounds (Mustapha et al., 2024; Parsons & Rock, 2013). Some *Bacillus* species synthesize proteins so this is why protein crude increased in the sample (Wang et al., 2022). The same explanation can be used to explain the case of the sweet potato tubers and mixed sample which improved on their crude protein content after fermentation process. While the biochemical properties of other samples especially the jackfruit seeds were negatively affected by the fermentation and the fact that they lost carbon content. May be microbes could consume proteins and lipids (Zhang et al., 2025) so they utilized them for their metabolism. Some *Bacillius* species such as *Bacillius* subtilis (Sahraei et al., 2019) utilize lipid and protein for energy hence reducing the carbon in the sample. Sweet potato and mixed samples lost their crude fat content after fermentation due to bacterial species that colonized the sample.

CONCLUSION

All the fermented samples ripe banana, jackfruit seeds, sweet potato tubers and mixed samples contained *Bacillus* since their microbial colonies displayed rod shaped cells and gram positive and they tested catalase.

Fermentation improves the biochemical properties of ripe bananas as evidenced by increase of crude proteins and fats and carbon. For samples jackfruit seeds, fermentation negatively affect the biochemical properties since they lost carbon, crude proteins and fat.

There is need to identify species of *Bacillus* present in each of fermented sample using molecular markers in fact there are over 20 species of *Bacillus* known so this necessitates the use of 16S rRNA gene. *Bacillus* species are used as biological pest control, probiotics in aquaculture and production of enzymes so requires experiments to try the *Bacillus* produced in these samples in these applications.

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Author's statements

Contributions

Conceptualization: B.R.N., M.S., S.K.; Data curation: B.R.N., L.N.; Formal Analysis: B.R.N., M.S., S.K.; Funding acquisition: A.S., C.W., J.K.; Investigation: B.R.N., M.S., S.K.; Methodology: B.R.N., C.M.; Supervision: B.R.N., M.S., S.K.; Validation M.S., S.K., C.M., A.S., C.W., J.K.; Writing – original draft: B.R.N., M.S., S.K.; Writing – review & editing: B.R.N.

Declaration of conflicting interest

The authors declare no competing interests.

Financial interests

The authors declare they have no financial interests.

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Data availability statement

No data were used for the current study.

AI Disclosure

The authors declare that generative AI was not used to assist in writing this manuscript.

Ethical approval declarations

Not applicable.

Additional information

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