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ORIGINAL RESEARCH ARTICLE

Effect of Cassava-Derived Microorganisms on Cyanide Detoxification and the Sensory Properties of Fermented Cassava Products

Tefera Tadesse [1]*, Betemariam Kebede[1], Mulatu Workie[1], Adaba Tilahun[1], Daniel Yimer[1], Tariku Abena[1], Sewunet Abera[1], Melaku Alemu[2], Tirsit Tibebu[1], Asab Alemnhe[1]

[1]Ethiopian Institute of Agricultural Research, National Agricultural Biotechnology Researcher Center, Microbial Biotechnology Research Center Holleta, Ethiopia.
[2]Ethiopian Institute of Agricultural Research.
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ABSTRACT

Cassava utilization as food and feed is constrained by its high levels of cyanogenic glycosides and low protein content. This study aimed to isolate lactic acid bacteria (LAB) capable of tolerating and degrading hydrogen cyanide (HCN) from fermented cassava pulp juice. From 131 isolates obtained from cassava pulp collected at seven sites in Southern Ethiopia, 67 were confirmed as LAB based on morphological, physiological, and biochemical characteristics. Five isolates exhibiting strong HCN tolerance were selected for further evaluation. Survival assessment on media containing 400–800 mg/L HCN showed that isolates La2 and Db2 had the highest tolerance, with survival rates of 47.85% and 44.12% at 800 mg/L. Cyanide detoxification at a 1-mL inoculum level ranged from 44.86%–68%, 50.62%–69.21%, and 84.25%–87% after 24, 48, and 72 hours of fermentation, respectively. At a 2-mL inoculum level, the greatest cyanide reduction after 72 hours was observed in isolates La_2 (89.33%) and Cd1 (88.77%). All isolates demonstrated moderate to high detoxification capacity. PCR and 16S rRNA sequencing identified the isolates as *Lactobacillus casei* (Cd_2), *Lactiplantibacillus plantarum* (La_2, Db_2), and *Lactobacillus plantarum* (La_6, Cd_1). Overall, *Lactiplantibacillus plantarum* strain OM108155.1 (Db_2) and *Lactobacillus plantarum* strain b36 (La_6, Cd_1) were the most effective cyanide-reducing strains after 72 hours, indicating their potential for improving the safety of cassava-based foods and feeds.

*Corresponding author
E-mail: teferatadesse@yahoo.com (Tefera Tadesse)
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1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a vital root crop that provides a rich source of carbohydrates for millions of people worldwide, particularly in Africa (FAO, 2023). It is considered the fifth most important food security crop after rice, maize, wheat, and sorghum. Cassava belongs to the order Malpighiales and the family Euphorbiaceae. The genus *Manihot* was domesticated more than 500 years ago in west-central Brazil and the Amazon region (Andrew, 2022). It has since spread and is now widely cultivated across tropical regions of Africa, Latin America, and Asia (Diallo, 2013). Globally, cassava production ranks it among the top five food crops. The roots contain approximately 20–25% starch but are deficient in proteins, fats, vitamins, and essential minerals (Murugan et al., 2012). In Africa, cassava is a priority crop, especially in drought-prone and food-insecure regions, where total production exceeds 57 million tons (Teshome et al., 2004). Cassava-based products serve as primary dietary staples in many developing countries, providing a major source of calories after cereals such as rice and maize (Amsalu, 2006). According to Gunawan et al. (2017), cassava supplies dietary energy for more than one billion people and supports the livelihoods of millions of farmers worldwide, underlining its importance in global food security.

In Ethiopia, cassava is cultivated in diverse agro-climatic zones, particularly in the southwestern parts of Western Oromiya, Sidama, Gambella, and southern Ethiopia. The crop was first introduced to drought-affected areas such as Amaro, Gamo Goffa, Sidama, Wolayta, and Gedeo by non-governmental organizations to mitigate food shortages (Sayre, 2011). At the household level, cassava is often grown as a sole crop or intercropped with taro, enset, maize, haricot bean, and sweet potato (Sayre, 2010). Its cultivation expanded and gained greater acceptance after the 1984 famine due to its high yield, drought tolerance, ability to grow in poor soils, and resistance to pests and diseases (Soryotha, 2010; Ahaotu, 2013). These characteristics make cassava essential for the survival and livelihood of low-income households.

Despite its benefits, cassava has significant nutritional and health limitations. It contains very low levels of protein and essential micronutrients such as iron, zinc, provitamin A, and vitamin E. Additionally, cassava contains toxic cyanogenic glycosides, mainly linamarin and lotaustralin, which can release hydrogen cyanide (HCN) when improperly processed (Bradbury et al., 2002). Poor processing may result in toxic residual cyanide that can cause severe health problems, including goiter, creatinism, tropical ataxic neuropathy, and even paralysis (Akoja & Mohammed, 2011; Hadiyat & Wahyudi, 2013; Girma et al., 2015). Concentrations above the WHO safe limit of 10 ppm pose a serious health risk (Smid & Hugenholtz, 2010).

The very low protein content of cassava also contributes to malnutrition among populations heavily dependent on cassava-based diets (Tariku, 2013). As a result, effective detoxification and nutritional improvement strategies are urgently needed. Among the various methods used to reduce cyanide levels such as drying, soaking, boiling, and fermentation microbial fermentation has proven to be the most promising. Fermentation utilizes naturally occurring cyanide-degrading microorganisms, particularly lactic acid bacteria (LAB) and yeasts, to break down toxic compounds with minimal technological and energy input (Caplice & Fitzgerald, 1999; Buckle et al., 1985). These microorganisms have the ability to utilize hydrogen cyanide as a carbon and nitrogen source and may significantly improve the protein content of fermented products through rapid microbial biomass generation.

Previous studies have reported significant reductions in cyanide content through microbial fermentation (Girma et al., 2014). In addition to detoxification, fermentation enhances the sensory properties of cassava products, improves protein content, and reduces cyanogenic glycosides to safe levels (Ogiehor et al., 2005; Rajkowska & Kunicka-Styczyńska, 2010). Lactic acid bacteria lower the pH to below 6, which inhibits linamarase activity and limits further hydrogen cyanide release. They also produce antimicrobial compounds that inhibit pathogenic microorganisms, improving product safety and shelf life (Barrow & Feltham, 1999). Although cassava is traditionally fermented in many communities for food and feed, the specific roles of fermenting microorganisms in detoxification, nutritional enhancement, and sensory improvement remain inadequately documented, particularly in Ethiopia. Therefore, this study aims to evaluate the effects of microbial fermentation on cyanide detoxification, protein enhancement, and sensory quality of fermented cassava flour. It also seeks to isolate and characterize elite lactic acid bacteria strains involved in cassava fermentation using molecular techniques, with the goal of developing a cost-effective and sustainable method to improve cassava safety and nutritional value.

2. MATERIALS AND METHODS

2.1 Sample collection and inoculum preparation

Freshly harvested sweet cassava varieties were collected from different geographical locations within three zones of the Southern Nations, Nationalities, and Peoples' Regional State (SNNPRS): Wolayta, Gamo Goffa, and Segen Areas. From each zone, one district and representative kebeles were selected from each site 5 fresh cassava tuber, a total of 15 fresh cassava root was collected. The sampling sites included: Areka District (7°4′N, 37°42′E), located approximately 300 km southwest of Addis Ababa;

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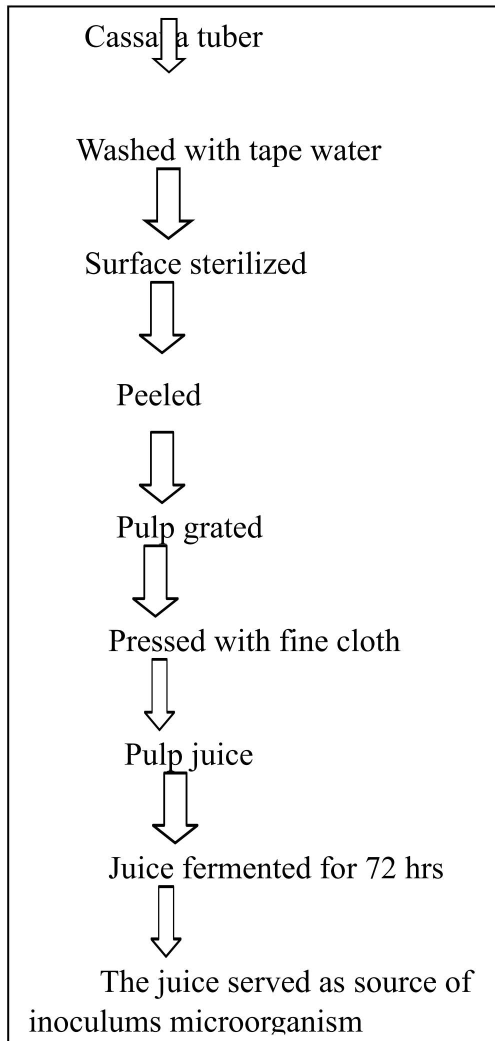
Mirab Abaya District (6°23′N, 37°44′E), about 458 km southwest of Addis Ababa; and Konso District (5°15′N, 37°29′E). These regions were selected because cassava is widely cultivated and consumed in southern Ethiopia.
Fresh cassava roots were collected in sterile 4-liter rubber containers packed with ice packs and transported within 24 hours to the National Agricultural Biotechnology Research Center (Microbial Biotechnology Laboratory) for microbiological analysis. Upon arrival, samples were kept in ice boxes to maintain freshness prior to processing. The fresh cassava tubers were manually sorted, cleaned, and prepared following the procedure described by Camargo *et al.* (2018) and as shown in Figure 1. The selected tubers were weighed, peeled manually using sterile laboratory knives, and the pulp was washed thoroughly with clean running water. The peeled cassava pulp was cut into pieces approximately 3 cm ± 0.03 m in length and soaked in 4L of tap water. The softened pulp mash was then squeezed through a clean fine cloth to obtain cassava pulp juice, which was allowed to ferment naturally for three days. This fermented juice served as the source of microbial inoculum for subsequent fermentation experiments.

A



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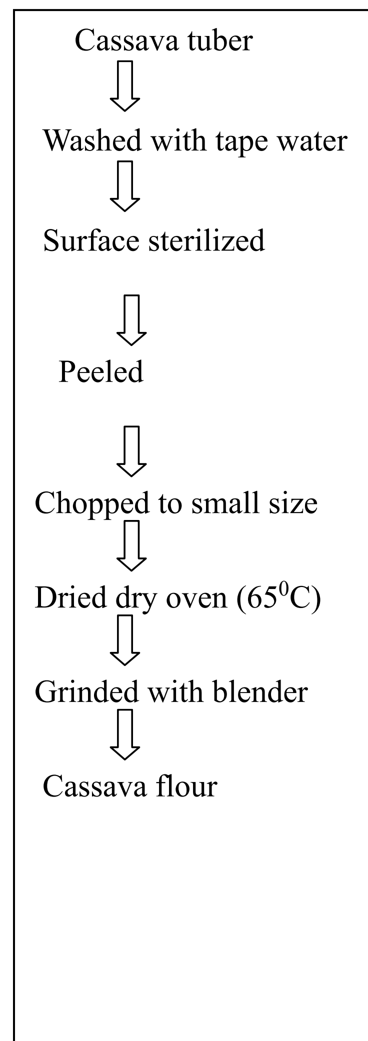

Fig. 1. Fresh cassava tubers were processed for cassava flour production and for the preparation of inocula from pulp juice. (A) Preparation of inocula from cassava pulp juice. (B) Preparation of cassava flour.

2.2 Experimental design

The experiment was conducted using a factorial arrangement of treatments consisting of five lactic acid bacterial (LAB) isolates, two inoculum volumes (1 mL and 2 mL), and three fermentation durations (24, 48, and 72 h). The treatments were arranged in a completely randomized design (CRD). Each treatment was replicated three times. Unfermented cassava flour without LAB inoculation was included as a control and was maintained under the same processing and environmental conditions as the fermented samples. All fermentation trials were carried out under identical laboratory conditions to minimize experimental variation.

2.3 Isolation and characterization of Lactic Acid Bacteria for cassava fermentation

Lactic acid bacteria were isolated using De Man, Rogosa, and Sharpe (MRS) agar. Serial dilutions were prepared up to 10^4–10^6 dilution factors. From each dilution, 0.1 mL of the sample was aseptically transferred onto sterile Petri dishes and pour-plated with molten MRS agar. The plates were incubated at 37°C for 24 to 48 hours. The isolated and identified LAB strains were selected as potential starter cultures for hydrogen cyanide detoxification. Each LAB isolate was inoculated into MRS broth and incubated anaerobically in Gas Pak jars (Gas Pak System, BBL) at 37°C for 24 hours. Following incubation, the cultures were centrifuged at 3,000 rpm for 4 minutes, and the supernatant was discarded. The resulting cell pellets were washed and resuspended in 0.9% sterile normal saline. The cell suspensions were standardized to an optical density of 0.5 or higher at 600 nm for use as starter cultures. For the fermentation experiments, 1 mL and 2 mL of each standardized starter culture (10^{-7}–10^{-8} CFU/mL) were inoculated into 20 g of cassava flour previously steeped in 350 mL of distilled water. The inoculated mixtures were incubated at 37 °C and allowed to ferment for 24, 48, and 72 hours. An uninoculated cassava sample was included as a control.

2.4 pH determination

The pH of the samples was determined according to the method described by Larmond (1991). To evaluate the growth performance of the isolates at different pH levels, 10 g of each fermented cassava sample was homogenized in 10 mL of distilled water. The pH of the resulting suspension was measured using a calibrated glass-electrode pH meter.

2.5 Temperature tolerance test

Temperature tolerance of the isolates was assessed using a modified method described by Rajkowska (Laguerre et al., 2017). Activated cultures adjusted to a concentration of 10^6–10^8 cfu/mL were inoculated into MRS broth and incubated at four different temperatures ranging from 25°C to 50°C for 24 hours. This analysis was performed to determine the optimal temperature range for bacterial activity, since low temperatures slow microbial growth and excessively high temperatures can cause cell death (Kumar et al., 2013). Bacterial growth was quantified using a spectrophotometer by measuring the optical density at 600 nm (OD_{600}), with distilled water serving as the blank.

2.5 Screening of isolates for hydrogen cyanide resistance

Screening for hydrogen cyanide resistance was performed following the method described by Mirizadeh *et al*. (2014), with slight modification. The samples were first inoculated into sterilized MRS broth at a ratio of 1 mL of sample to 100 mL of medium and incubated for 24 hours. Subsequently, 1 mL of the incubated MRS broth culture was streaked onto MRS agar supplemented with filter-sterilized potassium cyanide (KCN) at concentrations of 400, 600, and 800 mg/L. The inoculated plates were incubated at 35 ± 2 °C. After 48 hours of incubation, bacterial isolates capable of growing on the medium containing the highest KCN concentration (800 mg/L) were selected for further analysis. Individual colonies were then sub-cultured onto fresh MRS agar containing 800 mg/L filter-sterilized KCN and incubated for an additional 48 hours. The cyanide resistance of each isolate was assessed spectrophotometrically at 600 nm (OD_{600}), using distilled water as the blank. These interferences are removed at the laboratory prior to distillation by the addition of Sulfamic acid.

2.6 Determination of moisture content

Moisture content was assessed using the standard oven-drying method described by Tiwari et al. (2000). Samples were weighed before and after oven drying to a constant mass, and moisture content was calculated based on the loss in weight following drying.

2.7 Determination of hydrogen cyanide concentration

The hydrogen cyanide content of the fermented cassava samples was determined using an alkaline distillation and titration procedure adapted from Ben Omar et al. (2000). Ground cassava flour was subjected to acid treatment to release bound cyanide, followed by removal of interfering microbial metabolites. The liberated cyanide was distilled, captured in an alkaline solution, and subsequently titrated with silver nitrate. Cyanide concentration (mg/100 g wet weight) was calculated using the established equivalence factor, in which 1 mL of 0.02 N $AgNO_3$ corresponds to 1.08 mg of hydrogen cyanide. Indicators for argentometric titrations were selected to produce a color change at or near the equivalence point. Normally the indicator was selected to react with the added titrating agent, not the analyte.

2.8 Molecular characterization of cassava LAB

The five isolates showing the highest levels of cyanide reduction were selected for molecular identification based on their cyanide-reducing capacity, morphology, and biochemical characteristics. Genomic DNA was extracted from the selected isolates using a commercial purification kit in accordance with the manufacturer's protocol. Following extraction, DNA concentration and purity were assessed spectrophotometrically using absorbance readings at 260 nm and 260/280 nm, respectively.

2.8.1 Amplification of the 16S rRNA Gene

The 16S rRNA gene was amplified from the genomic DNA using universal bacterial primers targeting conserved regions of the gene. In addition, the intergenic spacer (IGS) region between the 16S and 23S rRNA genes was amplified using established primer sets described in the literature. Polymerase chain reaction (PCR) was performed using a standard reaction mixture prepared with commercial master mix reagents. Thermal cycling conditions followed conventional protocols commonly used for bacterial 16S rRNA gene amplification.

PCR products were separated by agarose gel electrophoresis and visualized using a nucleic acid stain under UV illumination to confirm the expected amplicon size. Purified PCR products were subsequently sequenced using capillary electrophoresis on an automated genetic analyzer.

2.8.2 Sequence Analysis

Resulting sequences were edited and assembled using standard bioinformatics tools. The consensus sequences were compared with reference sequences available in public databases through BLAST searches to determine the closest phylogenetic relatives of each isolate. Sequence similarity values and phylogenetic placement were used to assign the isolates to their respective lactic acid bacteria taxa. Phylogenetic trees were generated using established algorithms to illustrate the relationships between the cassava isolates and known LAB species.

2.9 Sensory Evaluation

Sensory attributes of the cassava cake were evaluated using a preference test as described by Wu et al. (2014). The ingredients used in the preparation of the cassava cake included 2 cups of grated cassava, 1 cup of coconut milk, 1/2 cup of sugar, 2 large eggs, 1/4 cup of melted butter, 1/4 teaspoon of salt, and 1 teaspoon of vanilla extract. All ingredients were blended for 2 minutes until a homogeneous batter was obtained, after which the mixture was baked in a preheated oven at 220 °C for 1 hour and 10 minutes, or until a golden-brown colour was achieved.

Cassava cakes prepared using these ingredients, along with fermented cassava flour inoculated with five pure cultures of lactic acid bacteria (in triplicate), were subjected to sensory analysis following the method of Wu *et al.* (2014). Panellists familiar with cassava-based foods were recruited from the National Agricultural Biotechnology Research Center. Ten panellists (four males and six females) evaluated the samples, which were presented in random order on the test day. Sensory attributes were rated using a 5-point hedonic scale. Samples receiving an overall quality score greater than 3 were considered acceptable.

2.10 Statistical Analysis

All analytical determinations were conducted in triplicate. The triplicate measurements for each treatment were evaluated to determine the effects of starter culture type, inoculum size, and fermentation time on pH, moisture content, cyanide content, and consumer acceptance of the cassava-based food product. Data were analyzed using Analysis of Variance (ANOVA). Where applicable, statistical analyses were performed using SPSS software (version 20.0), and mean comparisons were conducted using Tukey's HSD test at $P \leq 0.05$. Molecular data were analyzed using MEGA version 11.

3. RESULTS AND DISCUSSION

3.1 Isolation and screening of cyanide-tolerant microorganisms

A total of 131 lactic acid bacteria (LAB) isolates were obtained from cassava pulp juice fermented for three days. Among these, 67 were purified based on colony morphology, including size, shape, color, and texture (Table 1). Five isolates Cd2, La2, La6, Db2, and Cd1were selected for further characterization based on their physiological, biochemical, and cyanide-tolerance properties.

Morphological, physiological, and biochemical characterization showed that all five isolates were Gram-positive, non-motile, catalase-negative, and either spherical or rod-shaped. The isolates were capable of growing at temperatures ranging from 25–55 °C and at pH values between 2.5 and 4.5 (**Table**s 1 and **2**). These observations are consistent with the findings of Divisekera et al. (2019), who reported that LAB obtained from traditionally fermented foods were typically Gram-positive, catalase-negative, and exhibited rod or cocci cell morphology.

Table 1: Morphological characteristics of lactic acid bacteria screened from fermented cassava pulp juice

S.N	Isolate	Colony surface	Colony size	Colony margin	Colony colour	Cell morphology
1	La_2	rough	0.3-0.5 mm	entire	Dull white	Rods in pair
2	La_6	smooth	1 mm	Spherical	white	Long rods in chain
3	Db_2	smooth	1.1 mm	circular	white	Short rods, single/pair
4	Cd_1	smooth	1. 1mm	circular	Greyish white	Cocci in pair
5	Cd_2	rough	0.2-0.7 mm	spherical	white	Cocci in chain

La_2 La_6 (lactic acid bacteria isolated from cassava sample collected from location 2 and 6), Db_2 (Lactic acid bacteria isolated from cassava sample collected from Dellbo) Cd_1 and Cd_2 (lactic acid bacteria isolated from cassava sample collected from Darge) This table presents the morphological characteristics of lactic acid bacteria isolated from fermented cassava pulp juice, including colony appearance on MRS agar and cellular morphology observed by microscopic examination, which were used for preliminary identification of the isolates.

Table 2. Physiological characteristics of lactic acid bacteria isolated from fermented cassava pulp juice

S. N	Isolate	Gram Reaction	Oxidase test	Catalase test	Motility
1	La_2	+	-	-	-
2	La_6	+	-	-	-
3	Db_2	+	+	-	-
4	Cd_1	+	-	-	-
5	Cd_2	+	+	-	-

Gram Reaction: (+) Gram-positive, (-) Gram-negative **Oxidase Test**: (+) Positive for oxidase enzyme: (-) Negative for oxidase enzyme, **Catalase Test**: (+) Positive for catalase enzyme, (-) Negative for catalase enzyme, **Motility**: (+) Motile, (-) non-motile. The table presents the morphological and biochemical characteristics of lactic acid bacteria (LAB) isolates obtained from fermented cassava

3.2 pH tolerance of selected LAB isolates

The five selected isolates demonstrated the ability to survive at low pH conditions. Overall, isolates exhibited greater tolerance at pH 4.5 than at pH 3.5 or 2.5. Among them, isolate Db_2 showed the highest survival rate at pH 2.5 (78%), followed by Cd_2 (72%). All isolates exhibited their highest specific growth rates at pH 4.5, indicating that the optimal pH range for growth is between 3.5 and 4.5 (**Figure 2**). These results are in agreement with Amenu and Bacha (2023), who reported that several LAB strains remain viable at pH 2.5–3.5 after incubation. Similar findings were reported by Samedi and Charles (2019), who observed increased LAB viability with decreasing pH. The acid tolerance observed in the present study suggests that these isolates possess adaptive mechanisms that enable survival and colonization in acidic environments, as similarly described by Feng et al. (2017).

Figure 3 refers to a graphical representation showing how different LAB (Lactic Acid Bacteria) isolates withstand or grow at four different temperatures: 25°C, 35°C, 45°C, and 55°C. This test evaluated the temperature range in which the bacteria remain viable or active, indicating their adaptability and potential for various applications.

The growth of microorganisms is influenced by temperature bec ause changes in the environment can a limit and affect the stabil ity of microbial growth. Thus, each bacterial isolate adapts to an optimal range of temperatures in which it increases. Selected isolates showed the ability to adapt and grow in a wide spectrum of temperatures (25- 55^0C). Based on the bar graph presented in **Figure 3**, the highest count of bacteria, regardless of used media, was observed when cultures were conducted at 35^0C. The optimal growth conditions maintained at (35^0C) resulted in a better proliferation of isolates. Further increase in the incubation temperature had a negative effect on the growth dynamics and led to deceleration of growth. Microbial growth decreases with increasing temperature past the optimum due to irreversible denaturation of essential cellular proteins and enzymes, as well as the disruption of the cell membrane structure. In essence, while increasing temperature toward the optimum initially speeds up metabolic reactions, exceeding the maximum temperature causes catastrophic, irreversible damage to the cell's machinery and structure, leading to a rapid decline in the growth rate and eventually cell death.

This finding is consistent with the report of Ampe et al. (1994), who observed that the maximum growth of lactic acid bacteria

occurred at 35–37 °C, after which growth declined. The reduction in growth beyond the optimum temperature is attributed to increased energy expenditure for cellular maintenance, heat-shock responses, membrane leakage, and protein denaturation. A wide range of thermal tolerance is therefore an important trait for the industrial application of LAB. These results also agree with the findings of Abba and Khalil (2010), who reported that some cassava-fermenting lactic acid bacterial strains can grow at temperatures up to 55 °C.

3.3 Effect fermentation time and inoculums level on fermented cassava products

During fermentation, the pH was reduced from 6.00 for the unfe rmented control to 3.7 for the different inoculums and fermentat ion times (**Figure 2**).
Similarly, Tefera *et al*., (2004) reported the optimum pH for cas sava fermenting that lactic acid bacteriaare between 3.5- 4.5. Th ese findings show that the effect of microorganisms on cassava fermentation in a single or mixed culture result in a significant r eduction in pH, which result from the production of organic aci d by lactic acid bacteria on the carbohydrate content of cassava roots Tilahun *et al*., (2013) and Kostinek., (2007).There was a g eneral decrement of all samples over 24 to 72hrs of fermentatio n, which resulted in longer shelf lives andsafety of foods in cass ava fermentations, as it was stated By (Buckle *et al*., 2015 and Edward *et al*., 2011). In the same way Coulin., (2006) and Tann ock., (2004) suggests thatcyanide-degrading microrganisms hav e the potential to produce acid producers so that they can impro ve food Buckle *et al*., (2015), during cassava fermentation. This is because spoilaging bacteria and some members of *Enterobac teriaceae cannot be tolerated and* increase below pH (4.2). A d ecrease in pHdue to lactic acid bacteria inhibits the growth and multiplication of pathogenic organisms in fermented products K obawila *et al* 2004).

The selected Lactic Acid Bacteria (LAB) isolates demonstrated robust growth in high-cyanide environments and were identified as the dominant members of the microbial community during cassava fermentation. These findings are consistent with those of Sarawut et al. (2013), who reported that LAB are endogenous and dominant microorganisms during cassava fermentation. However, these results diverge from the findings of Izah et al. (2018), who reported the detection of Lactobacillus plantarum exclusively in cereal-based substrates. The results, depicted in **Figure 4**, indicate that all isolates exhibit a degree of tolerance to hydrogen cyanide (HCN) concentrations of 400, 600, and 800 mg/L; however, their tolerability potential decreased as the cyanide concentration increased. Isolates La2 and La6 demonstrated optimum tolerance potential at an HCN concentration of 400 mg/L, followed closely by isolate Db2. Both isolates La2 and Db2 maintained good tolerance potential at a concentration of 600 mg/L.

Generally, the tolerability of the isolates varies based on their individual capacity to resist HCN (**Figure 4**), and their growth performance consistently decreases with an increasing ratio of HCN concentration.

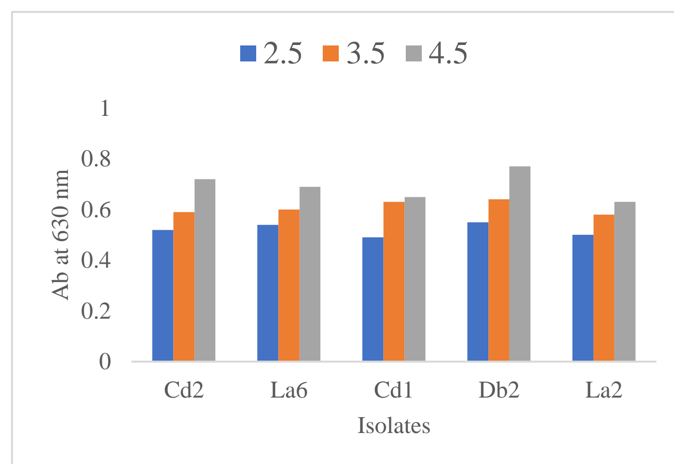

Fig. 2. LAB isolates pH tolerance test at different pH (2.5 3.5 and 4.5). X-axis isolates and Y axis-microbial growth absorbance reading at different pH. The results provide insights into the adaptability of these bacteria to acidic conditions and their growth survival at different pH levels. For all isolates triplicate samples was used.

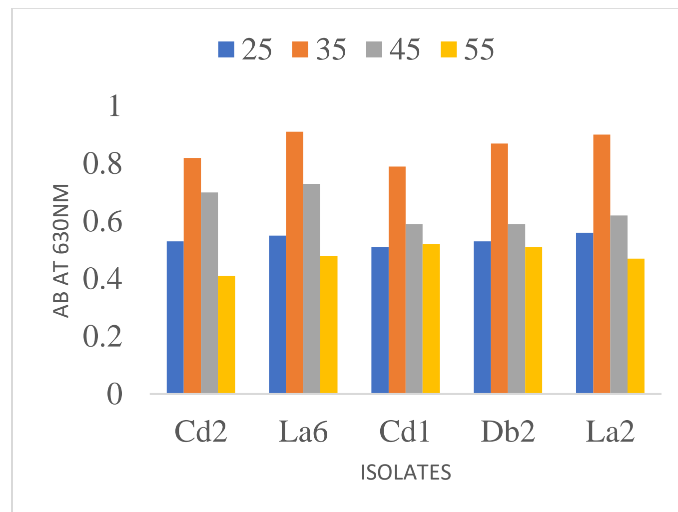

Fig. 3. Temperature tolerance of lactic acid bacterial (LAB) isolates at 25, 35, 45, and 55 °C. X- axis, isolates and Y- axis, microbial growth absorbance reading at 25, 35, 45, and 55 °C. For all isolates triplicate samples was used.

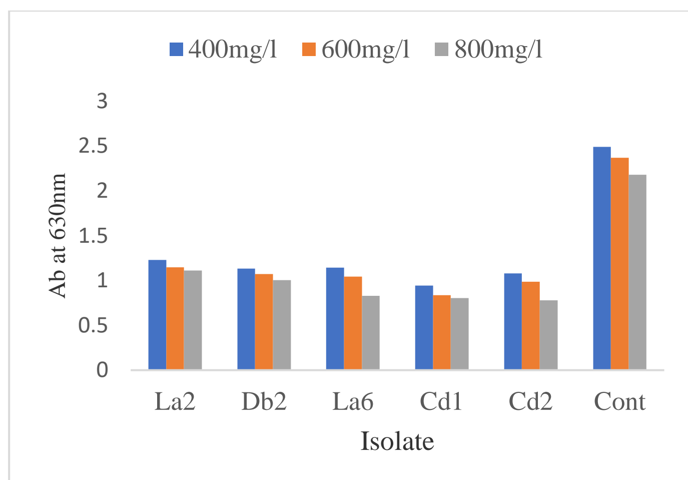

Fig. 4. Differences in HCN tolerability potential among LAB isolates at varying HCN concentrations (400–800 mg/g). X axis Isolates and Y axis microbial growth absorbance reading from different HCN concentration. For all isolates triplicate samples was used.

The high abundance of cyanide-utilizing bacteria in fermented cassava pulp juice indicates that these microorganisms are active degraders of cassava cyanogenic compounds and are capable of using cyanide as a carbon and nitrogen source (Enerijiofi *et al.*, 2017). Similar findings were reported by Kumar et al. (2013), who isolated cyanide-tolerant cassava microorganisms able to grow at concentrations of up to 780 mg/L, using cyanide as their sole carbon and nitrogen source.

Microorganisms, particularly Lactic Acid Bacteria (LAB), tolerate and degrade cyanide during cassava fermentation through a combination of enzymatic detoxification pathways, physiological adaptations (like alternative respiration and anaerobic metabolism), and the acidification of the fermentation medium. Thus the current findings indicate that the selected isolates were highly tolerant to up to a concentration of 800 mg L^{-1} of hydrogen cyanide (HCN). This tolerance level exceeds those reported in previous studies, such as the 700 mg L^{-1} by Uzoh et al. (2022) and 104 mg L^{-1} by Lacerda et al. (2005). Furthermore, their capacity for tolerability was greater than the threshold reported by Guira et al. (2016), who suggested that microorganisms generally cannot tolerate high cyanide concentrations exceeding 497 mg L^{-1}.

Microorganisms capable of surviving in such toxic environments typically develop specific enzymatic and physiological responses, enabling them to utilize cyanide as a substrate for growth and subsequent proliferation. This observation is consistent with earlier findings by Wilfrid et al. (2009) and Marco et al. (2014), which reported on the significant biodegradation potential of cassava mill effluents when using indigenous microorganisms.

3.4 Potential cyanide degradation of selected isolates

A significant difference was observed in the cyanide-reducing ability of the single bacterial starter cultures, particularly isolates La_2 and Cd_1, depending on inoculum level and fermentation duration. However, no significant difference was found between the cyanide reduction achieved by La_2 and Cd_1 when a 2 mL inoculum was applied for a 72hour fermentation period (**Table 3**).

Table 3: ANOVA value for single and interaction effect of isolate, time and inoculums level on % of cyanide reduction.

Source	DF	Anova ss	Mean square	F value	Pr > F
Isolate	5	11872.43569	2374.48714	525.51	<.0001
Inoculum	2	13145.62736	6572.81368	1454.66	<.0001
Time	3	24431.10745	8143.70248	1802.32	<.0001
iso*inoc*time	20	0.00000	0.00000	0.00	1.0000

Each factor individually (isolate, inoculum level, and time) significantly affects the percentage of cyanide reduction ($p < 0.0001$). The interaction between isolate, inoculum, and time shows no significant effect on cyanide reduction ($p = 1$), meaning their combined effect does not differ from what is expected based on their individual effects.

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The ANOVA results demonstrated that Isolate (Iso), Inoculum (Inoc), and Time had highly significant effects on the cyanide reduction ($p < 0.0001$) (Table 3). Specifically, isolates differed significantly in their effect ($F = 525.51$), indicating substantial variation among the tested isolates. Inoculum treatments also had a pronounced impact ($F = 1454.66$), suggesting that differences in inoculum type or concentration significantly influenced percent of cyanide reduction. Similarly, the effect of time was highly significant ($F = 1802.32$), reflecting considerable temporal changes in the cyanide reduction. In contrast, the three-way interaction among Isolate, Inoculum, and Time (Iso × Inoc × Time) was not significant ($F = 0.00$, $p = 1.0000$), indicating that the combined influence of these factors did not exceed their individual effects. These findings suggest that the main sources of variation in the percent of cyanide content are attributable to the independent effects of isolate, inoculum, and time rather than their interaction. Overall, the extent of cyanide reduction in all fermented samples was lower compared to the unfermented control. The degree of reduction varied with the type of microorganism used, inoculum size, and fermentation time. During fermentation, the initial cyanide content decreased by 24% to 87% (mg/100 g) across treatments, confirming that fermentation substantially reduces cyanide levels in fermented cassava. This result aligns with observations by Adeleke et al. (1994), who reported that the introduction of cyanide-tolerant starter cultures enhances cyanogenic compound reduction. Similar trends have been documented by Westby and Choo (1994), who demonstrated that starter cultures such as *Lactiplantibacillus plantarum* and *Bacillus subtilis* increase the rate of cyanide degradation during cassava fermentation.
Table 4 Mean % of HCN reduction through microbial fermentation

The highest cyanide reduction (90.80%) was recorded in samples fermented with La_6 using a 2 mL inoculum over a 72-hour period. This was followed by reductions of 88.82% and 88.59% in samples fermented with La_2 and La_6, respectively, under the same conditions. In contrast, the lowest reduction (62.81%) occurred in samples inoculated with La_6, after only 24 hours of fermentation. These results are consistent with findings by Ampe (1994), who reported that fermentation periods exceeding 48 hours generally result in cyanide reductions above 75.65%. The marked decrease in cyanide content observed in cassava flour samples may be attributed to increased microbial biomass capable of degrading cyanogenic glycosides (linamarin) and the production of extracellular linamarase, as described by Meitha et al. (2016). The addition of starter cultures also accelerates acidification of the fermentation matrix, which enhances the activity of enzymes that degrade the cellular structure, thereby facilitating linamarin hydrolysis by linamarase. This mechanism is in agreement with the findings of Aro (2008) and Odey (2019), who reported up to 75% reduction in hydrogen cyanide content in cassava roots following fermentation with appropriate microorganisms. Differences in the extent of cyanide reduction among isolates can be attributed to variations in fermentation time and inoculum size. Regardless of the specific isolate used, all treatments resulted in more than 60% cyanide reduction after 72 hours of fermentation. The variation in hydrogen cyanide degradation rates among isolates and fermentation durations is likely related to differences in their metabolic capacity to utilize cyanogenic compounds as sources of nitrogen and carbon (**Table 4**).

Table 4: Detoxification Potential of Lactic Acid Bacteria Isolates through Hydrogen Cyanide (HCN) Reduction"

% of HCN Reduction			
Code of Isolate	Inoc	Time	Mean STD Dve
CD1	1	24	42.540 ± 1.131^{fg}
CD1	1	48	68.723 ± 1.592^{de}
CD1	1	72	88.300 ± 0.987^{ab}
CD1	2	24	62.816 ± 3.078^{ef}
CD1	2	48	75.383 ± 4.524^{cd}
CD1	2	72	88.590 ± 0.655^{ab}
CD2	1	24	34.376 ± 3.481^{g}
CD2	1	48	74.746 ± 1.918^{de}
CD2	1	72	86.503 ± 1.157^{bcde}
CD2	2	24	73.703 ± 4.691^{cde}
CD2	2	48	80.307 ± 1.417^{bcd}
CD2	2	72	87.083 ± 2.954^{abcd}
DB2	1	24	58.297 ± 2.874^{ef}
DB2	1	48	79.033 ± 1.786^{cd}
DB2	1	72	87.953 ± 1.158^{abc}
DB2	2	24	76.193 ± 2.796^{cd}
DB2	2	48	81.350 ± 0.782^{bcde}
DB2	2	72	87.953 ± 1.158^{abc}
LA2	1	24	54.767 ± 3.619^{f}
LA2	1	48	80.770 ± 0.365^{bcde}
LA2	1	72	89.050 ± 1.390^{a}
LA2	2	24	75.907 ± 0.438^{cd}
LA2	2	48	80.887 ± 1.566^{bcde}
LA2	2	72	88.820 ± 2.158^{ab}
LA6	1	24	42.253 ± 0.820^{fg}
LA6	1	48	81.003 ± 0.364^{bcd}
LA6	1	72	89.340 ± 0.265^{a}
LA6	2	24	69.997 ± 2.435^{d}
LA6	2	48	69.997 ± 2.435^{d}
LA6	2	72	90.790 ± 0.917^{a}
Con	0	0	0.045 ± 0.064

This table presents the percentage reduction of hydrogen cyanide (HCN) by different isolates (coded as Cd_1, Cd_2, Db_2, La_2 and La_6) at two inoculum levels (1and 2ml) and time of fermentation (24, 48 and 72hours). For all isolates triplicate samples was used. Each row shows the mean percentage of HCN reduction along with the standard deviation (STD Dev). *a-g Values with different superscript letter in the same column are significantly different among samples ($p<0.05$).

Molecular identification results consistently indicated the prevalence of *Lactobacillus* species as the dominant microorganisms within the samples. The 16S ribosomal DNA

(rDNA) gene sequences obtained were compared with existing sequences available through the National Center for Biotechnology Information (NCBI) database. The accession numbers assigned were KX057606.1 for strains La_6 and Cd_1, GQ131152.1 for strain Cd_2, OR502303.1 for strain La_2, and OM108155.1 for strain Db_2. The five isolates (Cd_1, Cd_2, La_2, La_6, and Db_2) were characterized and identified as *Lactobacillus plantarum* strain, *Lactobacillus casei* strain, *Lactiplantibacillus plantarum* strain, and *Lactiplantibacillus plantarum* strain. All Lactic Acid Bacteria (LAB) isolated from the fermented cassava pulp juice were found to be members of the *Lactobacillaceae* family.

These findings align with previous reports by Orji and Ayogu (2018), which indicated that *L. plantarum* and *L. casei* strains are commonly found in both dairy and various fermented food products. The presence of *L. plantarum*, *L. fermentum*, and *L. lactis* during cassava fermentation has been independently confirmed by multiple authors, including Ojokoh and Udeh (2018), Egwim et al. (2013), and Tiwari et al. (2018). The consensus across numerous studies supports the frequent presence of *Lactobacillus plantarum* in traditional cassava fermentation processes. Molecular analysis based on 16S rRNA gene sequencing confirmed that isolate Cd_2 shared the highest similarity (approximately 98.62%) with *Lactobacillus casei* IMAU70036. Isolates La_6 and Cd_1 exhibited the highest similarities (99.27%) with *Lactobacillus plantarum* strain b36 whereas Db_2 exhibited 98.81 similarity with *Lactiplantibacillus plantarum* strain TMPC20734 (**Table 5**).

Table 5. Phylogenetic Neighbors of Cyanide-Degrading Lactic Acid Bacteria Based on Partial 16S rRNA Gene Sequence Similarity.

S/N	Isolate	Species of LAB Homologous	% Identity	Acc. Num
1	Cd_2	*Lactobacillus Casei* strain IMAU70036	98.62	GQ131152.1
2	La_2	*Lactiplantibacillus plantarum* strain HBUR51266	98.91	OR502303.1
3	La_6 and Cd_1	*Lactobacillus plantarum* strain b36	99.27	KX057606.1
4	Db_2	*Lactiplantibacillus plantarum* strain TMPC20734	98.81	OM108155.1

The isolates Cd_2, La_2, La_6/Cd_1, and Db_2 have been identified mostly as *Lactobacillus casei* or *Lactiplantibacillus plantarum* strains.High percentage identity values (above 98%) indicate strong genetic similarity, confirming accurate species identification. Some isolates (La_6 and Cd_1) share the same homologous species with 99.27% identity, suggesting they may be closely related or the same species. This table provides important taxonomic confirmation of the LAB isolates used in the study, supporting their characterization and potential applications.

These findings are also consistent with the observations of Wahyuni et al. (2023), who reported that the predominant lactic acid bacteria (LAB) associated with African indigenous cassava-fermented foods include *Lactobacillus* species such as *L. casei*, *L. paracasei*, *L. plantarum*, *L. brevis*, and *L. salivarius*. Similarly, Pongsub et al. (2022) identified *Lactobacillus plantarum* strain CQ2017ZC, *Pediococcus pentosaceus* strain 1931, and *P. pentosaceus* strain 5583 in cassava-based fermented foods.

In agreement with the present study, Gunawan et al. (2015) reported that *Lactobacillus casei* TH14 contributed to improvements in chemical composition, fermentation end products, and anaerobic stability during cassava pulp fermentation. In addition, studies on *puba*, a traditional Brazilian fermented cassava product, have documented the presence of *L. casei*, *L. plantarum*, and *L. fermentum*, while *Lactobacillus perolans* and *L. brevis* represented minor components of the microbial community. Across all stages of the fermentation process, *Lactobacillus pentosus* and *L. plantarum* were identified as the dominant bacterial spec ies.

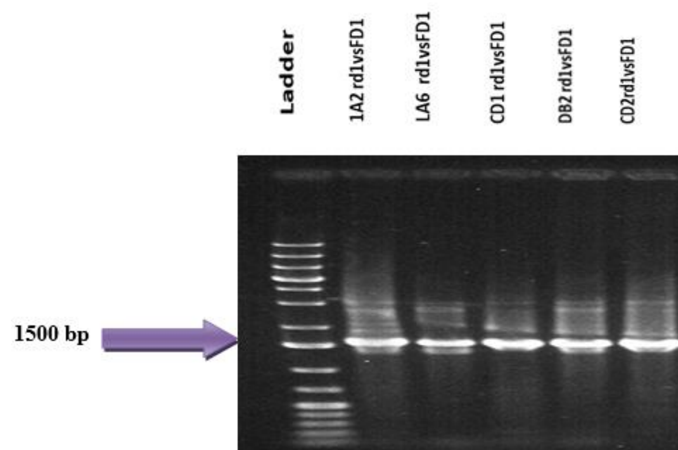

Fig.5. PCR product of 5 bacterial isolates obtained from cassava juice with amplification primer RD1/FD1 and ladder. The figure visually confirms successful amplification and allows assessment of PCR product size for identification. PCR products**:** The DNA fragments amplified using PCR (Polymerase Chain Reaction). Primer RD1/FD1**:** Specific primers used to amplify a target gene or DNA region in these bacteria. Ladder**:** A DNA size marker (molecular weight ladder) used as a reference to estimate the sizes of PCR products.

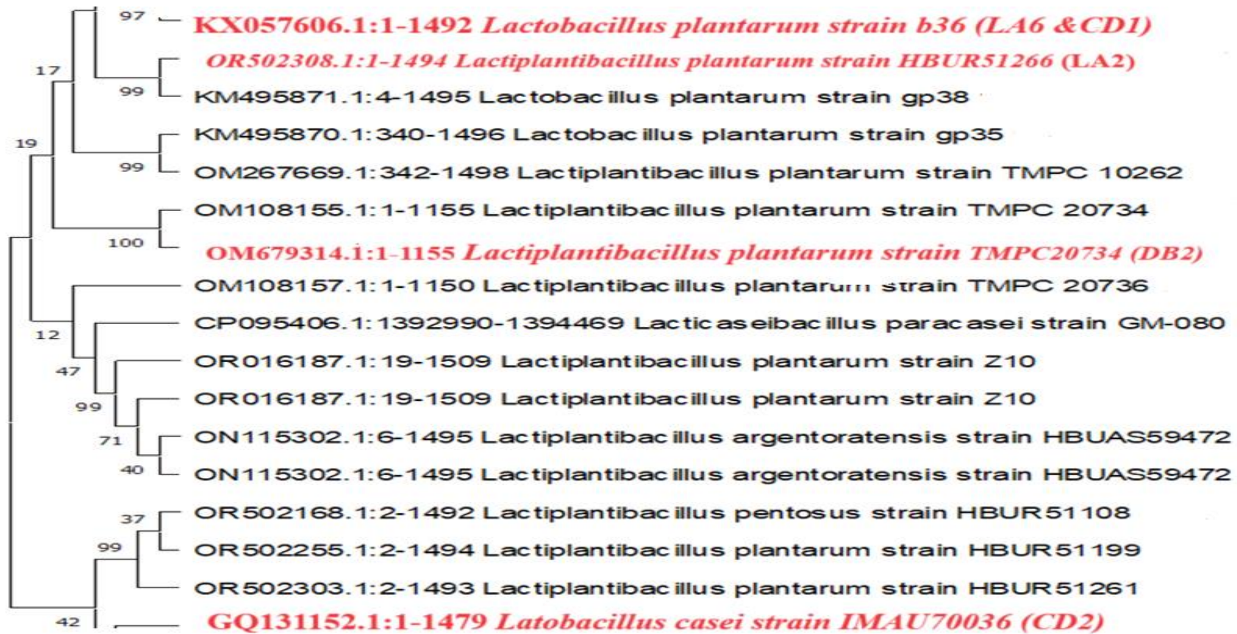

Fig. 6. PCR products of 5 bacterial isolates obtained from cassava pulp juice with amplification Primer RD1/FD1 and ladder" refers to an image (figure) showing the results of PCR amplification of DNA from five bacterial isolates sourced from cassava pulp juice

The moisture content of fermented cassava flour inoculated with selected isolates (Lactobacillus casei, *Lactiplantibacillus plantarum*, and *Lactobacillus plantarum*) for 24, 48, and 72 hours ranged between 9.41% and 12.65% (**Figure 7**). The results of the present study indicate that fermentation influences the chemical composition of cassava products, largely due to the utilization of available substrates by microorganisms for the production of various metabolites. This observation agrees with the findings of Agboregbe et al. (1995), who reported a progressive reduction in moisture content of fermented cassava samples as fermentation time increased. This trend may reflect the metabolic activity of microorganisms, which utilize water during growth and metabolite synthesis. Since moisture content below 12% is generally considered acceptable for suppressing microbial growth, the current findings fall within the recommended range, except for samples fermented with the *Lactobacillus plantarum* strain B36 (Cd_1) inoculated at 2 mL for 24 hours, which exceeded the threshold. This is consistent with Agboregbe (1995), who noted that low moisture content enhances the shelf life and storability of fermented cassava products.

The highest moisture content (12.74%) was recorded in the starter culture after 24 hours of fermentation, whereas the lowest value (9.41%) was observed in samples fermented with *Lactiplantibacillus plantarum* strains after 72 hours. The slight reduction in moisture content observed during fermentation may be attributed to microbial activity and water utilization for metabolic processes. Additionally, the introduction of microbial inocula into cassava flour may facilitate moisture loss during the fermentation process.

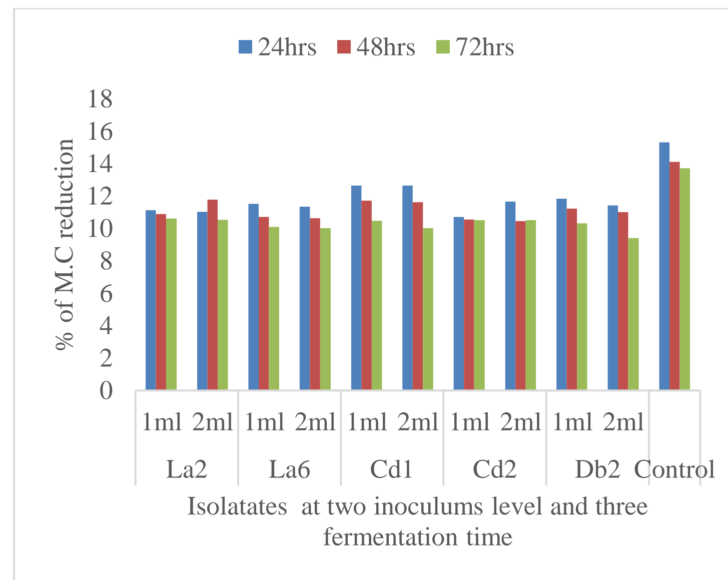

Fig. 7. Percentage moisture content of samples fermented with different lactic acid bacteria isolates over varying fermentation times. X- axis different isolates with two Inoculums level and Y-axis % of moisture content. For all isolates triplicate samples was used. La_2, La_6, Cd_1, Cd_2 and Db_2 microbial isolates, 1 and 2 ml – inoculumes level, fermentation time – 24, 48 and 72 hrs.

3.5 Sensory Evaluation of Fermented Cassava Cake

Sensory evaluation provides essential information on the organoleptic properties and consumer acceptability of cassava cake. Aroma, color, texture, and overall acceptability are the major sensory parameters influencing consumer preference (Andeta et al., 2018). In this study, the sample fermented with (La_6) recorded the highest scores across all four sensory attributes evaluated, each exceeding 3.5 on a five-point hedonic scale. In contrast, samples fermented with $Cd_1$and Cd_2 received the lowest scores for color, with values of 2.83 and 2.92, respectively (**Figure 8**).

Overall, samples fermented with La_2 and La_6 were the most preferred by panellists across all sensory parameters (**Figure 8**). This enhanced acceptability may be attributed to the ability of certain lactic acid bacteria such as *Lactobacillus plantarum* and *lactiplantibacillus plantarum* and *Leuconostoc mesenteroides*to dominate fermentation and produce metabolites that improve aroma, taste, and texture. Similar findings were reported by Chiang et al. (2018), who noted that lactic acid bacteria enhance the organoleptic qualities of fermented foods through the production of sugars, lactic acid, and acetic acid. The application of starter cultures therefore contributes to improved sensory attributes and increased consumer preference for fermented cassava products.

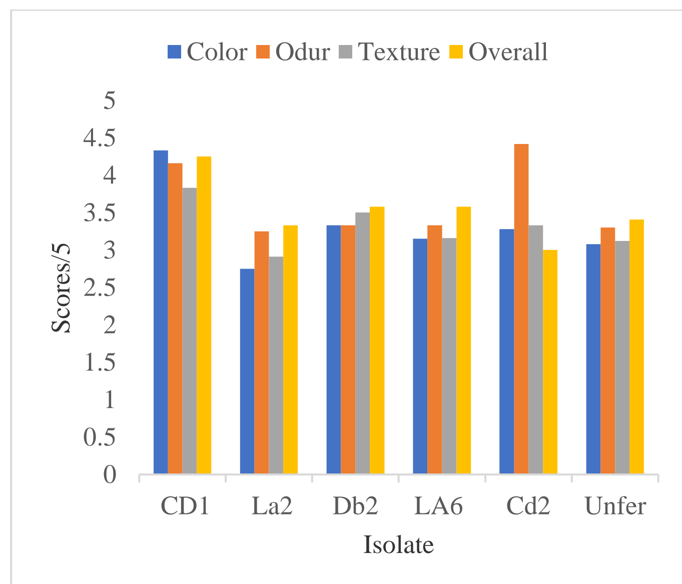

Fig. 8. Effect of fermentation with different lactic acid bacteria isolates on the sensory attributes of cassava cakes, including appearance, aroma, taste, texture, and overall acceptability. Y-axis hedonic scores, X axis sample fermented with different LAB isolates. For all isolates triplicate samples was used. Bars within a group show the four quality attributes. The isolates include CD1, La2, Db2, LA6, Cd2, and Unfermented.

CONCLUSION

This study examined the effects of isolate inoculum size and fermentation time on the physicochemical and sensory characteristics of fermented cassava flour and its derived products. The findings demonstrate the potential of native lactic acid bacteria (LAB) strains to improve the safety, nutritional quality, and overall acceptability of cassava-based foods. Increasing fermentation time and inoculum levels resulted in a reduction in pH, moisture content, and hydrogen cyanide concentration, indicating enhanced detoxification and improved product stability. Likewise, cassava cake prepared from flour fermented with *Lactobacillus plantarum* strain B36 exhibited superior scores across all tested organoleptic attributes, making it more acceptable to consumers compared to cakes prepared using other isolates or the unfermented control.

To further enhance the benefits of lactic acid fermentation, additional research is required to deepen understanding of preservation methods, technological optimization, and the socio-economic implications of adopting improved fermentation practices. The development and application of appropriate starter cultures will play a crucial role in advancing traditional lactic acid fermentation systems, ensuring the production of high-quality, safe, and culturally acceptable cassava-based products.

CONFLICT OF INTEREST

All authors declare that they do not have any conflicts of interest that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

The data used to support the findings of this study are available upon reasonable request from the corresponding author.

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Authors' contributions

Tefera, T., conceived the research idea involved in experimental works, field data collection and manuscript writing **Mulatu, W., Adeba, T**. and **Tariku, A**., involved in data analysis **Sewunet, A.,** and **Danil, Y**., participated in study design and conceptualization, All the authors read and approved the final manuscript.

REFERENCES

Abba, K. A., & Khalil, S. K. (2010). Modified starches and their usage in selected food products. *Journal of Agricultural Science, 2*(2), 90–100.

Adeleke, B. S., Akinyele, B. J., Olaniyi, O. O., & Agboola, Y. A. J. (2017). Effect of fermentation on chemical composition of fermented cassava peels. *Asian Journal of Plant Science and Research, 7*(1), 31–37.

Agboregbe, T., Mbome, L. I., Noubi, L., & Trèche, S. (1995). The effectiveness of cyanogens reduction during cassava processing into miondo. In E. Agbor, A. Brauman, D. Griffon, & S. Trèche (Eds.), *Transformation alimentaire du manioc* (pp. 307–318). ORSTOM.

Ahaotu, C. C., Ogueke, C. I., Owuamanam, N. N., & Nwosu, J. N. (2013). Fermentation of underwatered cassava pulp by linamarase-producing microorganisms: Effects on nutritional composition and residual cyanide. *American Journal of Food and Nutrition, 3*(1), 1–8.

Akoja, S. S., & Mohammed, A. O. (2011). Evaluation of the proximate, pasting and sensory characteristics of cassava flour (fufu) fortified with pigeon pea flour. *Pakistan Journal of Nutrition, 10*(2), 124–128.

Amenu, D., & Bacha, K. (2023). Probiotic potential and safety analysis of lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages. *Annals of Microbiology, 73*(1).

Ampe, F., Brauman, A., Trèche, S., & Agossou, A. (1994). Cassava retting: Optimization of traditional fermentation by an experimental research methodology. *Journal of the Science of Food and Agriculture, 65*, 355–361.

Amsalu, N. (2006). Phenotypic diversity of cassava (*Manihot esculenta* Crantz) in Ethiopia. In *Proceedings of the 12th Annual Conference of the Crop Science Society of Ethiopia* (Vol. 12, pp. 23–29). Addis Ababa, Ethiopia.

Andeta, A. F., Vandeweyer, D., Woldesenbet, F., Eshetu, F., Hailemicael, A., Woldeyes, F., Crauwels, S., Lievens, B., Ceusters, J., Vancampenhout, K., & Van Campenhout, L. (2018). Fermentation of enset (*Ensete ventricosum*) in the Gamo highlands of Ethiopia: Physicochemical and microbial community dynamics. *Food Microbiology, 73*, 342–350.

Andrew, W. (2002). Cassava utilization, storage and small-scale processing. In R. J. Hillocks, J. M. Thresh, & A. C. Bellotti (Eds.), *Cassava: Biology, production and utilization* (pp. 281–300). CABI Publishing.

Aro, S. O., Aletor, V. A., Tewe, O. O., Fajemisin, A. N., Usifo, B., & Adesida, J. A. (2008). Studies on the nutritional potential of cassava tuber waste collected from cassava starch factories. In *Proceedings of the 4th Annual Conference of FUTA/SAAT* (pp. 86–92).

Barrow, G. I., & Feltham, R. K. A. (1999). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge University Press.

Ben Omar, N., Ampe, F., Raimbault, M., Guyot, J.-P., & Tailliez, P. (2000). Molecular diversity of lactic acid bacteria from cassava sour starch (Colombia). *Systematic and Applied Microbiology, 23*, 285–291.

Bradbury, M. G., Egan, S. V., & Bradbury, J. H. (2002). Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *Journal of the Science of Food and Agriculture, 82*, 53–56.

Buckle, K. A., Edwards, R. A., Fleet, G. H., & Wootton, M. (2015). *Ilmu pangan*. Universitas Indonesia Press.

Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: Role of microorganisms in food production and preservation. *International Journal of Food Microbiology, 50*(1–2), 131–149.

Camargo, K. F., Leonel, M., & Mischan, M. M. (2008). Production of snacks from sour cassava starch and fiber: Effect of operational parameters on physical properties. *Food Science and Technology, 28*(3), 586–591.

Chiang, J. H., Loveday, S. M., Hardacre, A. K., & Parker, M. E. (2018). Effects of soy protein to wheat gluten ratio on the physicochemical properties of extruded meat analogues. *Food Structure, 19*, 100-102.

Coulin, P. (2006). Characterization of the microflora of attiéké, a fermented cassava product during traditional small-scale preparation. *International Journal of Food Microbiology, 106*(2), 131–136.

Diallo, Y. (2013). Importance nutritionnelle du manioc et perspectives pour l'alimentation de base au Sénégal. *Biotechnology, Agronomy, Society and Environment, 17*(4), 634–643.

Egwim, E., Amanabo, M., Yahaya, A., & Bello, M. (2013). Nigerian indigenous fermented foods: Processes and prospects. In *Mycotoxin and food safety in developing countries* (pp. 154–180). INTECH Open Science.

Food and Agriculture Organization of the United Nations. (2023). *FAOSTAT statistical database*. FAO.

Girma, G., Bultosa, G., & Abera, S. (2014). Effect of drying process and partial substitution of wheat flour with cassava flour on properties and antinutritional factors. *Basic Research Journal of Food Science and Technology, 1*, 38–45.

Girma, G., Bultosa, G., & Abera, S. (2015). Effect of cassava variety, drying process and blending ratio on compositional and sensory properties of cassava–wheat composite bread. *Direct Research Journal of Agriculture and Food Science, 3*, 38–47.

Gunawan, S., Istighfarah, Z., Aparamarta, H. W., Tankoano, A., & Savadogo, A. (2016). African cassava traditional fermented foods: Microorganisms' contribution to nutritional and safety values—A review. *International Journal of Current Microbiology and Applied Sciences, 5*(10), 664–687.

Gunawan, S., Widjaja, T., Zullaikah, S., Istianah, N., Aparamarta, H. W., & Prasetyoko, D. (2015). Effect of fermenting cassava with selected microorganisms on

flour chemical composition. *International Food Research Journal, 22*(3), 1280–1287.

Hadiyat, M. A., & Wahyudi, R. D. (2013). Integrating steepest ascent for the Taguchi experiment: A simulation study. *International Journal of Technology, 3*(2), 280–287.

Izah, S. C., Bassey, S. E., & Ohimain, E. I. (2018). Impacts of cassava mill effluents in Nigeria. *Journal of Plant and Animal Ecology, 1*(1), 14–32.

Kobawila, S. C., Louembe, D., Keleke, S., Hounhouigan, J., & Gamba, C. (2005). Reduction of cyanide content during fermentation of cassava roots and leaves. *African Journal of Biotechnology, 4*, 689–696.

Kostinek, M. (2007). Characterization and biochemical properties of predominant lactic acid bacteria from fermenting cassava for starter culture selection. *International Journal of Food Microbiology, 114*(3), 342–351.

Kumar, V., Kumar, V., & Bhalla, T. C. (2013). In vitro cyanide degradation by *Serratia marcescens* RL2b. *International Journal of Environmental Sciences, 3*, 1985–1995.

Lacerda, C. A., Miranda, R. L., Borelli, B. M., Nunes, A. C., Nardi, R. M. D., Lachance, M. A., & Rosa, C. A. (2005). Lactic acid bacteria and yeasts associated with spontaneous fermentation of sour cassava starch. *International Journal of Food Microbiology, 105*, 213–219.

Laguerre, G., Mazurier, S. I., & Amarger, N. (2018). Plasmid profiles and restriction fragment length polymorphism of *Rhizobium leguminosarum* bv. *viciae* in field populations. *FEMS Microbiology Ecology, 101*, 17–26.

Larmond, E., Linda, M., Deborah, A., & Mackie, G. (1991). *A laboratory method of sensory evaluation of foods* (2nd ed.). Canada Communication Group Publishing Centre.

Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., & De Vuyst, L. (2014). The international scientific association for probiotics and prebiotics consensus statement. *Nature Reviews Gastroenterology & Hepatology*.

Meitha, A., Bindar, Y., & Kresnowati, M. T. A. (2016). Effects of cassava chips fermentation conditions on flour properties. *ASEAN Journal of Chemical Engineering, 16*, 51–59.

Mirizadeh, S., Yaghmaei, S., & Nejad, Z. G. (2014). Biodegradation of cyanide by a newly isolated strain under alkaline conditions. *Journal of Environmental Health Science and Engineering, 12*(1), 1.

Murugan, K. Y., Sekar, S., & Al-Sohaibani, S. (2012). Detoxification of cyanide in cassava flour by linamarase of *Bacillus subtilis* KM05. *African Journal of Biotechnology, 11*, 7232–7237.

Odey, G. N., & Lee, W. (2019). Evaluation of the quality characteristics of flour and pasta from fermented cassava roots. *International Journal of Food Science and Technology*, 1–10.

Ogiehor, I. S., & Ikenebomeh, M. J. (2005). Extension of shelf life of garri by hygienic handling and sodium benzoate treatment. *African Journal of Biotechnology, 4*, 744–748.

Ojokoh, A. O., & Udeh, E. N. (2014). Effects of fermentation and extrusion on proximate composition and organoleptic properties of sorghum–soya blends. *African Journal of Biotechnology, 13*(40), 4008–4018.

Padonou, W. S., Nielsen, D. S., Hounhouigan, J. D., Thorsen, L., Nago, M. C., & Jakobsen, M. (2009). Microbiota of lafun, an African traditional cassava food product. *International Journal of Food Microbiology, 133*, 22–30.

Pongsub, S., Suntara, C., Khota, W., Boontiam, W., & Cherdthong, A. (2022). Chemical composition, fermentation end products, and aerobic stability of cassava pulp silage. *Veterinary Sciences, 9*(11), 617.

Rajkowska, K., & Kunicka-Styczyńska, A. (2010). Probiotic properties of yeasts isolated from chicken feces and kefirs. *Polish Journal of Microbiology, 59*(4), 257–263.

Sayre, R. (2010). Research news: Researchers get to the root of cassava's cyanide-producing abilities.

Sayre, R., Beeching, J. R., Cahoon, E. B., Egesi, C., Fauquet, C., Fellman, J., Fregene, M., Gruissem, W., Mallowa, S., Manary, M., Maziya-Dixon, B., Mbanaso, A., Schachtman, D. P., Siritunga, D., Taylor, N., Vanderschuren, H., & Zhang, P. (2011). BioCassava Plus program: Biotechnology.

Smid, E. J., & Hugenholtz, J. (2010). Functional genomics for food fermentation processes. *Annual Review of Food Science and Technology, 1*, 497–519.

Soryotha, S., Kyu, K. L., & Ratanakhanokchai, K. (2010). Detoxification of cassava starch by plant cell wall–degrading enzymes. *Journal of Bioscience and Bioengineering, 109*, 9–14.

Tannock, G. W. (2004). A special fondness for lactobacilli. *Applied and Environmental Microbiology, 70*(6), 3189–3194.

Tariku, H. (2013). Research trends in modern food fermentation biotechnology. *Journal of Science and Sustainable Development, 1*, 71–86.

Tefera, T., Ameha, K., & Bruhtesfa, A. (2014). Cassava-based food fermentation using single starter cultures. *International Food Research Journal, 21*, 1751–1756.

Teshome, S., Demel, T., & Sebsebe, D. (2004). Ecological study of vegetation in Gamo Gofa Zone, southern Ethiopia. *Journal of Tropical Ecology, 45*, 209–221.

Tilahun, A., Shimelis, A., Gulelat, D., & Tilahun, B. (2013). Effect of processing on physicochemical composition and antinutritional factors of cassava grown in Ethiopia. *International Journal of Science Innovations and Discoveries, 3*(2), 212–222.

Tiwari, H., Chen, S. W., Kim, R., & Jha, R. (2018). Supplemental effects of xylanase and mannanase on nutrient digestibility and gut health of nursery pigs.

Tiwari, U., Gunasekaran, M., Jaganmohan, R., & Alagusundaram, K. (2011). Quality characteristics and

shelf life of deep-fried snacks from rice brokens and legume by-products. *Food and Bioprocess Technology, 4*, 1172–1178.

Uzoh, C. V., Orji, J. O., Okeh, C. O., Nworie, C. O., Igwe, P. C., & Uwanta, L. I. (2022). Assessment of probiotic potentials of lactic acid bacteria from locally fermented foods. *Asian Journal of Food Research and Nutrition, 1*(1), 11–16.

Wahyuni, S., Dewi, N. D. P., Pato, U., Susilowati, P. E., Khaeruni, A., & Fatahu, F. (2023). Identification of lactic acid bacteria in spontaneous fermented cassava food products.

Westby, A., & Choo, B. K. (1994). Cyanogen reduction during lactic fermentation of cassava. *Acta Horticulturae, 375*, 209–215.

Wu, L., Seo, D. J., Demargne, J., Brown, J., Cong, S., & Schaake, J. (2014). Ensemble precipitation forecast generation using meta-Gaussian models. *Journal of Hydrology, 399*(3–4), 281–298.