Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science Applied and Health Science, Anchor University Lagos
URL: journal.aul.edu.ng
In AJOL: https://www.ajol.info/index.php/aujst
Vol. 7 No 1, January 2026, Pp. 55-62
ISSN: 2736-0059 (Print); 2736-0067 (Online)

Physicochemical and Bacterial Characterization of Cassava Steeping Water

[1]Adeosun, Tobi and [2]Ubakah, Divine

Kanmi Alo Interlink Polytechnic, Ijebu-Jesa, Osun State.

***Corresponding author Email**: Adeosuntobi995@gmail.com

Submitted 22 July, 2025
Accepted 25 September, 2025

Competing Interests.
The authors declare no competing interests

ABSTRACT

Background: Cassava (*Manihot esculenta*) is a tropical root crop native to South America and Africa. Cassava is often fermented during processing to reduce toxic compounds and improve food safety hence, studying the physicochemical and bacteriological characteristics of its steeping water is essential for ensuring safe consumption and understanding microbial dynamics in traditional foods. **Objective:** This study aims to understand the physicochemical and bacteriological properties of cassava steeping water. **Methods:** Ten (10) cassava tubers with each weighing 200g were peeled and immersed in 5 litres of distilled water for four days. The physicochemical analysis of the steeping water was done by using a digital meter (Extech model Do 700). The electrode of the meter was immersed into steeping water and readings were taken. The bacteriological analysis was done by plating 0.1 ml of the cassava steeping water on already solidified agar media such as nutrient agar, de mann-rogosa sharpe's agar and mannitol salt agar. **Results:** The pH of the steeping water sample ranges from 7.61 to 4.10 on the fourth day, the readings of temperature ranges from 39.2 °C on day one to 28 °C on day 4, conductivity values increased from 603 to 4164 (µS/cm) while total dissolved solid ranges from 284 mg/l to 2081 mg/l. The total heterotrophic bacterial count ranged from 50 CFU/ml to 10 CFU/ml, the total lactobacillus count ranged from 2 CFU/ml to 7 CFU/ml while the staphylococcal count reduced from 2CFU/ml to 7CFU/ml. The results from Pearson correlation reveals that there exists a significant negative relationship between total bacterial count and pH ($r = -0.601$, $p < 0.01$), temperature and pH (($r = 0.975$, $p < 0.01$), Conductivity and total bacterial count ($r = -0.601$, $p < 0.01$) and pH and conductivity ($r = -0.461$, $p < 0.01$). The organisms isolated from the cassava steeping water include *Lactobacillus fermentum, Klebsiella* spp, *Pseudomonas aeruginosa, Lactobacillus* spp, *Bacillus* spp, *Proteus* spp and *Staphylococcus aureus.* **Conclusion**: Findings from this research reveals the existence of bacterial diversity in cassava steeping water.

Keywords. Cassava, Fermentation, Lactobacillus, steeping water, physicochemical parameters

1. Introduction

Cassava (*Manihot esculenta*) is a tropical root crop native to South America, also known as yuca, manioc, or mandioca. It's a perennial shrub that grows up to 3 meters tall, with edible roots that can weigh up to 10 kg (Adebayo-Oyetoro, *et al.* 2013; Adeleke *et al.,* 2021*)*. Cassava roots are rich in carbohydrates, fiber, and minerals. They can be consumed fresh either boiled, mashed, or fried; they can also be processed into flour, starch, or tapioca and used as animal feed for livestock (Simonyan, 2015). Cassava is a good source of Carbohydrates, Fiber, Minerals, Vitamins *(*Oyewole *et al.,* 2017*).* Also, it is a vital crop for food security and income generation in many developing countries. It's also an important source of revenue for small-scale farmers and rural communities (Montagnac *et al.,* 2009).

Fermentation is a metabolic process in which microorganisms, such as bacteria, yeast, or mold, convert sugars into acids, gases, or other compounds. This process involves the breakdown of organic matter, such as carbohydrates, proteins, or fats, into simpler substances *(*Adebowale *et al.,* 2013*)*. Microorganisms involved in fermentation include bacteria such as *lactobacillus*, and *Escherichia coli*; Yeast such as *saccharomyces, Candida*, and *Geotrichum* species; Molds such as *Aspergillus, Penicillium* and *Rhizopus* species. *(*Oyewole *et al.,* 2017*).* The factors affecting fermentation consist of

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Temperature, pH, availability of nutrients, oxygen levels and the type of fermentation (Adebowale *et al.*, 2013).

Cassava fermentation is a process that involves the breakdown of cassava roots or leaves by microorganisms, such as bacteria or yeast, to produce lactic acid, ethanol, or other compounds. This process can improve the nutritional value, texture and flavor of cassava (Burns *et al.,* 2012).

Microorganisms involved in cassava fermentation are *Lactobacillus*, *Leuconostoc,* and *Pediococcus* species, fungi such as *Saccharomyces, Candida* and *Geotrichum* species (Oyewole *et al.*, 2017). Cassava steeping water is the liquid byproduct obtained from soaking cassava roots in water, typically during fermentation or detoxification. This process is crucial for reducing cyanogenic glycosides, natural compounds in cassava that can release toxic hydrogen cyanide if not properly processed (Montagnac *et al.,* 2009). Steeping allows these compounds to leach into the water, and microbial activity further breaks them down, making the cassava safer for consumption (Obilie *et al.,* 2004). This method is commonly used in traditional processing techniques, such as gari and fufu production in West Africa, where fermentation enhances the flavor, texture, and safety of cassava products (Amoa-Awua *et al.,* 2006).

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Cassava samples were collected from a local cassava processing plant in Lagos state. The samples were stored in sterile containers and transported to Kanmi Alo Interlink microbiology laboratory for analysis. Ten (10) cassava tubers with each weighing 200g were peeled and immersed in 5 litres of distilled water for four days as reported by Balogun *et al.* (2021).

2.2 Physicochemical Analysis of cassava steeping water

2.2.1. pH

The pH values were determined using digital meter (Extech model Do 700). The electrode was rinsed in distilled water and wiped with tissue paper, the electrode was then immersed into the cassava steeping water sample for reading and the pH value was read directly by the figure displayed. The electrode was carefully wiped before and after each measurement (Fubara *et al.,* 2022). The process was repeated daily during the period of fermentation.

2.2.2 Electrical Conductivity (µS/cm)

The conductivity values were determined using digital meter (Extech model Do 700). The electrode was rinsed in distilled water and wiped with tissue paper, the electrode was then immersed into the cassava steeping water sample for reading and the conductivity value was read directly by the figure displayed. The electrode was carefully wiped before and after each measurement (Fubara *et al.,* 2022). The process was repeated daily during the period of fermentation.

2.2.3 Temperature (°C)

The temperature values were determined using digital meter (Extech model Do 700). The electrode was rinsed in distilled water and wiped with tissue paper, the electrode was then immersed into the cassava steeping water sample for reading and the temperature value was read directly by the figure displayed. The electrode was carefully wiped before and after each measurement (Fubara *et al.,* 2022). The process was repeated daily during the period of fermentation.

2.2.4 Total dissolved solid

The total dissolved solid values were determined using digital meter (Extech model Do 700). The electrode was rinsed in distilled water and wiped with tissue paper, the electrode was then immersed into the cassava steeping water sample for reading and the total dissolved solid value was read directly by the figure displayed. The electrode was

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carefully wiped before and after each measurement (Fubara *et al.,* 2022). The process was repeated daily during the period of fermentation.

2.3 Fermentation and bacteriological analysis

Cassava tubers were peeled, washed, cut into cylindrical portions of about 3-5 cm. Two hundred grams (200g) of the washed tubers was steeped in five litres of sterile water for a period of four days. Bacteriological analysis was carried out daily by pipetting 1 ml of the cassava steeping water into a sterile water, this process was repeated until the appropriate dilution was reached which was 10^4 as reported by Balogun *et al.* (2021). A 0.1 ml each was pipetted from the selected dilution and dispensed onto the surface of a solidified nutrient agar, De mann rogosa sharpe's agar and mannitol salt agar plate. Each plate was incubated at 35 °C for 24 hours in an inverted position. The process was repeated daily during the period of fermentation. Colony forming unit per ml is calculated by using the formula below

$$\text{CFU/ml} = \frac{Colony\ counted\ x\ dilution\ factor}{Volume\ plated}$$

2.4 Biochemical Characterization of bacteria isolated from cassava steeping water

2.4.1 Gram staining

A sterile inoculating loop was used to pick a thin smear from the distinct colony on the media and placed on a clean grease-free slide containing a drop of sterilized water introduced aseptically. The smear was allowed to dry and heat fixed gently by passing it over blue gas flame. The heat fixed smear was then flooded with crystal violet solution for 60 seconds and rinsed with distilled water. The smear was again flooded with Lugol's iodine for 60 seconds and rinsed with distilled water, 95 % alcohol was poured on the slide until crystal violet has been completely washed off; it was then counter-stained with Safranin for another 30 seconds and was air dried. The smear was observed under oil immersion objective of the microscope as described by Olusola-Makinde (2019).

2.4.2 Sugar Fermentation Test

Sugar fermentation test was carried out to determine the ability of organism to ferment sugars with the production of acid and/or gas. Sugar indicator broth prepared using peptone water medium containing 0.5% fermentable sugar and 0.01% phenol red to note colour change. Ten millilitres of prepared sugar indicator broth dispensed into the test tubes each and Durham tube which would trap gas if produced was inverted carefully into each of the test tubes in such a manner that no air bubble was found. Each of the test tubes was properly covered. The test tubes were sterilized in the autoclave at 121 $^{\circ}$C for 15 minutes. After sterilization a loop full of 24 hours culture of the test organisms was introduced aseptically into the test tube, a test tube assigned to each labelled organism with respect to assigned sugar. A test tube without inoculated organism served as control. The set up was incubated at 37 $^{\circ}$C and observed for acid and gas production as described by Fawole and Oso (2001). Yellow colouration indicated acid production while gas production was indicated by displacement of the medium in Durham tube. The sugars used were glucose, fructose and sucrose.

2.4.3 Catalase Test

Three drops of 3% hydrogen peroxide was added to the test bacterium on a clean glass slide according to the method of Cheesbrough (2006). Effervescence, bubble formation indicated a positive catalase test.

2.4.4 Endospore Staining

Endospore staining was carried out to determine if the isolated organism is endospore positive. A smear of the organism was made on a grease free slide, the slide was flooded with malachite green stain while steaming for 5 minutes, the slide was rinsed and then flooded with safranin stain followed by blot drying. Under the microscope, endospore positive cells have green colour

https://dx.doi.org/10.4314/aujst.v7i1.7

while endospore negative cells appear pink (Cheesbrough, 2006).

2.5 Data Analysis

Data obtained from physicochemical analyses were analyzed using Graph pad prism version 8.02(263)

3.0 RESULTS AND DISCUSSION

3.1 pH and temperature of cassava steeping water

Figure 1 shows the pH and temperature values of cassava steeping water. The pH value of the steeping water dropped from 7.61 on day 1 to 4.10 on day 4 while the temperature values dropped from 39.2 °C on the first day of fermentation to 28 °C on the fourth day of fermentation. Findings from this work revealed that the pH value of the steeping water drops as the number of days increase, this is probably due to the fermentation process, this finding has been corroborated in Adegbehingbe *et al.* (2017). However, this is against the findings of Balogun *et al.* (2021) who reported that the temperature values remain constant throughout the days of fermentation. The temperature value of the steeping water also dropped as the days of fermentation increase this may be due to decreased microbial activity leading to reduced heat production (Adegbehingbe *et al.,* 2017; Akinrinlola *et al.,* 2024).

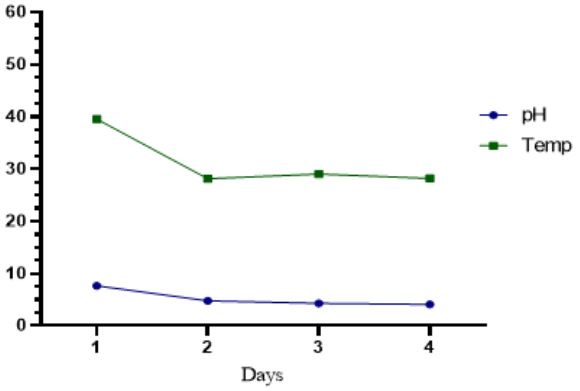

Figure 1: pH and temperature of cassava steeping water

3.2 Conductivity and Total dissolved solid of cassava steeping water

Figure 2 shows the conductivity and total dissolved solid values of cassava steeping water. The conductivity value of the steeping water increased from 603 µScm^{-1} on day 1 to 4164 µScm^{-1} on day 4 while the total dissolved solid values increased from 284 mg/l on the first day of fermentation to 2081 mg/l on the fourth day of fermentation. Findings from this study also revealed that the values of conductivity are directly proportional to the number of days, this finding has also been corroborated by Lawal *et al.* (2018). This may be as a result of release of ions into the steeping water by microbial metabolism. It was also observed from the result that the values of total dissolved solid also increased with the number of days, this observation may be as a result of release of soluble metabolites into the steeping water or breakdown of organic matter.

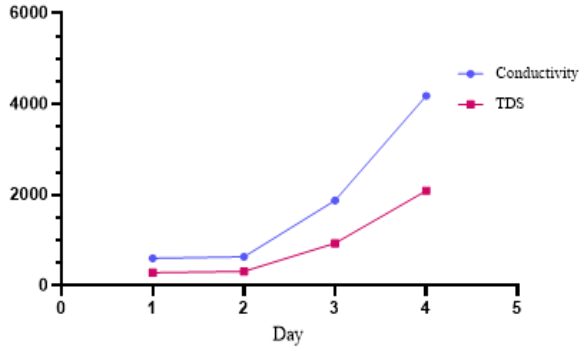

Figure 2: conductivity and total dissolved solid of cassava steeping water

3.3 Pearson correlation coefficient of cassava steeping water samples

Table 1 shows the Pearson correlation values between the physicochemical parameters to determine the strength and direction of relationships between the parameters. There exist a strong positive relationship between temperature and pH ($r = 0.975$, $p < 0.01$), There exists a negative relationship between pH and conductivity ($r = -0.461$, $p < 0.01$), There also exist a negative association between temperature and conductivity ($r = -0.494$, $p < 0.01$), there also exist a negative association between temperature and total dissolved solid ($r = -0.489$, $p < 0.01$) and also a moderately

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negative association between temperature and Total dissolved solid ($r = -0.522$, $p < 0.01$), there is a perfect positive interaction between conductivity and total dissolved solid ($r = 0.999$, $p < 0.01$) , temperature and total bacterial count ($r = 0.32$, $p < 0.01$), Findings from this work revealed that there exists a strong positive relationship between temperature and pH, this observation may be as a result of the fact that higher temperatures lead to increased microbial activity. In addition, there exists a negative relationship between pH and conductivity, this may be because of conductivity which is mainly influenced by total dissolved solid.

The population of total heterotrophic bacteria decreased with the number of days. This is probably due to the production of harmful metabolites by the *lactobacillus* spp. This finding has also been corroborated by Adeleke *et al.* (2021). It was also observed that the bacterial population of *lactobacillu*s spp increased with the number of days, this is probably because the bacterium is able to survive in acidic environments such as the one in a fermenting medium, this is also similar to the findings of Adeleke *et al.* (2021). Furthermore, this result revealed the ability of *Bacillus* spp to survive in a fermenting medium. This is also similar to the findings of Ezedima *et al.* (2006). This is may be as a result of its amylolytic activity. The findings from this study also revealed the ability of *Klebsiella pneumonia* to exist in a fermenting medium, this is also similar to the findings of Adeleke *et al.* (2021). The presence of *Staphylococcus aureus* in the fermenting medium may be as a result of improper handling originating from human contact.

3.4 Bacterial count of cassava steeping water sample

The total heterotrophic bacterial count ranges from $50x10^4$ CFU/ml to $10x10^4$ CFU/ml as the number of days increase however the lactobacillus count increases from 2 CFU/ml to 3 CFU/ml from day 1 to day 2 and it flattens out as from day 3. The total staphylococcal count also decreases from 3 CFU/ml to 0 CFU/ml as the number of days increase. The population of total heterotrophic bacteria decreased with the number of days. This is probably due to the production of harmful metabolites by the *lactobacillus* spp. This finding has also been corroborated by Adeleke *et al.* (2021). It was also observed that the bacterial population of *lactobacillu*s spp increased with the number of days, this is probably because the bacterium is able to survive in acidic environments such as the one in a fermenting medium, this is also similar to the findings of Adeleke *et al.* (2021).

Table 1: Pearson correlation coefficient of the physicochemical parameters of cassava steeping Water.

	pH	Temp	Conductivity	Total dis-	Total bacterial
pH	1	0.975	-0.461	-0.489	-0.601
Temp		1	-0.494	-0.522	0.32
Conductivity			1	0.999	0.27
Total dissolved solid				1	0.13
Total bacterial count					1

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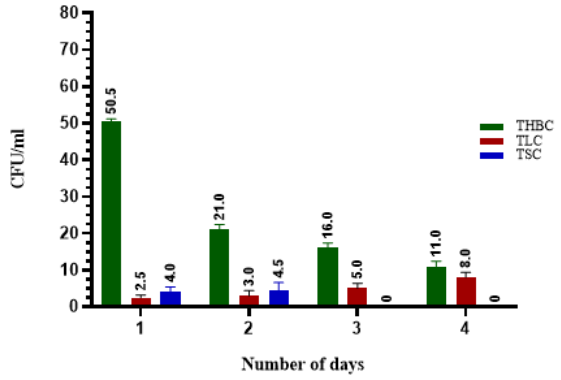

Figure 3: Bacterial count of cassava steeping water

Key: THBC: Total heterotrophic bacterial count; TLC: total lactobacillus count; TSC: total staphylococcal count.

3.4 Presumptive identities of bacteria isolated from cassava steeping water

The presumptive identities of bacteria isolated from cassava steeping waster are shown in Table 2. The result revealed the presumptive identities of the bacteria isolated from cassava steeping water with *Lactobacillus* spp as the most abundant bacterium. Findings from this work revealed the ability of *Bacillus* spp to survive in a fermenting medium. This is also similar to the findings of Ezedima *et al.* (2006). This is probably as a result of its amylolytic activity. The findings from this study also revealed the ability of *Klebsiella pneumonia* to exist in a fermenting medium, this is also similar to the findings of Adeleke *et al.* (2021). The presence of *Staphylococcus aureus* in the fermenting medium may be as a result of improper handling originating from human contact.

Table 2: Biochemical characteristics of bacteria isolated from cassava steeping water

Isolate code	Gram's reac-tion	Cata-lase	Spore staining	Sucrose	Lactose	Fructose	Probable organisms
A1, B2	-	-	-	-	-	-	*Lactobacillus fermentum*
A2	+	+	-	+	+	+	*Klebsiella spp*
B1	+	-	-	-	-	-	*Pseudomonas aeruginosa*
B3, D2	+	-	-	-	-	-	*Lactobacillus spp*
C1	+	+	+	+	+	+	*Bacillus spp*
C3	+	-	-	-	-	-	*Proteus spp*
D1	+	+	-	+	+	+	*Staphylococcus aureus*

https://dx.doi.org/10.4314/aujst.v7i1.7

Conclusion

Cassava steeping water supports diverse microbial populations with lactic acid bacteria emerging as key players in fermentation. These findings provide insights into microbial dynamics that contribute to safer and improved cassava products.

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