

Molecular Characterization and Taxonomic Profiling of Bacteria Isolates from Naturally Fermented Banana Wine

Akindele S. T. Okoh O. A.* Obafebi T. O. and Raufu T. T.

Department of Science Laboratory Technology, Abraham Adesanya Polytechnic,
PMB 1020, Ijebu-Igbo, Ogun State, Nigeria.

* Corresponding author: agboladeoa@gmail.com

Abstract: Wine is a product of fermentation of fruits by microbes. Banana is a general term embracing a number of species or hybrid in the genus *Musa* of the family Musaceae. It is a seasonal and highly perishable fruit, which can be available all year round. This study was designed to identify the organisms responsible for the fermentation of wine. One species of banana was gotten from Ushin market in Ijebu-Mushin, Ogun-State and taken to the laboratory where they were fermented. A total number of 12 isolates were isolated from the fermented banana wine and identified molecularly. The isolated organisms were identified as *Enterobacter* spp (D1mcb), *Erwinia billingiae* (D4b), *Erwinia* spp (D3Bb), and *Pantoea agglomerans*, (D5ym). The antimicrobial sensitivity test of the identified organisms revealed their susceptibility pattern to Amoxicillin, Ceftriaxime, Ceftriaxone Sulbactam, Cefixime, Levofloxacin, Ciprofloxacin, Imipenem, Cefuroxime, Ofloxacin, Erythromycin, Gentamycin and Azithromycin are more sensitive to Ofloxacin (100%). *Enterobacter* species were sensitive to majority of the tested antibiotics. The *Erwinia billingiae* show sensitivity to Levofloxacin, Ofloxacin, Cefixime, Imipenem/Cilastatin and Azithromycin (100%). *Pantoea agglomerans* shows sensitivity to Levofloxacin, Ciprofloxacin, Imipenem/Cilastatin, Ofloxacin, Gentamycin, and Azithromycin but more sensitive to Ofloxacin (100.00%). The result of this study revealed that the bacteria isolated plays significant roles in the fermentation process, contributing to the conversion of sugars to wine and other by products, and as well influencing the taste and quality of the wine. However, their presence might also be as a result of contamination during the fermentation process.

Key word: Antimicrobial susceptibility, Bacteria, Banana wine, Fermentation process.

INTRODUCTION

Wine is a product of fermentation of fruits by microbes. Banana is a general term embracing a number of species or hybrid in the genus *Musa* of the family Musaceae. It is a seasonal and highly perishable fruit, which can be available all year round. One of the most well-known high-value fruit products is wine, which is the result of yeast fermenting the juice of mature grapes or any fruit that contains a significant amount of wine. It can also be utilized as a substrate in the production of vinegar, which is a byproduct of wine. In Nigeria, banana (*Musa sapientum*) represents a significant starchy staple food (Oboh *et al.*, 2022). Fruits offer dietary and nutritional value and are very significant. Over the past few decades, there has been a remarkable growth in fruit product consumption of over 30 %. The fruit has a long history of use in Nigeria as a seasonal crop and staple meal due to its short shelf life in tropical regions with typical temperatures and humidity levels. It is an extremely perishable, seasonal fruit that is

available throughout the year. Research has been conducted on the generation of banana juice from ripe banana pulp in an effort to prevent significant financial loss caused by the bananas' quick degradation. According to Evans *et al.* (2020), banana wine is a fruit wine that is created only from bananas. Any application that results in a marketable, value-added product will boost the economies of banana farms and solve the significant environmental issue that banana waste poses. Because of its flavour and perfume, bananas might then compete on the market, either as banana juice or as blends with other juices (Liu *et al.*, 2023). They are also prized in temperate regions for their year-round availability, flavour, and nutritional content (Sampath Kumar *et al.*, 2021). Banana is a very popular fruit due to its low price and high nutritive value. It is consumed in fresh or cooked form both as ripe and raw fruit. Bananas have lot of nutritional benefits, and are consumed by patients who have low potassium because of its impressive potassium content (Evans *et al.*, 2020). The flowers are used for

bronchitis and dysentery and on ulcers, cooked flowers are given to diabetics, the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers; the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India (Kumoro *et al.*, 2021).

Nigeria produces a lot of bananas and plantains, which have the potential to be used in the industrial production of wine. In my region of the country, Ogun, Ondo, Kogi, and River State are home to banana plantains. As a result, the economies of banana farms will be enhanced by the production of a marketable, value-added product, increased competitiveness in the market for banana juice and juice blends, and the resolution of the significant environmental issue posed by banana waste (Kandhare *et al.*, 2023). The peel and pulp of completely ripe bananas possess antifungal and antibacterial properties. They also contain norepinephrine, dopamine, and serotonin. The first two increase blood pressure, whereas serotonin stimulates the smooth muscle of the intestines and suppresses gastric output. Both antifungal and antibacterial properties are present in peel and pulp. The neurotransmitters dopamine and norepinephrine have also been found to be present in these structures (Dahab *et al.*, 2018). Banana juice can also be used to make wine, but before it is sold, it needs to be clarified because it is typically quite viscous, turbid, and gray in color. It also tends to settle over time. The polysaccharides in banana juice, such as pectin and starch, are primarily responsible for the turbidity and viscosity of banana wine, which makes the process of clarifying more difficult (Tamrakar *et al.*, 2020). It is crucial to apply pectinase and α -amylases,

which have an impact on wine quality, to enhance the banana wine production process. Although the methods required in producing wine are quite simple, it is difficult to produce a product that is marketable. Nearly any fruit can be used to make the very acceptable wines. Yeast that grows naturally in grapes can be used to ferment wine; however, in nations where grapes are not grown, other fruits are typically preferred for winemaking. While the color stability and flavour profiles of certain soft fruits from temperate and tropical regions are comparable to those of any wine made from grapes, grape wine suffers from a lack of intensive study and development. According to Ofoedu *et al.* (2022), reports on tropical fruit wines have mostly focused on exotic species including bananas, pineapples, citrus fruits, mangos, pawpaws, apples, strawberries, and so on. Wine is a calorie- and vitamin-rich beverage that is safe and beneficial. In an era when life was frequently demanding, it provided solace and pain relief. The work was carried out to isolate, identify and determine the antibiotic susceptibility of the organisms associated with fermentation of banana wine.

MATERIALS AND METHODS

Sample Collection: Ripe banana fruits (*Musa sapientum*) used in the study were purchased from Ushin Market in Ijebu-Mushin Ogun state and taken to the laboratory for further processing.

Banana Juice Preparation and Fermentation: The banana were thoroughly washed in distilled water, peeled and weighed. Upon weighing 600 g of the banana was selected and grounded using a mortar and pestle. After grinding, the sample was put into a clean glass jar and distilled water was added to level up with the banana. The mixture was left for five days at room temperature to ferment. In order to assess the microbial community participating in the fermentation process, an aliquot of the sample was regularly cultivated.

Molecular Identification/Characterization of Isolates: This was carried out using Zymo kit according to the manufacturers' instruction. The method of Anandika (2013) was used for isolation of genomic DNA. Genome DNA was amplified with the method described by (Mehrotra, wang & Johnson, 2000) with modification (Ezeamagu *et al.*, 2018). Agarose Gel Electrophoresis was carried out following the method of (Ezeamagu *et al.*, 2018). DNA Sequencing- DNA was extracted according to the protocol in (Zymo research Mini Kit, USA). Phylogenic Analysis was carried by inferring the evolutionary history using the Maximum Likelihood method used on the Tamura-Nei model. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

Antimicrobial Susceptibility Test: According to the Clinical Laboratory Standard Institute (2021), antimicrobial susceptibility discs containing various medications were used for the antibiogram. These drugs included ceftazidime, nalidixic acid, ciprofloxacin, imipenem, vancomycin, tetracycline, ampicillin, gentamicin, cefixime, nitrofurantoin, and ciprofloxacin. The same criteria were applied while interpreting the antibiogram.

RESULTS AND DISCUSSION

Several microorganisms were identified from the wine. However, the most prevalent ones were identified. The isolated organisms were identified by using 16S RNA molecular markers. DNA chromatogram sequencing was confirmed by using NCBI database. The phylogenetic tree was constructed by using MEGA 6, it was shown that the isolates were *Enterobacter* spp., *Enterobacter* spp., *Erwinia billingiae*, *Erwinia* spp., *Enterobacter* spp., and *Pantoea agglomerans* as showed in figure 4.1. The use of 16S rRNA molecular markers for identifying isolated organisms is a common and effective method in microbiology. The 16S rRNA gene is highly conserved across bacterial species but also contains variable regions that can be used

for species-specific identification. The process involves extracting the DNA from the isolated organisms, amplifying the 16S rRNA gene, and then sequencing it to obtain a DNA chromatogram. Confirmation of the DNA chromatogram sequencing through the NCBI database is a crucial step in the identification process. The NCBI database contains a vast collection of genetic information, and comparing the obtained sequences with entries in the database helps validate and assign taxonomy to the isolated organisms. The phylogenetic tree provides a visual representation of the evolutionary relationships between different species. The identified isolates in this study is a genus of bacteria commonly found in various environments, including soil and water. *Erwinia* spp. and *Erwinia billingiae* are also bacterial genera known for their diversity and presence in different ecological niches. *Pantoea agglomerans* is another bacterium that has been associated with plants and plant surfaces. The phylogenetic tree shows OQ608068 1:386-797 *Enterobacter* sp. strain LI16 16S ribosomal RNA gene partial sequence: This appears to be a partial sequence of the 16S ribosomal RNA gene from *Enterobacter* sp. strain LI16. The numbers "1:386-797" likely represent the specific region of the gene that has been sequenced. KP708587.1:351-762 *Enterobacter* sp. INT27-42 16S ribosomal RNA gene partial sequence: Similar to the first entry, this is another partial sequence of the 16S ribosomal RNA gene, but from *Enterobacter* sp. INT27-42. The numbers "351-762" likely indicate the sequenced region. CP031695.1:468286-468710 *Erwinia billingiae* strain TH88 chromosome complete genome: This entry represents a complete genome sequence of *Erwinia billingiae* strain TH88. It seems to be distinct from the previous entries, as it involves the entire genome rather than just a partial sequence. OR472911.1:272-696 *Erwinia* sp. strain Sh1.1 16S ribosomal RNA gene partial sequence: This is another partial sequence of the 16S ribosomal RNA gene, but from *Erwinia* sp. strain Sh1.1. Similar to

the first two entries, it covers a specific region ("272-696") of the gene. MF033498.1 *Enterobacter* sp. strain MM034 16S ribosomal RNA gene partial sequence: another partial sequence of the 16S ribosomal RNA gene, this time from *Enterobacter* sp. strain MM034. KY495782.1:325-753 *Pantoea agglomerans* strain SR6 16S ribosomal RNA gene partial sequence: This entry represents a partial sequence of the 16S ribosomal RNA gene from *Pantoea agglomerans* strain SR6. The isolated organisms were completely resistant to Cefotaxime (CTX), Erythromycin (ERY), Amoxicillin/clavulanic acid (AUG), and Cefuroxime (CXM). The test on *Enterobacter* spp., *Erwinia billingiae*, *Erwinia* spp., and *Pantoea agglomerans* revealed their susceptibility pattern to Amoxicillin, Ceftriaxime, Ceftriaxone, Sulbactam, Cefixime, Levofloxacin, Ciprofloxacin, Imipenem/Cilastatin, Cefuroxime, Ofloxacin, Erythromycin, Gentamycin and Azithromycin. *Enterobacter* species were sensitive to majority of the antibiotics, Amoxicillin, Ceftriaxime, Ceftriaxone, Sulbactam, Cefixime, Levofloxacin, Ciprofloxacin, Imipenem/Cilastatin, Cefuroxime, Ofloxacin, Erythromycin, Gentamycin and Azithromycin but more sensitive to Amoxicillin, Ceftriaxone, Levofloxacin, Ofloxacin and Azithromycin (100.00 %) respectively. *Erwinia billingiae* was sensitive to Levofloxacin, Ofloxacin, Cefixime, Imipenem/Cilastatin and Azithromycin (100%). *Pantoea agglomerans* was sensitive to Levofloxacin, Ciprofloxacin, Imipenem/Cilastatin, Ofloxacin, Gentamycin, and Azithromycin but more sensitive to Ofloxacin (100.00%). The 16S rRNA gene sequencing-identified bacterial isolates provide unambiguous proof that molecular characterisation is a completely reliable method for identifying bacterial isolates. The species' sequencing data have been added to GenBank (NCBI: NC_009436, accession numbers: EF688010, EF688009). All of the bacteria that were obtained were similar to the corresponding

species in GenBank, as demonstrated by the phylogenetic trees, underscoring the significance of the sequencing method in microbial taxonomy. The study's bacterial species' pairwise nucleotide similarity values fell within the permissible range for *Pantoea agglomerans* and species identification. For the first time in the history of banana wine production, *Enterobacter* spp. isolated from wastes related to banana wine production were reported; other research along these lines have reported on the involvement of other bacteria in the creation of traditional banana wine (Bunani *et al.*, 2020). In this study, isolated bacteria from banana wine were investigated. Antimicrobial Sensitivity was used to determine the level of their pathogenicity. The result from this study revealed that banana wine contained four bacterial which are *Enterobacter* spp. (D1mcb), *Erwinia billingiae* (D4b), *Enterobacter* spp. (D3sa), and *Pantoea agglomerans* (D5ym). One of the isolated organisms had also been reported by Bunani *et al.* (2020) that work on the microbiological analysis of traditional banana wine prepared by local populations in North and East regions (Case Study Ngoma and Musanze) revealed the presence of many microbes from different species including *E. coli*, *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *Enterobacter*, *Clostridium*, and *Bacillus*. In this study, the isolated bacterial species include, *Enterobacter* spp. is a genus of Gram-negative bacteria that is commonly found in the environment. Some *Enterobacter* species have been isolated from fermented foods, including wine. These bacteria can produce a variety of enzymes, such as amylases, that are involved in the fermentation process. They can also produce a number of other compounds, such as organic acids, that can affect the taste and quality of the wine. *Enterobacter* species are commonly found in the environment and are known for their ability to ferment sugars. In the banana wine, *Enterobacter* spp. play a role in the fermentation process by converting sugars into various byproducts,

including alcohol. *Enterobacter* spp is also reported by Oluwawapelumi *et al.* (2020) in the study of Bacteriological assessment of tropical retail fresh-cut, ready-to-eat fruits in south-western Nigeria. *Erwinia billingiae* and *Erwinia* spp. are species of bacteria within the Enterobacteriaceae family. It is a common contaminant of fruit and has been found in a variety of fermented foods, including wine. It has been known to produce a number of different enzymes, including amylases and lipases that can affect the fermentation process. *Erwinia* species are often associated with plant pathogens, and their presence in banana wine could be a result of contamination from the fruit's surface or the environment during the fermentation process. While some *Erwinia* strains can be harmful to plants, the impact of their presence in banana wine on

human health would depend on the specific strain and its characteristics. *Pantoea agglomerans* has been found in banana wine. Like *Erwinia billingiae*, *Pantoea agglomerans* can produce a number of enzymes, including amylases and lipases that can affect the fermentation process. In addition, *Pantoea agglomerans* has been shown to produce a number of flavour compounds, such as esters and ketones that can influence the taste of the wine. *Pantoea* species are commonly found in various environmental niches, including plants. In fermentation, *Pantoea agglomerans* contribute to the development of specific flavours or other characteristics in the wine. However, like *Erwinia*, its presence might also be a result of contamination during the fermentation process.

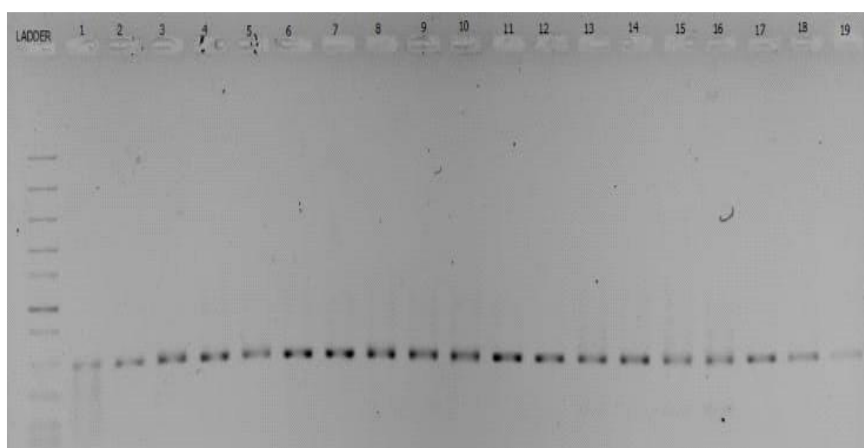


Figure 1: Gel Electrophoresis of Isolated Bacteria from Banana



Figure 2: Phylogenetic Tree of the Bacterial Isolates Showing their Closest Relatives in Genbank
(Note: The evolution history was inferred using the Neighbor-joining method and distance were computed using the Jukes-cantor method. All the isolates were within four cluster)

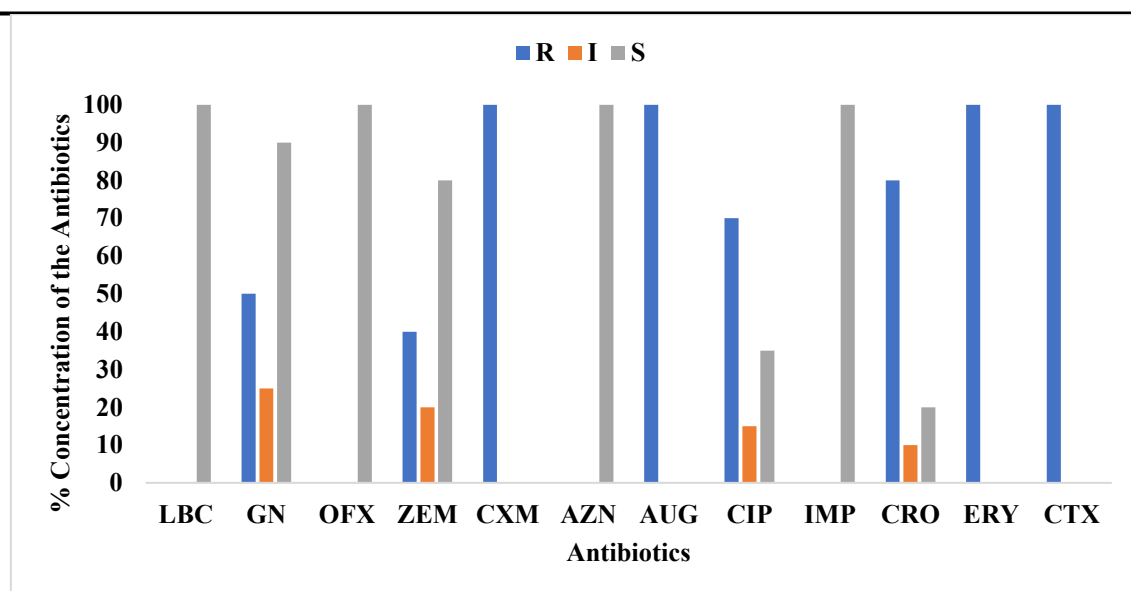


Figure 3: Antibiotic Susceptibility of the Isolated Bacteria from Banana

Keys: AUG: Amoxicillin, CTX: Cefotaxime, CRO: Ceftriaxone Sulbactam, ZEM: Cefixime, LBC: Levofloxacin, CIP: Ciprofloxacin, IMP: Imipenem/Clistatin, CXM: Cefuroxime, OFX: Ofloxacin, ERY: Erythromycin, GN: Gentamycin, AZN: Azithromycin

CONCLUSION

The bacterial species found in this study which includes; *Enterobacter spp.*, *Erwinia billingiae*, *Erwinia spp.*, and *Pantoea agglomerans*. Antimicrobial sensitivity and molecular characterisation were used to assess the pathogenicity of these isolates. These microorganisms are important to the fermentation process because they help turn carbohydrates into alcohol and other byproducts while also affecting the wine's flavour and quality. On the other hand, contamination that occurs during fermentation could potentially be the cause of their presence.

Based on the findings the following were recommended: more studies are needed to

understand the specific strains of these bacteria present in banana wine and their characteristics. This could help in assessing their potential impact on human health; implementing stringent quality control measures during the fermentation process could help in minimizing contamination from these bacteria; regular microbial analysis of the banana wine could help in early detection of any potential harmful strains of these bacteria; as these bacteria contribute to the development of specific flavours in the wine, further research could explore how to harness these bacteria to create unique flavour profiles in banana wine.

REFERENCES

- Anandika, D. (2013). DNA Extraction and Purification. *Materials and Methods*. DOI: 10.13070/mm.en.3.191.
- Bunani Parfait, K. B., Serge, I. R., & Patrick, I. (2020). Microbiological Analysis of Traditional Banana Wine Prepared by Local Population with Feet and Hands in North and East (Case Study Ngoma and Musanze). *International Journal of Pharma*
- Medicine and Biological Sciences* Vol. 9, No. 3.
- Clinical Laboratory Standard Institute (2021). M100- Performance Standards for Antimicrobial Susceptibility Testing.
- Dahab, A. A., Nady, H. N., & El-Salam, H. S. A. (2018). The potential of some plants extract as bio-stimulants for enhancing growth and biochemical constituents of banana

- plantlets. *Middle East Journal of Agriculture Research*, 7(3), 904-914.
- Evans, E. A., Ballen, F. H., & Siddiq, M. (2020). Banana production, global trade, consumption trends, postharvest handling, and processing. *Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition*, 1-18.
- Ezeamagu C, Imanatue I, Dosunmu M, Odeseye A, Baysah G, Aina D, Odutayo F, Mensah-Agyei G. (2018). Detection of methicillin resistant and toxin- associated genes in *Staphylococcus aureus*, Beni- Suef University Journal of Basic and Applied Sciences 7, 1, 92-97.
- Kandhare, K. S., Gaikwad, R. S., & Pate, S. S. (2023). Production and evaluation of wine prepared from banana (*Saccharomyces cerevisiae*).
- Kumoro, A. C., Damayanti, A., Bahlawan, Z. A. S., Melina, M., & Puspawati, H. (2021). Bioethanol production from oil palm empty fruit bunches using *saccharomyces cerevisiae* immobilized on sodium alginate beads. *Periodica Polytechnica Chemical Engineering*, 65(4), 493-504.
- Liu, H., Ni, Y., Yu, Q., & Fan, L. (2023). Evaluation of co-fermentation of *L. plantarum* and *P. kluyveri* of a plant-based fermented beverage: Physicochemical, functional, and sensory properties. *Food Research International*, 172, 113060.
- Mehrotra M, Wang G, Johnson WM. (2000). Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *Journal of Clinical Microbiology*, 38,3 p.1032-5.
- Oboh, G., Oluwakemi, A. S., Ayokunle, A. O., & Oyeleye, I. S. (2022). Comparative Studies on Anti-Diabetic Properties of Commonly Consumed Musa Cultivars (*M. paradisiaca*, *M. sapientum*, and AAB Group Hybrid) Flour. *Tropical Journal of Natural Product Research*, 6(12).
- Ofoedu, C. E., Ofoedu, E. O., Chacha, J. S., Owuamanam, C. I., Efekalam, I. S., & Awuchi, C. G. (2022). Comparative evaluation of physicochemical, antioxidant, and sensory properties of red wine as markers of its quality and authenticity. *International Journal of Food Science*, 2022.
- Sampath Kumar, N. S., Sarbon, N. M., Rana, S. S., Chintagunta, A. D., Prathibha, S., Ingilala, S. K., ... & Dirisala, V. R. (2021). Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies. *AMB Express*, 11, 1-9.
- Tamura K, Stecher G, Peterson, D, Filipski A, Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *The Society for Molecular Biology and Evolutions Molecular Biology and Evolution*, 30, 12, p.2725-2729.
- Tamrakar, K., Lama, A., Dhakal, B., Adhikari, L., Shrestha, M., & Amatya, J. (2020). Qualitative analysis of wine prepared from banana and orange. *Int J Food Sci Nutr*, 5(1), 60-63.