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(RESEARCH ARTICLE)

Impact of yeast fermentation on cyanide detoxification, physicochemical properties, and protein enrichment of cassava flour

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Abstract

Cassava production has increased steadily in Ethiopia; however, its utilization is limited due to the presence of toxic hydrogen cyanide and its low protein content. Therefore, appropriate processing techniques are required to reduce cyanide levels and improve the nutritional quality of cassava prior to consumption. In this study, cassava flour was prepared and fermented using yeast isolates obtained from fermented cassava pulp juice. Two inoculum levels (1 and 2 mL containing 1.07×10^{7}to1.2×10^{8}CFU/mL) and three fermentation periods (24, 48, and 72 h) were evaluated. Changes in pH, moisture content, and crude protein content of the fermenting cassava flour were analyzed. The results showed that fermentation significantly reduced pH and moisture content, with pH decreasing from 6.72 to 4.07 and moisture content from 12.07% to 6.18%. Cassava flour fermented with yeast isolates F9 and F12 at a 2 mL inoculum level for 24 h showed the highest improvement in crude protein content, increasing by 85.52% and 78.38%, respectively. Overall, all fermented samples exhibited a notable increase in protein content, ranging from 24.35% to 85.52%, compared with the unfermented control (7.24%). In conclusion, yeast fermentation of cassava flour effectively reduces factors associated with cyanide toxicity while substantially enhancing protein content, indicating its potential as a simple and affordable processing method to improve the nutritional quality and safety of cassava-based foods.

Key words: Cassava flour; Yeast fermentation; Cyanide Tolerance; Protein enrichment; Nutritional quality

1. Introduction

Cassava (*Manihot esculenta*) is an extensively cultivated crop and a staple food for millions of people in the tropical regions of Africa, Latin America and Asia. Globally, in terms of annual production, it is the fifth most important food crop after), maize, rice, wheat and potato (FAOSTAT, 2011). This plant is growing well under critical conditions of climate and soil, and flexible havesting date, allowing farmers to keep the roots in the ground until needed. Therefore, its production has increased consistently over the last ten years. Cassava is one of the most important tropical root crops in developing countries. Its starchy roots are a majorsource of dietary energy for more than 500 million people (IITA, 2020). It is known to be the highest producer of carbohydrates among staple crops. However, cassava is carbohydrate rich but low in protein and of all its toxicity.

In Ethiopia, this cassava has been cultivated in the southern and southwestern regions for decades as an alternative food insecurity crop (Dada., 2016). Cassava is one of the underutilized root crops in the country mainly to tackle seasonal food shortage in northern areas of Ethiopia. Traditionally cassava roots are processed in a number of ways that vary from one region to another resulting in different end products like Gari, Fufu, Lafun, Farinha, Pande, Yucca. Despite all the usefulness of cassava, its uses as food source are limited by its perishability, its low protein content and its potential toxicity (Andrew, 2002).

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1.1. Hydrogen Cyanide Toxicity and Protein Limitation of Cassava

Among the various anti-nutritional factors present in cassava, hydrogen cyanide (HCN) is of major concern, as its concentration in cassava roots and by-products often exceeds the World Health Organization (WHO) safe limit for human consumption (10 ppm) (Hadiyat & Wahyudi, 2013). Chronic consumption of inadequately processed cassava has been associated with konzo, an irreversible neurological disorder linked to cyanide exposure (Bradbury, 2006). Therefore, effective detoxification processes are required to reduce cyanogenic compounds and ensure the safe consumption of cassava based foods. Cassava contains a cyanogenic glucoside known as linamarin, which is enzymatically hydrolyzed by linamarinase to release hydrogen cyanide. Nearly all parts of the cassava plant, except the seeds, contain cyanogenic glucosides (CGs). The total cyanogenic glucoside content varies depending on cultivar type, conditions, agronomic practices, and plant maturity (Kenyon et al., 2006).

Another major limitation of cassava tubers is their low protein content. Cassava roots consist predominantly of starch (approximately 20 25%) but contain very limited amounts of protein, fats, vitamins, and minerals (Kosugi et al., 2009). This nutritional deficiency contributes to protein–energy malnutrition in populations that rely heavily on cassava as a staple food. Consequently, improving the protein content of cassava could significantly enhance its nutritional value and contribute to better protein–calorie balance in resource-limited communities.

A practical and cost effective approach to improving the protein content of cassava is microbial fermentation, during which microorganisms synthesize proteins while growing on cassava substrates. Fermentation has been shown to enhance the nutritional quality of foods through the biosynthesis of vitamins, essential amino acids, and microbial proteins, as well as by improving protein digestibility and fiber utilization. Additionally, fermentation facilitates the degradation of anti nutritional factors, including cyanogenic compounds (Adewusi et al., 2009). Microorganisms such as algae, fungi, bacteria, and yeasts have been widely studied as alternative protein sources due to their high protein content, rapid growth rates, and ability to utilize diverse substrates (Araujo, 2006). Among these, yeasts are the most widely accepted for food and feed applications, owing to their favourable amino acid profile, particularly their high lysine content (Ijah et al., 2004). Previous studies have demonstrated the effectiveness of yeast fermentation in protein enrichment of cassava. For example, Campanile et al., (2008) reported that Candida utilis grown on enzymatically hydrolyzed cassava in submerged culture produced a product containing up to 35% crude protein on a dry weight basis. Similarly, Iyayi and Losel (2001) identified fungal and yeast fermentation as inexpensive and efficient methods for increasing protein content under solid state fermentation conditions.

Previous research on cassava processing has primarily focused on spontaneous or natural fermentation rather than controlled fermentation using selected yeast isolates. Besides that, most studies emphasized detoxification of cassava (hydrogen cyanide removal) without adequately addressing improvement in nutritional quality, particularly protein enhancement. Some studies have often centered on lactic acid bacteria, with comparatively little attention given to the role of specific yeast strains in improving cassava flour. Although cassava is known to be low in protein, limited studies have quantified the extent to which controlled yeast fermentation can significantly enhance crude protein content.

The use of microorganisms for single-cell protein production is attractive due to their fast growth rates in semi-solid and solid media, high protein yield, good nutritional quality, and potential for genetic improvement to optimize growth on specific substrates. Therefore, developing an affordable fermentation-based processing method that simultaneously reduces cyanide toxicity and improves protein content could enhance the suitability of cassava for food and feed applications.

The purpose of this study was to evaluate the effectiveness of yeast fermentation in improving the safety and nutritional quality of cassava flour. Specifically, the study aimed to determine the effect of selected yeast isolates on reducing factors associated with cyanide toxicity. Evaluate the improvement in crude protein content of cassava flour at different inoculum levels and fermentation periods and identify the optimal fermentation conditions (inoculum level and time) for enhancing protein enrichment. With this gap and purpose the study was designed to improve the protein content of cassava flour through fermentation using yeast isolates obtained from cassava pulp juice at different inoculum levels and fermentation durations.

2. Materials and methods

2.1. Study Area and Sample Collection

Cassava tuber samples were collected from Wolaita, Gamo, and Goffa Zones, as well as Konso Woreda, located in southern Ethiopia, where cassava is commonly cultivated and consumed. Fresh, mature cassava roots free from visible defects were selected and transported to the laboratory for processing and analysis.

2.2. Experimental Design

A completely randomized design (CRD) with a 5×3×2 factorial arrangement was employed. The experimental factors included five selected pure cultures of cassava-fermenting yeast isolates, three fermentation durations (24, 48, and 72 h), and two inoculum levels (1 mL and 2 mL). Unfermented cassava flour served as the control treatment for all experiments.

2.3. Inoculum Preparation

Fresh cassava tubers were peeled, washed, grated, and pressed using a clean fine cloth to obtain cassava pulp juice. The extracted juice was allowed to undergo spontaneous fermentation at ambient temperature (26–32 °C) for 72 h. The fermented juice served as the source of yeast inoculum for subsequent fermentation experiments.

2.4. Cassava Flour Preparation

Fresh cassava tubers (2kg) were peeled, washed, and cut into cylindrical pieces. The pieces were steeped in 4L of sterile distilled water for 72 h to allow softening. The softened tubers were manually pulverized and sieved using a 1.0 mm mesh sieve. The resulting slurry was allowed to sediment for 12 h, after which the supernatant water was decanted. The sedimented mash was transferred into a jute bag and pressed to remove excess water. The wet cake was then spread in a thin layer and dried in a cabinet dryer at 65 °C for 48h. The dried product was milled into flour using a laboratory blender and stored in airtight containers at 4 °C until further fermentation and analysis.

2.5. Isolation and Inoculum Preparation

Identification of yeast isolates was carried out based on morphological and physiological characteristics following standard yeast identification techniques described by (Kurtzman et al., 2011). Selected yeast starter culture candidates were harvested by aseptically adding 10 mL of sterile peptone water to the respective agar slants. The resulting cell suspensions were adjusted using sterile peptone water and a spectrophotometer to obtain a final concentration of 10^6–10^7 CFU mL^{-1}. These standardized suspensions were subsequently used as inocula.

2.6. Screening of Yeast Isolates for Cyanide Resistance

Test tubes containing 5 mL of screening medium (2% w/v glucose in distilled water) were prepared and sterilized by autoclaving at 121 °C for 15 minutes. After cooling, aliquots (0.1 mL) of potassium cyanide (KCN) solutions at concentrations of 400, 600, and 800 mg L^{-1}, sterilized by tyndallization as described by Parmar et al., (2012), were aseptically added to the screening medium.

Each yeast isolate was inoculated into the respective test tubes and incubated at room temperature (28 ± 2 °C) for 48 hours. The sensitivity or resistance of the isolates to cyanide was assessed by measuring optical density at 530 nm using a spectrophotometer, with distilled water serving as the blank. Yeast isolates exhibiting growth at the highest cyanide concentrations were considered resistant and selected as predominant yeast strains associated with cassava fermentation.

2.7. Physicochemical Analysis of Fermented Cassava Flour

2.7.1. Determination of pH

Ten grams of fermenting cassava pulp were homogenized with 20 mL of sterile distilled water and filtered through Whatman No. 1 filter paper. The pH of the filtrate was measured using a calibrated pH meter.

2.7.2. Determination of Moisture Content

A metallic dish was dried in an oven at 80 °C for 20 minutes, cooled in a desiccator, and weighed. Five grams of the sample were placed in the dish and weighed. The dish containing the sample was then dried in an oven at 80 °C for 24

hours until a constant weight was achieved. After drying, the dish was transferred to a desiccator, allowed to cool, and weighed immediately. Moisture content was calculated as the loss in weight of the sample during drying.

2.7.3. Determination of Protein Content

Protein content of fermented and unfermented cassava peel samples was determined using the micro-Kjeldahl method, applying a nitrogen-to-protein conversion factor of 6.25, as described by AOAC (2000). Protein content was calculated using the formula:

$$\{\text{Protein content (\%)} = \%N \times 6.25\}.$$

2.8. Molecular Identification of Yeast Isolates

Genomic DNA was extracted from pure yeast cultures using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region between the 18S and 28S rRNA genes was amplified using the primer pair ITS1 and ITS4 (Pintilie., 2011).

PCR amplification was performed with an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 1 minute, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. The PCR products were sequenced using the same primers on an ABI 3730XL Genetic Analyzer

Primer sequences**:**

ITS1:5′TCCGTAGGTGAACCTGCGG-3′

ITS4:5′TCCTCCGCTTATTGATATGC-3′

2.9. Data Analysis

Statistical analyses were performed using SAS version 11. Analysis of variance (ANOVA) was used to determine significant differences among treatments, and differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

A total of 108 yeast isolates were obtained from naturally fermented cassava pulp juice collected from different locatio ns in southern Ethiopia. Morphological and biochemical characterization revealed that yeast colonies were generally spherical, creamy, smooth, raised or flat, with some colonies appearing shiny (Table 1).

Table 1 Morphological characteristic of yeast isolates from fermented cassava pulp juice

Isolate	**Cy_4**	**Cy_6**	**Cy_7**	**F_9**	**F_{12}**
Color	cream	white	cream	cream	white
Elevate	raised	flat	raised	raised	flat
Edge	lobed	entire	lobed	lobed	lobed
Cell Shape	oval	spherical	oval	oval	spherical

The biochemical characterization of the isolates (Cy4, Cy6, Cy7, F9, and F12) showed that all isolates were Gram positive and capable of utilizing multiple carbon and nitrogen sources, indicating metabolic versatility. Most isolates utilized sucrose, glucose, lactose, maltose, and dextrin, although variability was observed among isolates, reflecting strain specific carbohydrate metabolism. Nitrogen assimilation tests revealed that the majority of isolates could utilize urea, potassium nitrate (KNO_3), and ammonium sulfate ($(NH_4)_2SO_4$) as nitrogen sources (Table. 2). Growth at different temperatures demonstrated that all isolates grew well between 20 35°C, with several isolates maintaining growth up to 40 45 °C, indicating good thermal tolerance. Overall, these biochemical traits suggest that the isolates are physiologically robust and adaptable, supporting their potential use in fermentation and other biotechnological applications. Out of the 108 isolates, five isolates (F9, F12, Cy7, Cy6, and Cy4) demonstrated notable hydrogen cyanide tolerance and evaluated for their ability to grow in the presence of cyanide at concentrations of 400, 600, and 800 mg

L^{-1} (Fig.1). Finally, isolates that exhibited growth at the highest cyanide concentration were selected for further analysis of their protein enhancement potential (Figure 1).

Table 2 Biochemical characteristics of yeasts isolated from fermented cassava pulp juice

Isolat	Gram rex	Carbon utilization						N assimilation	
		sucro	gluco	lacto	malto	dextro	Urea uti	KNO_3	$NH_4SO_4^+$
Cy_4	+	+	+	+	-	-	-	+	+
Cy_6	+	+	+	-	+	+	-	+	+
Cy_7	+	-	+	+	+	-	+	-	+
F_9	+	+	+	+	+	+	-	-	+
F_{12}	+	+	+	+	-	+	-	+	-

The bar graph (Fig 1) illustrates the growth response of different yeast isolates (F9, CY7, F12, CY4, and CY6) under increasing hydrogen cyanide (HCN) concentrations (400, 600, and 800mg/L), measured as absorbance at 530nm. Overall, yeast growth decreased progressively with increasing HCN concentration, indicating a dose dependent inhibitory effect of cyanide on microbial growth. Among the isolates, F9 and CY6 showed relatively higher absorbance values across all HCN levels, suggesting greater tolerance to cyanide stress. This finding is in agreement with Edem and Sanni, (2008) also employed similar procedure in selection of starter cultures for fermented cassava bread. The existence of microbes in cyanide containing media was due to anaerobic metabolism, or by using an aerobic respiration chain as an alternative path way which resulted with the production of end products like formamide, formic acid and ammonia as an outcome of cyanide degradation (Ebbs., 2004). In contrast, CY7 and CY4 exhibited comparatively lower absorbance, indicating higher sensitivity to elevated HCN concentrations. The control sample (without HCN) recorded the highest absorbance, confirming that cyanide negatively affects yeast growth. The ability of some isolates to maintain moderate growth even at 800mg/L HCN demonstrates their potential cyanide tolerance and possible detoxification capability, making them promising candidates for application in cassava fermentation and cyanide reduction processes.

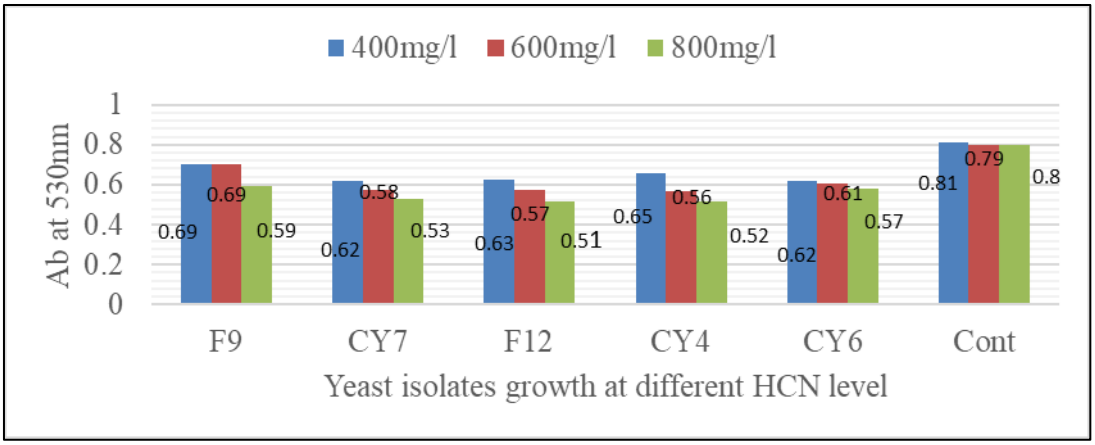

Figure 1 Growth performance of yeast isolates at different HCN concentration of (400, 600 800mg/l)

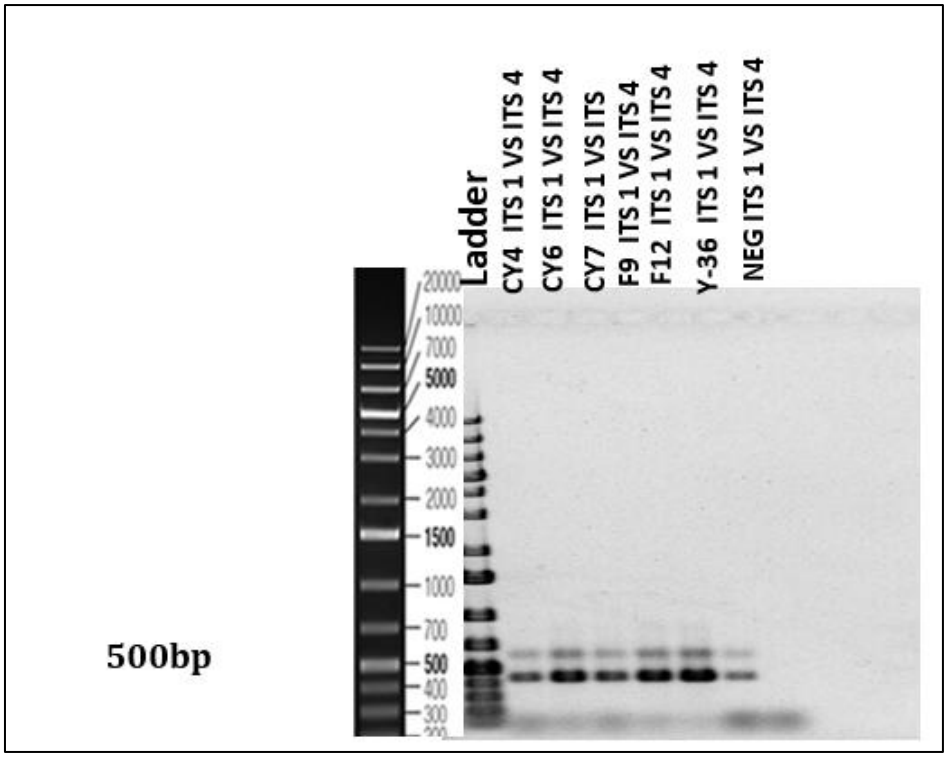

Figure 2 Isolate Electropherograms and reference standards. L: Ladder; Cy_4, Cy_6, Cy_7, F_9 and F_{12}: yeast strains to be identified

Phylogenetic analysis based on sequence homology revealed that all yeast isolates (Cy4, Cy6, Cy7, F9, and F12) clustered closely with Pichia fermentans reference strains deposited in the GenBank database. The isolates Cy4, Cy6, and F9 showed 100% sequence identity with established P. fermentans strains, confirming their taxonomic placement within this species. Isolate Cy7 exhibited a slightly lower similarity (99.75%), while F12 showed 99.26% identity, indicating minor nucleotide variations but still falling well within the species-level threshold.

The high percentage similarity among all isolates suggests a common evolutionary origin and strong genetic relatedness, with small intra-species genetic diversity. These minor variations may reflect strain-level adaptations to different ecological niches or functional traits, such as cyanide tolerance and fermentation performance. Overall, the phylogenetic results confirm that the isolates belong to the species Pichia fermentans, supporting their suitability for comparative evaluation in fermentation and cyanide detoxification studies. The isolates from the present study (Cy4, Cy6, Cy7, F9, and F12) clustered closely with reference strains retrieved from GenBank. The degree of sequence similarity exceeded 99%, indicating a high level of genetic relatedness and confirming their taxonomic identity as Pichia fermentans (Singh., 2020).

Table 3 Molecular identification of yeasts isolated from fermented cassava pulp juice based on the similarity to the partial ITS rRNA sequence

No	Isolate Code	Species of yeast Homolg	Ident (%)	Acce No
1	Cy_4	*Pichia fermentans strain* M0169	100	EU789405.1
2	Cy_6	*Pichia fermentans culture* CBC 2060	100	KY104550.1
3	Cy_7	*Pichia fermentans culture* 6662	99.75	KY104538.1
4	F_9	*Pichia fermentans culture* CBC 4550	100	KY104539.1
5	F_{12}	*Pichia fermentans strain* Y-23	99.26	OR534243.1

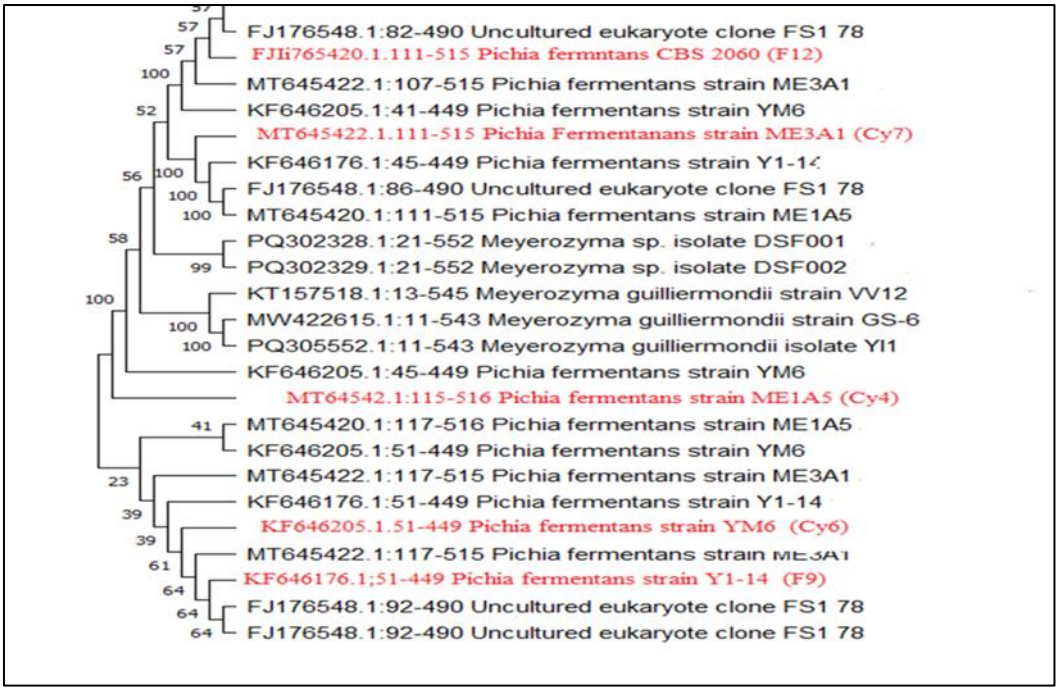

Figure 3 Phylogenetic tree showing multiple sequence alignment of ITS2 sequence of yeasts isolated from cassava pulp juice and close relatives from database

Temperature has been considered as one of the most important environmental factors positively and negatively affecting yeast growth and cassavabased food fermentation processes. In similar way Aysun and Ahmet (2016) reported that temperature has a marked influence on fermentation process during cassava fermentation. Similar findings reported that suitable temperature in fermentation process is the good condition for the yeast to react and grow. Low temperature slows down yeast activity and too high temperature kills yeast, thus, to keep a specific range of temperature is important; in this study three different tempertur were considere. Fig 4 shows all the yeast isolates could tolerate and grow at wide range of temperature (25, 35 and 45 ℃). This finding is consistent with the report of Kurtzman et al. (2011), who observed that yeasts involved in cassava fermentation can tolerate a wide range of However, it contradicts the study by Ma'aruf et al. (2011), which reported that yeast isolates could only 2tolerate temperatures up to 37 °C. All five yeast isolates shwed optimum growth at 35 ℃ (Fig.3), However, growth gradually decreased as the temperature increased to 45 ℃.

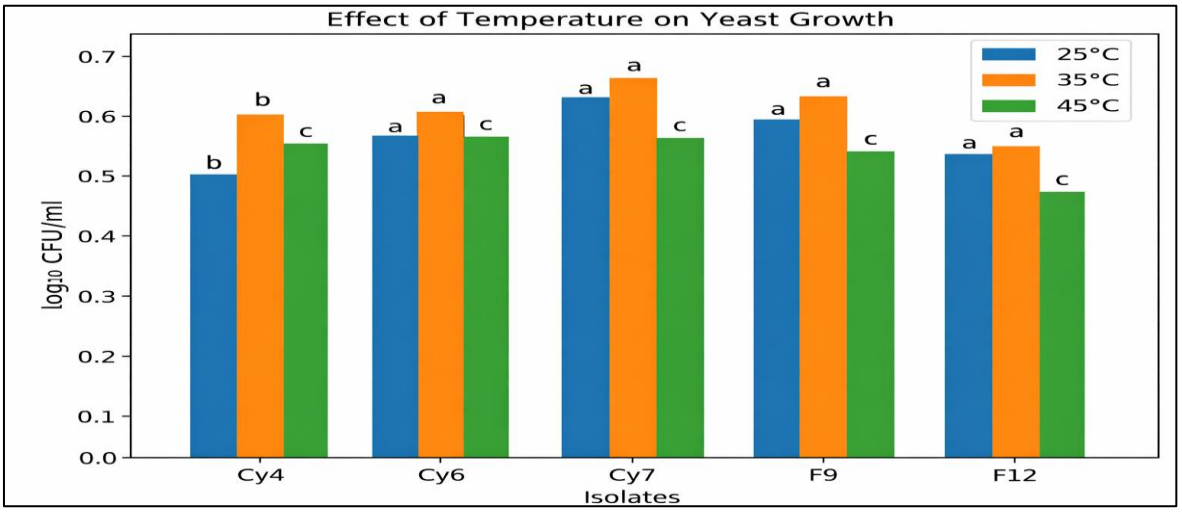

Figure 4 Yeast isolates growth performance at different temperature range of yeast growth

The gradual reduction of yeast growth rate has been observed after optimum point of temperature due to alteration of the cellular composition of yeasts under O2 limited conditions enzyme denaturation, and cellular membranes damage

(Serra et a.l 2005). In line with this result Bai, and Zhao, (2012) reported that the optimum temperature for yeast cell growth and its fermentation processes have been at range of 30 37 ℃ but it is strain dependent and more tolerate above 37 ℃

3.1. Proximate Composition Changes during Fermentation

The proximate composition of cassava samples fermented with different yeast isolates (Cy_4, Cy_6, Cy_7, F_9, and F_{12}) at varying fermentation times (24–72h) and inoculum levels (1and 2 ml) is presented in (Table 4).

3.1.1. Changes in pH During Fermentation

The results of pH measurements for all samples during the fermentation period are presented in Table 4. The pH of all samples fermented with different isolates decreased progressively with increasing fermentation time. Samples inoculated with isolate F12 showed the greatest reduction in pH, reaching a value of 4.07 at the 2 ml inoculum level after fermentation, compared to the unfermented control, which had a pH of 6.72. In contrast, the sample fermented with isolate Cy4 at the 1 ml inoculum level exhibited the least reduction in pH, recording a value of 6.52 after 24 hours of fermentation. Overall, due to the combined effects of inoculum level and fermentation time, the pH decreased from 6.72 in the unfermented control on the initial day to 4.07 after 72 hours of fermentation. This reduction in pH is attributed to the production of organic acids by fermenting microorganisms. Similar findings have been reported by Odoet al, (2016) during gari fermentation. Comparable observations were also made by Oyewole (2001) and Ogunnaike et al. (2013), who reported that acid production during fermentation is primarily responsible for the release and accumulation of organic acids, leading to a reduction in pH and improved food stability and safety. Karki and Kharel (2010) reported pH values as low as 3.37 for finger millet jand, while jand produced from different cereals ranged between 3.9 and 4.17. Therefore, the pH values observed in the present study are consistent with those reported in previous studies.

3.1.2. Moisture Content of Fermented Samples

The moisture content of all samples fermented for 24, 48, and 72 hours at different inoculum levels are presented in (Table.5). The results revealed that moisture content ranged from 6.69–11.65% at 24 hours, 6.31–9.86% at 48 hours, and 6.18–8.67% and 6.49–7.72% at 72 hours for 1 ml and 2 ml inoculum levels, respectively. The highest moisture content (11.65%) was observed in samples fermented with isolate Cy4 at the 1 ml inoculum level, while the lowest moisture content (6.18%) was recorded in samples fermented with isolates F9 at 2 ml inoculum levels after 72 hours of fermentation. The unfermented control sample exhibited a moisture content of 12.07%.

The moisture content of all fermented samples at both inoculum levels across all treatments fell within the range generally considered acceptable for dry products to ensure an extended shelf life (Sriroth et al., 2000). This reduction in moisture content may be attributed to changes in the chemical composition of cassava flour during fermentation, which leads to moisture loss (Meitha et al., 2016). Additionally, during fermentation, starch degradation occurs, resulting in the release of water from cassava cells. These moisture values are considered acceptable, as moisture contents above 12% may promote microbial growth. Therefore, the relatively low moisture content of the fermented cassava flour observed in this study is favorable and contributes to improved shelf life.

The moisture content recorded in this study was within the maximum level recommended for cassava flour by previous authors. Eke et al. (2007) reported a maximum acceptable moisture content of 14% for cassava flour. Therefore, both fermented samples in the present study exhibited good microbial safety due to their relatively low moisture content, which minimizes the risk of growth and proliferation of spoilage and pathogenic microorganisms.

3.1.3. Ash Content (% Ash)

The ash content of unfermented flour was 1.97% while that of fermented flour for 24hrs, 48hrs and 72hrs were range between 1.69 to 2.81%. The ash content increased as fermentation period increased except for sample fermented for 24 and 48hrs with F9. This observed increase in ash content of the flour is similar to the observation by Ishiwu et al (2015) who stated that total ash increases with fermentation period of castor oil seed. The high ash content of fermented cassava flour compared with the unfermented (control) could be due to loss of dry matter caused by the activities of enzymes and microorganism during fermentation (Onwurafo et al.,2014). However, this is contrary to the result reported by Igbabul et al (2014) on the effect of fermentation decreased proximate composition of ash content.

3.2. Effect of Fermentation on Crude Protein Content

The changes in crude protein content of cassava samples fermented for 24, 48, and 72 hours are presented in Table 4. The results demonstrate the effect of fermentation with selected protein enhancing yeast isolates on the crude protein content of cassava flour. Crude protein content increased from 0.386% in the unfermented control sample to values ranging from 1.68% to 4.94% in the fermented samples. This corresponds to an increase of 24.35% to 85.53% in crude protein content over a 72 hour fermentation period. These findings are consistent with the reports of Khurshida et al. (2025), who observed that fermentation of cassava peels with pure cultures of Saccharomyces cerevisiae increased protein content from 2.4% in unfermented cassava to 14.1% in fermented products. Similarly, Khurshida et al, (2025), reported that fermentation of cassava flour using pure cultures of Saccharomyces cerevisiae improved protein content from 2.4% in unfermented to14.1% in fermented products. Similarly, Oboh and AKindahunsi (2005) reported that fermentation of cassava flour using pure cultures improved protein content from 3.3% to 10.9%. The results further indicate that both inoculum level and fermentation time significantly influenced the crude protein content of cassava flour compared with samples without inoculum (control). The sample fermented with isolate F9 at a 2 ml inoculum level for 24 hours exhibited the highest crude protein content (4.94%), representing an 85.53% increase compared to the unfermented control, which showed only a 7.24% increase (0.39%). In the present study, the maximum crude protein enhancement was observed in cassava samples fermented with yeast isolates F9 and F12, which recorded incensement of 85.51% and 78.38%, respectively, at a 2 ml inoculum level after 24 hours of fermentation. These results are comparable with findings reported by Thongkratok et al. (2019), indicating that fermentation with selected yeast isolates can significantly improve the protein content of cassava flour.

3.3. Effect of Fermentation Time and Inoculum Level

Overall, increasing fermentation time from 24 to 72h and increasing inoculum level from1 to 2ml resulted in: lower pH values, reduced moisture content, and increased ash content and markedly improved protein content. These effects were isolate dependent, with Cy7, F9, and F12 showing superior performance in nutritional enhancement compared to Cy4 and Cy6. Among the isolates, Cy7 and F9 demonstrated the greatest improvement in protein enrichment and acidification efficiency, suggesting their suitability as starter cultures for cassava fermentation. Cy4 and Cy6 showed moderate improvements, while F12 exhibited balanced performance in moisture reduction and mineral enrichment. The observed difference in protein content between fermented cassava flour and the unfermented control can be attributed to the activity of microorganisms involved in the fermentation process. The protein content of the control (non-fermented) cassava flour was consistently lower than that of cassava flour inoculated with selected yeast isolates, suggesting that microbial activity plays a significant role in enhancing protein levels. Oboh and Elusiya (2007) reported that the increase in protein content of fermented cassava flour may result microorganisms that degrade cassava substrates and secrete extracellular enzymes, which contribute to the protein pool. Similarly, Bonnop et al. (2009) noted that the growth and proliferation of yeast biomass during fermentation, particularly in the form of single-cell proteins, may account for the apparent increase in protein content observed in fermented cassava flour.

Table 4 Physicochemical changes of cassava fermented with different yeast isolates at various time points and inoculum levels, with Statistical Annotations

Isolate	Time (hr)	Inocu (ml)	pH	% M.C	% Ash	% P.C
Cy4	24	1	6.52^{a}	11.65^{a}	1.00^{a}	20.78^{i}
	24	2	5.58^{b}	7.66^{b}	3.17^{b}	26.42^{hi}
	48	1	5.48^{b}	7.23^{b}	2.99^{b}	25.23^{hi}
	48	2	4.33^{c}	7.12^{b}	2.36^{b}	27.60^{hi}
	72	1	4.30^{c}	7.08^{b}	2.44^{b}	27.29^{hi}
	72	2	4.29^{c}	6.64^{c}	2.67^{b}	32.86^{gh}
Cy6	24	1	6.43^{a}	11.40^{a}	2.82^{b}	27.60^{hi}
	24	2	5.34^{b}	9.03^{b}	2.42^{b}	32.67^{gh}
	48	1	5.27^{b}	8.29^{b}	2.72^{b}	23.47^{hi}
	48	2	4.31^{c}	7.61^{b}	2.86^{c}	24.35^{hi}
	72	1	4.22^{c}	6.92^{c}	2.91^{b}	27.35^{hi}

	72	2	4.14^{c}	6.79^{c}	2.05^{b}	33.05^{gh}
Cy7	24	1	6.41^{a}	9.15^{a}	1.97^{a}	72.13^{abc}
	24	2	5.73^{b}	6.31^{b}	2.31^{b}	71.70^{abc}
	48	1	5.29^{b}	6.44^{b}	2.75^{b}	72.76^{abc}
	48	2	4.55^{c}	6.49^{b}	2.65^{b}	53.31^{de}
	72	1	4.49^{c}	6.52^{b}	2.02^{b}	66.32^{bcd}
	72	2	4.37^{c}	6.45^{b}	2.31^{b}	55.62^{de}
F9	24	1	6.37^{a}	9.00^{a}	1.95^{a}	65.87^{bcd}
	24	2	5.31^{b}	8.72^{a}	1.76^{a}	85.52^{a}
	48	1	5.07^{b}	8.67^{a}	1.99^{a}	53.49^{de}
	48	2	4.27^{c}	7.37^{b}	1.69^{a}	60.44^{cd}
	72	1	4.19^{c}	6.70^{b}	2.39^{b}	74.70^{abc}
	72	2	4.09^{c}	6.29^{c}	2.94^{b}	47.37^{ef}
F12	24	1	6.13^{a}	6.64^{b}	2.09^{b}	74.30^{abc}
	24	2	5.03^{b}	9.86^{a}	2.37^{b}	78.39^{ab}
	48	1	5.41^{b}	8.45^{a}	2.59^{b}	48.41^{ef}
	48	2	4.60^{c}	7.19^{b}	2.56^{b}	30.17^{h}
	72	1	4.13^{c}	6.58^{b}	2.11^{b}	39.42^{fg}
	72	2	4.07^{c}	6.56^{b}	2.31^{b}	37.92^{fg}
Control	0	0	6.67^{a}	12.07^{a}	1.97^{a}	7.24^{j}

Note: numbers in the same column followed by the same letter are not significantly different within 5%

4. Conclusion

The study demonstrated that cassava flour samples fermented with different yeast isolates derived from cassava pulp juice exhibited reduced pH and moisture content, alongside a considerable improvement in protein content. Cassava flour was successfully fermented with yeast, with pH values decreasing from 6.72 in the unfermented control to between 6.52 and 4.07 after 72 hours of fermentation. Similarly, moisture content declined from 12.07 in the control to values ranging between 11.65 and 6.18. This reduction in moisture content is beneficial, as it inhibits the growth of spoilage microorganisms and thereby extends storage time while maintaining product quality. In addition, the crude protein content of cassava flour increased during yeast fermentation, resulting in significantly higher values compared to the unfermented control. This enhancement in protein content can be attributed to microbial activity, including enzyme secretion and yeast biomass proliferation. Furthermore, the specific yeast isolate used for fermentation influenced the magnitude of protein enrichment, indicating that microbial source selection plays a critical role in determining the nutritional quality of the fermented product.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no Conflict of interest.

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