

# Impact of *Xylopia aethiopica* powder on microbiological safety, biochemical characteristics, and organoleptic properties of *Kunnu gyada* produced from groundnut and rice

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## Abstract

*Kunnu gyada* is a nutritious beverage usually produced in small household quantities because of its poor keeping quality. This study investigated the preservative effect of *Xylopia aethiopica* powder on *Kunnu gyada* stored at refrigeration temperature (4°C) for 21 days. *Xylopia aethiopica* was added to *Kunnu gyada* at 2%, 3%, 5% and 7% concentrations to extend its shelf life. Microorganisms associated with the products were enumerated, isolated and identified, pH and sugar were evaluated, and organoleptic properties were assessed using standard methods. The results showed that total viable count (0-8.62 log CFU/mL), LAB count (0-7.47 log CFU/mL) and total fungi count (0-8.51 log CFU/mL) generally increased during storage and decreased with an increase in the concentration of *Xylopia aethiopica* added to the drink. *Pseudomonas fluorescens*, *Bacillus subtilis*, *Micrococcus luteus*, *Lactobacillus plantarum*, *Candida albicans*, *Aspergillus niger*, *Kluyveromyces marxianus*, and *Fusarium oxysporum* were isolated during storage. Total sugar, total reducing sugar and pH also decreased with an increase in concentration of *Xylopia aethiopica*. Results of sensory analysis of *Kunnu gyada* showed that the drink that contained 3% of *Xylopia aethiopica* powder was more accepted by the panelists than the others. Thus, *Xylopia aethiopica* could serve as a suitable bio-preservative in extending the shelf life of *Kunnu gyada* and could also encourage large-scale production for commercial purposes thereby making nutritious food available, accessible and affordable.

## 1. Introduction

*Kunnu gyada* is a thin cereal-legume beverage similar to kunu zaki. It is produced in limited daily quantities because of its short shelf life. It is different from the popular kunnu zaki which is mainly produced from cereal such as maize, sorghum and millet (Bolarinwa *et al.*, 2015) because it contains both cereal and legume (rice and groundnut). It is usually consumed as weaning food or breakfast and beverages for special occasions such as weddings and naming ceremonies. It is usually produced in limited daily quantity and might spoil in a few hours if not properly refrigerated. However, it can only last for four days at refrigeration temperature (Halilu *et al.*, 2022). Legumes such as groundnut and others improve the protein content of traditional cereal-based foods because they are high in protein and essential amino acids needed for the growth and proper functioning of the body and also reduce

malnutrition and nutritional-related diseases (Malomo and Abiose, 2019).

Groundnut (*Arachis hypogaea* L.) is majorly produced for its protein and oil content (Yadachi *et al.*, 2012). It is the second most important legume after soybeans. It is a cheap source of essential amino acids and fatty acids. The seed contains about 25-36% protein and 47-53% oil depending on the species (Prasad *et al.*, 2011). Its oil is rich in essential fatty acid which is free of trans fat and cholesterol. The oil is also rich in unsaturated fatty acids "oleic acid (52%) and linoleic acid (52%)" with a low amount of saturated fatty acid (Özcan, 2010). Groundnut serves as a rich source of calcium, phosphorus, magnesium, zinc, potassium and vitamins A, B and E and also contain antioxidants and phytochemicals that are beneficial to the body (Gradawa *et al.*, 2019).

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Rice is one of the most important cereals in the world with about twenty varieties. The two important species are *Oryza sativa* and *Oryza glaberrima*. It contains carbohydrates, protein, calcium, magnesium, and phosphorus with traces of iron, copper, zinc and manganese (Okon and Ugwu, 2011). Rice flour/starch has gained attention as a stabilizer in food due to its hypoallergenic properties, gluten-free nature and bland flavour which might not interfere with the organoleptic properties of the products (Bao and Bergman, 2004). Rice will provide energy while groundnut will supply protein, fat, fat-soluble vitamins and minerals. Groundnut and rice are important in the production of a nutritious beverage known as *Kunnu gyada* which is usually produced in daily household quantity due to its short shelf life (Adbulrahman et al., 2014). Groundnut has high nutritional value and provides an affordable alternative to protein which has been exploited for combating malnutrition due to protein deficiencies in children. The use of locally available legumes in food processing will increase the nutritional and health status of the population, especially in those who cannot afford animal protein (Malomo et al., 2020). High digestibility and organoleptic acceptability have been reported in beverages produced from blends of legumes and cereal by all groups (Elsamani and Ahmed, 2014).

The addition of preservatives has improved the shelf life of many foods and beverages globally. The knowledge of the negative effects of chemical preservatives has led to an increase in the use of bio-preservatives such as spices in food (World Health Organisation (WHO), 2002). *Xylopiya aethiopiya* also known as Ethiopian pepper or African guinea pepper is commonly used as a flavouring agent, preservative and food seasoning because of the presence of aromatic substances (Marcel et al., 2010; Imo et al., 2018). Chemical compound found in the seed are flavonoids, alkaloids, cineol, phytosterols, tannins, saponins, glycosides, carbohydrates, terpenes, paradol, cryptone, limonene and myrtenol which give the seed antioxidative, anti-inflammatory, laxative and antimicrobial properties (Ogbonna et al., 2013; Anumudu et al., 2020). These properties also qualify it as functional food since it has many health benefits (Ogbonna et al., 2013).

It is important to improve the consumption of protein in developing countries with plant proteins that are available, accessible, and affordable because of the high cost of animal protein such as meat, milk, egg, and fish as this will improve the nutritional status and reduce malnutrition, especially undernutrition and micronutrient deficiencies (Malomo et al., 2019). This research generated information on the preservative effect of

*Xylopiya aethiopiya* on the microbiological characteristics and physicochemical properties of *Kunnu gyada* and encouraged the production of this nutritious beverage on a large scale. Several studies have been conducted on the production, chemical properties and microbiological characteristics of *Kunnu zaki* which is produced from maize, millet or sorghum with low protein content (Gaffa et al., 2002; Essien et al., 2009; Ogbonna, et al 2011; Sosanya et al., 2021) but there is still a dearth of information on *Kunnu gyada*. Hence, this study determined the effect of *Xylopiya aethiopiya* on the microbiological characteristics, safety and shelf life of *Kunnu gyada* to increase its production on a large scale and combat micronutrient deficiencies.

## 2. Materials and methods

### 2.1 Sources of materials

Groundnut (*Arachis hypogaea* L.), rice (*Oryza glaberrima*) and *Xylopiya aethiopiya* were obtained from Sabo market in Ile-Ife, Nigeria. The media and chemicals used were of analytical grade.

### 2.2 Preparation of *Xylopiya aethiopiya*

The method of Anumudu et al. (2020) was used to prepare the fruit. *Xylopiya aethiopiya* fruits were sorted, cleaned, and dried in a hot air oven at 65°C for 24 hrs. The dried spice was cooled to room temperature, milled using a hammer mill and stored in an airtight container for further use.

### 2.3 Production of *Kunnu gyada*

Groundnut was cleaned, sorted, roasted and winnowed. It was ground in a blender (USHA mixer grinder, Model: MG:2053 N) by mixing with water at the ratio of 3:8 (w/v) and the thin slurry obtained was sieved and boiled for 10 mins. Rice was cleaned, steeped in water for 8 hrs, drained and rinsed in potable water. Water was added to rice at a ratio of 1:4 (w/v), ground in a blender (USHA mixer grinder, Model: MG:2053 N) and boiled for 10 mins to obtain thin porridge. Groundnut and rice porridge were mixed together at a ratio of 4: 1 (v/v) and boiled for 30 mins (Modified method of Abdulrahman et al., 2014). The mixture was divided into five portions and *Xylopiya aethiopiya* powder was added to the mixture at 0%, 2%, 3%, 5% and 7%. A- *Kunnu gyada* with no preservative; B- *Kunnu gyada* with 2% *Xylopiya aethiopiya* powder; C- *Kunnu gyada* with 3% *Xylopiya aethiopiya* powder; D- *Kunnu gyada* with 5% *Xylopiya aethiopiya* powder; E- *Kunnu gyada* with 7% *Xylopiya aethiopiya* powder. The mixtures were allowed to cool, dispensed into sterile plastic bottles and stored at refrigeration temperature (4°C).

## 2.4 Microbiological analysis

*Kunnu gyada* samples treated with 0%, 2%, 3%, 5% and 7% of *Xylopiya aethiopiya* powder were subjected to microbiological analysis during the period of storage. Total viable count, lactic acid bacteria count, and fungi count were assessed at 7-day intervals for 21 days using the pour plate method. Each *Kunnu gyada* sample (5 mL) was dispensed into a stomacher bag and homogenized with 45 mL of diluent (peptone water). Serial dilution was carried out, and the representative dilution was dispensed into a sterile petri dish. Molten agar was poured, and the petri dish was rocked gently clockwise and anticlockwise for even distribution of growth. The total viable count was enumerated using Nutrient Agar (NA), lactic acid bacteria using de Man Rogosa and Sharpe (MRS) Agar and fungi count using Potato Dextrose Agar (PDA) (Harrigan, 1998) and plates were incubated at 35°C for 24 hrs, 35°C for 72 hrs, and 25°C for 3 to 5 days, respectively (Harrigan, 1998). The colonies formed were counted using Gallenkamp colony counter and representative colonies were streaked on the appropriate agar and kept in the refrigerator for identification. All isolates were viewed under the microscope (Leica DM500 Model 13613210) to determine their size, shape and arrangement. Bacteria isolates were identified using cultural, morphological characteristics and biochemical tests. (Harrigan and McCance, 1976; Woods and Holzappel, 1995). Yeasts in *Kunnu gyada* samples were isolated based on cultural characteristics, cell size, shape, arrangement, reproduction, and ability to assimilate carbon and nitrate (Barnett et al., 2000). Mould isolates were identified using the cultural characteristics and microscopy characteristics such as spore head, hyphae and presence of special structures (Malomo et al., 2018).

## 2.5 Determination of total sugar

Anthrone reagent Method of Morris (1948), described by Malomo et al. (2021), was used for the determination of the total sugar content of *Kunnu gyada*. The filtrate (1 mL) was dispensed into the test tube, and 4 ml of anthrone reagent was added. The mixture was heated in a boiling water bath (Gallenkomp, HH-S6, England) for 10 min and rapidly cooled in cold water. Absorbance was read at 620 nm against a reference blank in a spectrophotometer (Spectrumlab 752S, YM1206PHB2, China). The quantity of sugar was extrapolated from the standard curve based on known concentrations of glucose (10-100 mg/L).

## 2.6 Sensory analysis

Coded samples of *Kunnu gyada* were presented to 20 panelists to score for colour, taste, aroma, flavour and

overall acceptability using a 9-point Hedonic scale where 1 was the lowest score and 9 was the highest (Yangilar and Yildiz, 2017).

## 2.7 Statistical analysis

Data were analyzed using ANOVA on SPSS (17 incorporations Chicago, USA), Principal component analyses and Agglomerative Hierarchical Clustering were conducted using XLSTAT (2014).

## 3. Results and discussion

### 3.1 Microbial count of *Kunnu gyada* during storage

The total viable count (TVC) of *Kunnu gyada* is shown in Figure 1. The count generally increased from week 0 (0-3.28 CFU/mL) to week 2 (3.33 to 7.44 log CFU/mL) and decreased in week 3 (2.94-6.08 log CFU/mL). The results show that the TVC decreased with an increase in addition to *Xylopiya aethiopiya* and the count was highest in sample A with 0% *Xylopiya aethiopiya* throughout refrigeration storage. There was no TVC at week zero in Sample D and E which contain 5% and 7% *Xylopiya aethiopiya* respectively at the beginning of storage. Anumudun et al. (2020) also reported no count in orange juice preserved with *Xylopiya aethiopiya* at the beginning of storage. The decrease in microbial load with an increase in the concentration of *Xylopiya aethiopiya* could be a result of antibacterial activity exerted by the chemical compound present in *Xylopiya aethiopiya* (Fleisher et al., 2008).

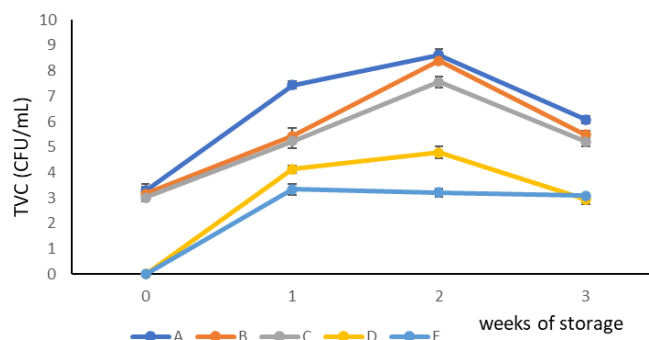


Figure 1. Changes in total viable count during storage of *Kunnu gyada*. The error bars indicate  $\pm$  standard error.

Lactic acid bacteria (LAB) count decreased with an increase in the addition of *Xylopiya aethiopiya* powder (Figure 2). There were no viable lactic acid bacteria in Week 0 in samples B, C, D and E treated with *Xylopiya aethiopiya* but LAB started growing in these samples at week 1 (3.49-5.72 log CFU/mL) and progressed to week 3 (3.19-6.47 log CFU/mL). The higher the concentration of *Xylopiya aethiopiya* powder, the lower the LAB growth. The reduction in the growth of LAB in the samples may be due to the antibacterial activities of

dried *Xylopi*a *aethi*o*p*i*c*a powder added to *Kunnu gyada* drink. This is in line with the report of Ogbonna et al. (2013) who reported the effectiveness of *Xylopi*a *aethi*o*p*i*c*a in inhibiting both spoilage and pathogenic bacteria. *Xylopi*a *aethi*o*p*i*c*a fruit has been reported to possess an anti-microbial effect against Gram-positive bacteria. *Xylopi*a *aethi*o*p*i*c*a seed powder has been an important ingredient in Nigerian traditional cuisines from time immemorial, especially in the preparation of Nigerian pepper soup which helps in the treatment of cough, fever and headache and also serves as an anti-inflammatory, antiplasmodial and antidiabetic agent (Jin et al., 2019; Onwuzuruike et al., 2023) It is also an essential flavour component in fermented cereal gruel and could be used as preservative in the paste because of its antimicrobial properties (Onwuzuruike et al., 2023).

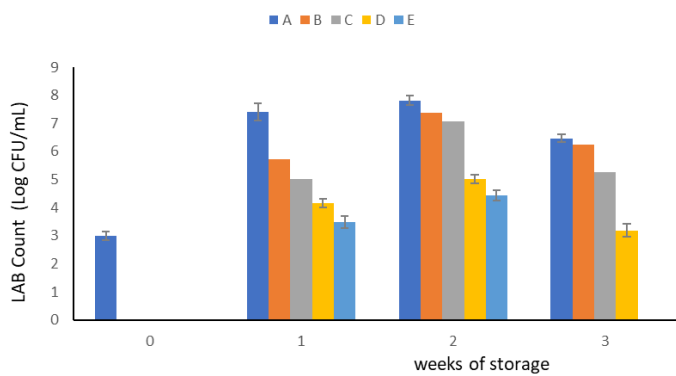


Figure 2. Changes in lactic acid bacteria count (LAB) during storage of *Kunnu gyada*. The error bars indicate  $\pm$  standard error.

At the beginning of storage, the fungi count was higher in sample A which was not treated with *Xylopi*a *aethi*o*p*i*c*a powder (Figure 3). There was no count in Samples D and E containing 5% and 7% *Xylopi*a *aethi*o*p*i*c*a powder respectively at week zero. This could be due to the inhibitory action of *Xylopi*a *aethi*o*p*i*c*a against fungi such as *Aspergillus*, *Fusarium* and *Penicillium* as reported by Chinonye et al. (2022).

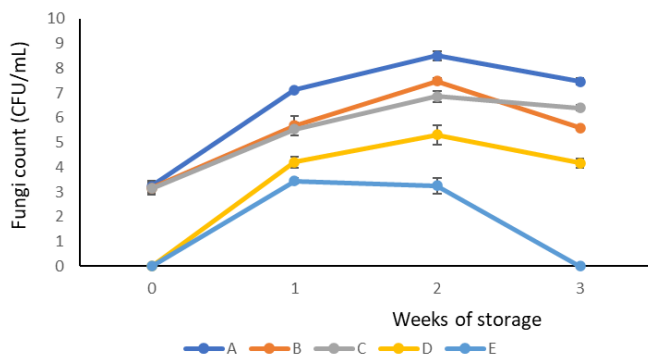


Figure 3. Changes in fungi count during storage of *Kunnu gyada*. The error bars indicate  $\pm$  standard error.

### 3.2 Identification of microorganisms in *Kunnu gyada*

Microorganisms isolated from *Kunnu gyada* are

*Micrococcus luteus*, *Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Candida albicans*, *Kluyveromyces marxianus*, *Aspergillus niger* and *Fusarium oxysporum* (Figure 4). The population of these organisms decreased with an increase in the concentration of *Xylopi*a *aethi*o*p*i*c*a powder.

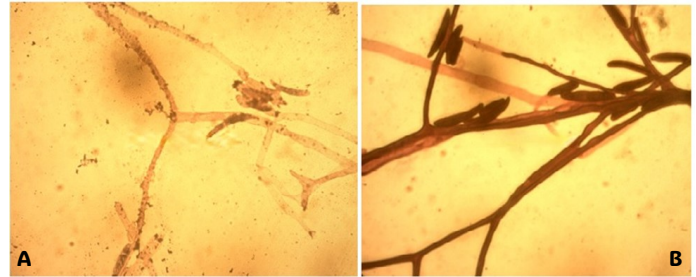


Figure 4. Pictures of *Fusarium oxysporum* isolated from *Kunnu gyada* without *Xylopi*a *aethi*o*p*i*c*a (Magnified 600 $\times$ ). A. *Fusarium oxysporum* with hyphae that has lost some cell constituent, B. *Fusarium oxysporum* with true hyphae.

*Lactobacillus plantarum* was present in all *Kunnu gyada* samples throughout storage and it was the most prominent bacteria. *Bacillus subtilis* was present in samples A and B and *Pseudomonas fluorescens* were only present in sample A. Inhibition of bacteria by *Xylopi*a *aethi*o*p*i*c*a could be due to the

action of mono- and ses-qui-terpene hydrocarbons that are present in *Xylopi*a *aethi*o*p*i*c*a on the bacteria (Karioti et al., 2004). *Xylopi*a *aethi*o*p*i*c*a extract has been reported to have inhibitory effect on *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas* and *Bacillus subtilis* and some *Lactobacilli* at low concentrations (Ijeh et al., 2005; Ogbonna et al., 2011; Chinonye et al., 2022).

*Candida albicans* and *Kluyveromyces marxianus* were present in all *Kunnu gyada* samples throughout storage but their population decreased with an increase in the concentration of *X. aethi*o*p*i*c*a. *Fusarium oxysporum* was the least as it was only isolated from sample A accounting for 8% of total microorganisms. *Aspergillus niger* was present in samples A and B throughout storage but was not detected in samples C, D and E. This shows that *Xylopi*a *aethi*o*p*i*c*a is effective against *Fusarium oxysporum*. The presence of *Fusarium oxysporum*; a mycotoxin-producing mould has been reported in groundnut and groundnut products such as kulikuli, donkwa, peanut butter, yaji and roasted groundnut (Odeniyi et al., 2019). *Aspergillus*, *Penicillium* and *Fusarium* have been reported to be the most dominant mould in rice (Nagede et al., 2022). It was reported that the essential oil of *Xylopi*a *aethi*o*p*i*c*a acts as an antifungal and it is effective against *Fusarium* and *Aspergillus* (Ogbonna et al., 2011).

### 3.3 Total sugar content of *Kunnu gyada*

Table 1 shows the total sugar content of *Kunnu gyada*. The range of total sugar was between 25.68 and 50.85 mg glucose/mL and generally decreased in all samples during the period of storage. *Xylopi aethiopic a* did not have a significant effect ( $p < 0.05$ ) on the samples at week 0 but did from week 1 to week 3. It was highest in sample A which had no *Xylopi aethiopic a* powder (44.81-50.85 mg glucose/mL) and lowest in sample E which contained 7% *Xylopi aethiopic a* powder (34.88-50.57 mg glucose/mL) throughout storage. The addition of *xylopi aethiopic a* generally reduced the total sugar content probably due to the dilution factor. *Xylopi aethiopic a* contains high levels of phytochemicals such as tannin that are associated with astringency and bitterness (Ma et al., 2014). The decrease in total sugar during storage may be due to the activities of the microorganisms that break down sugar into organic acids such as lactic acid and acetic acid (Adepoju et al., 2016; Malomo et al., 2021).

### 3.4 Principal component analysis of the sensory properties of *Kunnu gyada*

The principal component analysis (PCA) categorized the sensory parameters into four principal components with PC1 having the highest variability of 98.68% followed by PC2 with 0.88% (Figure 5). The Biplot showed that sample A had the highest score for taste (7.60), texture (7.53), thickness (7.70), Appearance (8.13), colour (8.27) and overall acceptability (8.13) followed by sample B. Samples A and B are represented on the positive axis of PC1 while C, D and E are represented on the positive axis of PC2. Sample C only had a positive correlation with appearance, and thickness but had a negative correlation with taste, colour and overall acceptability. The plot also showed that samples D and E had a negative correlation with all the sensory parameters. This showed that the addition of *Xylopi aethiopic a* powder to *Kunnu gyada* reduced the scores for sensory parameters. The strong aroma which could be objectionable to some people in some food could be considered as the limitation of these spices (Ogbonna et al., 2013).

Table 1. Total sugar content of *Kunnu gyada*.

Samples	0	1	2	3
A	50.85±0.42 <sup>a</sup>	49.52±0.14 <sup>a</sup>	51.27±0.10 <sup>a</sup>	44.81±0.14 <sup>a</sup>
B	50.83±0.17 <sup>a</sup>	45.52±0.17 <sup>b</sup>	46.29±0.3 <sup>b</sup>	36.52±0.57 <sup>b</sup>
C	50.83±0.28 <sup>a</sup>	44.89±0.25 <sup>c</sup>	46.62±0.54 <sup>b</sup>	36.33±0.30 <sup>b</sup>
D	50.74±0.23 <sup>a</sup>	43.63±0.48 <sup>d</sup>	43.98±0.28 <sup>c</sup>	36.26±0.14 <sup>b</sup>
E	50.57±0.40 <sup>a</sup>	43.55±0.18 <sup>d</sup>	43.27±0.16 <sup>c</sup>	34.88±0.10 <sup>c</sup>

Values are presented as mean±standard error of triplicate. Values with different superscripts in the same column are statistically significantly different ( $p < 0.05$ ).

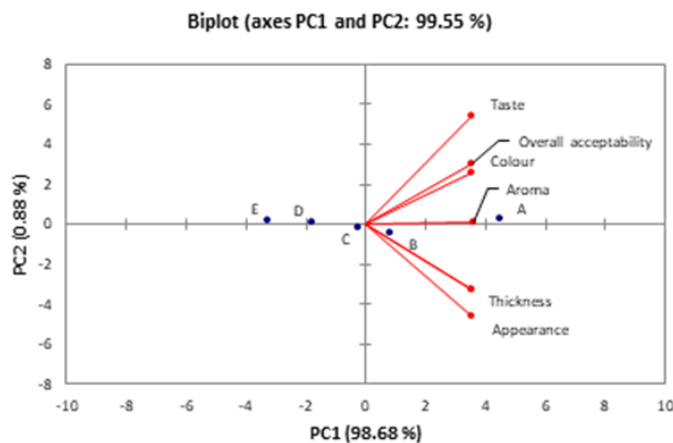


Figure 5. Principal component analysis of the sensory properties of *Kunnu gyada*.

## 4. Conclusion

The use of *Xylopi aethiopic a* powder as a bio-preservative in *Kunnu gyada* at 2% and 3% concentration extends its shelf life with minimum effect on the sensory characteristics. It also ensures the safety of this product by inhibiting toxin-producing *Fusarium oxysporum*. This could increase the production of *Kunnu gyada* on a commercial basis and empower women who are the major producers. Production of *Kunnu gyada* in large quantities will reduce malnutrition and provide a cheap and accessible nutritious diet for both children and adults.

## Conflicts of interest

The authors declare no competing interests.

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