

Research Article

Physicochemical, Dough Volume and Microbial Growth during Leavening of Cassava-wheat Composite Dough

*Amapu, T. Y.¹, Dapiya, H. S.², Onyimba, I. A.¹, Njoku, A. I.¹, Gwanzhi, D.³ and Mathew, C. L.⁴

¹Department of Science Laboratory Technology, University of Jos, Nigeria

²Department of Microbiology, University of Jos, Nigeria

³Department of Science Laboratory Technology, Plateaus State Polytechnic, Jos, Nigeria

⁴Department of Nutrition and Dietetics, College of Health Technology Zawan, Jos, Nigeria

*Corresponding Author's email: tarfenaa@gmail.com

ABSTRACT

This study determined the impact of cassava inclusion on the physicochemical properties, dough volume, and microbial growth kinetics of cassava-wheat composite dough during leavening. A high-quality cassava flour (HQCF) proportions (10%, 20, 30%,40 and 50%) partially substituted the wheat flour for bread dough production were evaluated for pH, titratable acidity, dough volume, and microbial growth kinetics at 1-hour intervals. Overall, the dough volume progressively decreased with cassava inclusion which peaked at 84.00, 80.50, 74, 72.50, and 69.00 in 10%, 20%, 30%, 40%, and 50% cassava-wheat inclusion respectively during leavening. Cassava-wheat composite dough leavened with commercial yeast had the highest gas retention (85.50 cm³), followed by wheat dough co-fermented with amylolytic *L. plantarum* (AMz5), while cassava dough had the least (66.00 cm³). The 10% cassava-wheat composite dough had a dough volume comparable ($p > 0.05$) to the 100% wheat dough. The dynamics of dough-specific volume decreased with cassava inclusion in the order of 10% < 20% < 30% < 40% < 50%. The pH decreased from 4.75 to 3.60 in the wheat dough and from 5.30 to 4.50 in the composite dough during proofing. Moreover, the continuous growth of lactic acid bacteria decreased with cassava flour inclusion. Similarly, the baker's yeast exhibited steady growth in the cassava-wheat composite dough with fermentation. This data indicates that using 10% or 20% cassava flour inclusion will provide dough with the leavening kinetics necessary for acceptable bread production.

Keywords: Cassava-wheat flour, Composite dough, Dough leavening, Microbial growth, physicochemical properties

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INTRODUCTION

Today, Nigeria is one of the largest producers of cassava tubers with over 63 million tons per annum globally. It is projected that by 2025 about 62% of global cassava will be produced in sub-Saharan Africa (FAOSTAT, 2020). Despite this, Nigeria has not fully capitalized on the industrial potential of cassava, as it is mostly used for human consumption as a food crop. However, there have been identified opportunities for utilizing cassava tubers in the industrial sector, particularly in the production of high-quality cassava flour (HQCF) for bread, biscuits and ethanol. One

significant development has been the establishment of the cassava bread chain, which has boosted the industrial production of composite flour (Abideen, 2022). Accordingly, efforts towards cassava flour substitution in bread have been the focus of several researchers (Chisenga *et al.*, 2019; Amapu *et al.*, 2019; Abideen, 2022; Asadu and Chukwu, 2024). Bread-making is primarily a fermentation process in which Co₂ production accompanies the metabolic activity of yeast for dough development (Tucker, 2021). The fermentation of abundant mono- and disaccharides by baker's yeast through an energy

metabolic process resulted in the release of CO₂ that inflated the dough matrix during leavening (Tucker, 2021). In dough development, the gluten protein forms a network that facilitates gas holding, elasticity, and extensibility of the dough (Ribeiro *et al.*, 2018; Akintayo *et al.*, 2020).

However, cassava flour is low in protein (1 – 2%) and lacks gluten (Dendy, 1992; Manano *et al.*, 2017), which influence the rheological structure of the dough and bread quality when substituted for wheat flour (Eriksson *et al.*, 2014; Amapu *et al.*, 2019; Chisenga *et al.*, 2020). The cassava inclusion into the wheat dough is expected to reduce gluten levels and weaken the composite flour functionality for bread making. Improving the rheological qualities of the cassava-wheat composite dough is crucial since the dough volume is critically affected by the dough composition (Oyeyinka *et al.*, 2023). So far, approaches to improve the rheological properties of the cassava-wheat composite dough include treatments such as adding protein body-free maize zein (Bugusu *et al.*, 2002).

Understanding the kinetics of leavening in the cassava-wheat composite dough is necessary for predicting the final characteristics of bread (Ziobro *et al.*, 2016; Vidaurre-Ruiz *et al.*, 2019). While the properties of gluten containing doughs have been extensively studied, limited focus has been placed on the effect of cassava inclusion on the rheological properties of wheat dough. This study aimed to determine the impact of cassava inclusion in wheat flour on the physiochemical properties, dough volume and microbial growth kinetics of cassava-wheat composite dough during leavening.

MATERIALS AND METHODS

Collection of Wheat and Cassava Flour

High-quality cassava flour (HQCF) was purchased from the Federal Institute of Industrial Research Oshodi (FIRO) Nigeria. The industrially produced wheat flour was purchased from a retail shop markets of Samaru market, Zaria, Nigeria. This research was conducted at the Food and Industrial Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Sample Preparation

The flour obtained was sifted through a 0.25 sieve, then packed in low-density polythene bags, and stored in a dry place at room temperature (27±5°C) until used.

Collection of Starter

Strain of amylolytic *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) were stock cultures previously identified based on cultural

morphology and physiological characteristics using API 50 CHL and API 20 C AUX kit BIOMERIEUX in the study by Amapu *et al.* (2016). The isolates were maintained in agar slants and stored at 4 °C.

Cultivation of Starter Cultures

The culture of the amylolytic *Lactobacillus plantarum* (AMz5) was inoculated in de Man-Rogosa-Sharpe (MRS) broth (Difco™, Becton, Dickinson and Co, Le Point de Croix, France) and incubated anaerobically at 37°C for 24 hours. The bacterial cells were then harvested by centrifugation at 12,000 ×g at 4°C for 10 min and washed three times with sterile peptone solution (0.1% w/v). The cell concentrations were then adjusted to 10⁷ CFU/ml using 0.5 Mac-Farland turbidly standard.

The *Saccharomyces cerevisiae* (YSg2) was propagated on a basal medium consisting of 2% (w/v) glucose, 0.5% (w/v) yeast extract, 1% (w/v) peptone, 0.1% (w/v) ammonium sulfate and 0.1% (w/v) magnesium sulphate at pH 5.6 (Ameh and Umaru, 2000). Afterward, the cells were harvested by centrifugation at 4000×g for 30 min. The yeast pellets were then rinsed twice with sterile distilled water and stored at 4°C.

Preparation of Composite Flour

A modified method of Aboaba and Obakpolor (2010) was adopted for composite flour formulation. The cassava flour inclusion ration of 10, 20, 30, 40, and 50 % which are equivalent to 10, 20, 30, 40, and 50 g were separately mixed with 90,80,70,60 and 50g of wheat flour respectively.

Bread Dough Preparation

The dough was prepared by mixing various proportions of the composite flour (400g) with 32mL each of standardized YSg2 (10⁶ cells/mL) and AMz5 (10⁸ cells/mL), sugar (16.00 g), salt (4.00 g) and water (258 mL as adopted by Eddy *et al.*, (2007). Controls dough was developed using commercial baker's yeast as a leavening agent on composite flour and 100% wheat flour.

Determination of Dough Rising Ability of Isolates and Leavening profile

The dough was prepared by mixing the composite flour (50 g), 4mL each yeast cell (10⁶ cells/mL), ALAB cells (10⁸ cells/mL), sugar (2 g), salt (0.5 g), and water (32mL) in a beaker as specified by Eddy *et al.* (2007). Samples of the dough developed were loaded into measuring cylinders greased with vegetable oil, covered with foil paper, and incubated at 30°C for 4 h. The leavening profile of the dough was determined by measuring height displacement from the cylinder graduation at 30-minute fermentation time intervals.

Dough pH and total titratable acidity Determination

Ten (10g) of dough was homogenized in 90 mL of distilled and the calibrated pH meter electrode was inserted. The readings displayed were appropriately recorded to the nearest one decimal place for each sample.

The suspension obtained above was then poured into a 100 mL conical flask and titrated with 0.1M NaOH using 0.1 mL of 0.5% phenolphthalein as an indicator. Total titratable acid was expressed as the amount of NaOH used at the pink color indicator endpoint.

$$\% \text{ Total titratable acidity} = \frac{\text{Titre value}}{\text{Weight of sample}} \times 100$$

Determination of Amylolytic *L. plantarum* (AMz5) Count

Ten grams of the fermenting dough was suspended in 90 mL sterile distilled water, and the resultant stock was then tenfold serially diluted. Aliquots of 0.1mL of the diluted sample were inoculated on de Man-Rogosa-Sharp agar (Difco™, Becton, Dickinson and Co, Le Point de Croix, France) plates and incubated anaerobically at 30°C for 48 hours. The discrete colonies formed were enumerated and expressed as colony-forming units per gram of sample (CFU/g).

Viability Yeast Count

Yeast viability counts were determined using the spread plate technique as described by Gerez *et al.* (2009). The inoculated Sabouraud Dextrose Agar modified with 50 ppm chloramphenicol (250mL) was then incubated at 30°C for 48 hours. Discrete colonies formed were enumerated as colony-forming units per gram (CFU/g).

Data Analysis

The values obtained were expressed as mean ± standard error of the mean of triplicate determination. One-way ANOVA was carried out to determine the quality of performance of the test organisms using the Statistical Analysis System (SAS) package. Differences among samples were considered significant at $P \leq 0.05$.

RESULTS

Dough Rising and Leavening Profiles

The effect of varying proportions of cassava flour in cassava-wheat composite dough during leavening is presented in Figure 1. According to the results obtained, as the level of substitution for cassava flour increased, the dough volume decreased progressively with leavening. Dough volume (cm³) of the cassava composite flour peaked at 84, 80.5, 74, 72.5, and 69 for 10, 20, 30, 40, and 50 % cassava inclusion respectively in decreasing order during leavening,

Comparatively, wheat dough volume leavened with commercial yeast (85.5cm³) had the highest gas retention followed by wheat dough co-fermented with amylolytic *L. plantarum* (AMz5) while dough from cassava only (66 cm³) had the least. Notably, cassava-wheat composite dough at a 10 % cassava inclusion rate, recorded a dough volume similar ($p > 0.05$) to the 100% wheat dough.

Figure 1 presents a dough volume expansion curve characterized by a longer lag that increased with the cassava flour inclusion rate. In this study, the wheat dough (36 min) exhibited a shorter leavening lag time, followed by 10% cassava-wheat composite dough (90 min) while cassava dough (150 min) exhibited the highest. Dynamics of dough-specific volume per time similarly decreased with increased cassava inclusion at 10 < 20 < 30 < 40 < 50 in increasing order.

Change in pH during Dough Fermentation

Typically, a decrease in pH was observed during the leavening of the cassava-wheat composite dough. Accordingly, a higher pH decrease occurred from 4.75 to 3.6 in the wheat dough than 5.30 to - 4.5 observed in the composite dough proofing. Moreover, acidification increases with decreasing cassava flour proportioning during the 240-minute leavening period. Thus, the rate of pH change occurred in 10 > 20 > 30 > 40 > 50% composite dough with decreasing pattern.

Total titratable acidity (TTA) of fermenting dough

In this study, the total titratable acidity of composite doughs increased with leavening but, decreased with cassava flour proportioning (Figure 3). Comparatively, wheat dough leavened with commercial baker's yeast experienced the highest total titratable acidity (7.49±0.84 % lactic acid) increase followed by dough co-cultured with *L. plantarum* (AMz5) and baker's yeast (6.66±0.90). The composite dough leavening curve revealed that after 30 min lag time, acidification of all the composite doughs increased, however at a rate that reduced with increased cassava inclusion. Notably, dough with 10% cassava-wheat composite flour and wheat flour (Wf) co-cultured with amylolytic *L. plantarum* (AMz5) and baker's yeast did not vary significantly ($p > 0.05$).

Kinetics of Microbial Growth

The growth kinetics of LAB in cassava-wheat composite dough is presented in Figure 3. Here, a continuous increase in LAB viability in cassava-wheat composite dough after 30 min lag was observed during leavening. Comparatively, 10% cassava inclusion dough enhanced the LAB count similar to the control and decreased with proportioning. Similarly, the baker's yeast exhibited steady growth in the cassava-wheat composite dough with

fermentation (Figure 4). The growth profile showed that the continuous increase in yeast counts but, varied relatively with increased cassava flour inclusion.

DISCUSSION

Wheat bread dough preparation for bread making is an ancient practice however, knowledge of the effect of cassava inclusion on the kinetic of microbial growth and dough volume is still novel. In this study, the dough volume and microbial viability were found to be strongly determined by the level of cassava inclusion in the composite dough. In the development of most composite and gluten-free doughs, this scenario is a fundamental behaviour (Aboaba and Obakpolor, 2010; Vidaurre-Ruiz *et al.*, 2021).

Overall, dough volume and microbial growth rate kinetics were found to be affected by cassava inclusion during the leavening process. The starchy cassava could result in low availability of nutrients for metabolic activity contributing to the reduction in CO₂ production by fermentation of yeast. This assertion is within the background that the availability of fermentable substrates is essential to support optimal gassing power by yeast cells during leavening. Additionally, the functionality of the gluten proteins network is highly dependent on the specific dough recipe, such as water, salt, and other typical dough components. Beyond all these considerations, the gluten proteins play a key role in the development of dough volume by the entrapment of carbon dioxide evolved from yeast respiration during fermentation. Findings by several researchers have revealed that nongluten flours adversely affected dough rising due to the absence of gluten protein (Oladunmoye *et al.*, 2010; Abdelghafor *et al.*, 2011; Viswanathan and Ho, 2014).

Even though, dough volume reduction occurred significantly with cassava inclusion, however, 10 and 20% levels compared to the control. The maintenance of dough volume similar to the control at cassava inclusion below 20 % is consistent with the reports of coworkers (Shittu *et al.*, 2007; Aboaba and Obakpolor, 2010). It is therefore certain from this study that composite flour beyond 10 and 20% non-wheat flour rapidly impaired the dough volume (Aboaba and Obakpolor, 2010).

One important aspect of our research focused on the use of amylolytic lactic acid bacteria and yeast as starter leavening agents. It is evident from this study that the co-culture of specific strains of amylolytic *L. plantarum* (AMz5) and *Saccharomyces cerevisiae* resulted in higher dough volume compared to the control using commercial baker's yeast. This indicates

that the co-culture of *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) enhanced dough volume than the control (commercial baker's yeast). This conforms to the fact that coculturing will enhance the hydrolysis of cassava starch by amylolytic *L. plantarum* (AMz5) resulting in a faster fermentation process (Putri *et al.*, 2011). The ability of amylolytic *Lactobacillus amylopylus* NBRC 15881, to ferment carbohydrates into simple sugars and organic acids changed cassava starch molecular structure, which resulted in a better dough swelling power (Putri *et al.*, 2011).

Furthermore, this study investigated pH, total titratable acid, and increased viability of lactic acid bacteria and yeast during dough fermentation. Our results revealed a correlation between pH, viable counts of yeast and lactic acid bacteria, and the accumulation of organic acids. The decrease in pH and viable counts during fermentation suggested a strong fermentation activity and the production of organic acids. This finding indicates the accumulation of organic acids (lactic acid, acetic acid) which resulted in the change in pH and total titratable acids. The acidic environment is favorable to fermentative organisms and creates optimum pH for dough texture improvement (Gobbetti *et al.*, 2005; Rehman *et al.*, 2006). The decrease in pH and the increase in viable counts during fermentation suggested a strong fermentation activity, which will influence the texture of the fermented dough (Meignen *et al.*, 2001; Rehman *et al.*, 2006). The dough pH values (5.3 to 3.45) obtained in this study may contribute to the safety of fermented foods as affirmed by researchers (Kostinek *et al.*, 2007; Gerez *et al.*, 2009). Our findings suggest that the inclusion of cassava in wheat bread dough affects the kinetics of microbial growth, physicochemical changes, and dough volume compared with the control. In this study, the LAB and yeast counts differ considerably among the blends, however, a continuous increase in the viability of the starter cultures with leavening was observed. This is in concert with the expectation that the microbial population of LAB and yeast increases during fermentation (Asmahan and Muna, 2009). In addition, the viability of LAB (3.50×10^8 to 3.80×10^8 CFU/g) and yeast (2.1×10^6 to 3.6×10^6 CFU/g) meets the criteria that in a good bakery practice, dough should contain metabolically active LAB at 10^8 to 10^9 CFU/g and yeast at 10^6 to 10^7 CFU/g as reported by De Vuyst and Neyens, (2005).

CONCLUSIONS

This study provides the kinetics of leavening in cassava-wheat composite dough and its effect on

dough volume, physicochemical changes, and growth of starter cultures. Typically, the cassava inclusion ratio decreased the kinetics of microbial growth, physicochemical changes, and dough volume with cassava inclusion ratio (%) at levels that 10 < 20 < 30 < 40 < 50 in increasing order. Overall, dough volume reduction occurred significantly with increased cassava inclusion however, 10 and 20% levels compete with the control dough. This finding, therefore, provides that adopting 10 or 20% cassava flour inclusion will provide dough with the kinetics of leavening for the production of the bread of acceptable quality.

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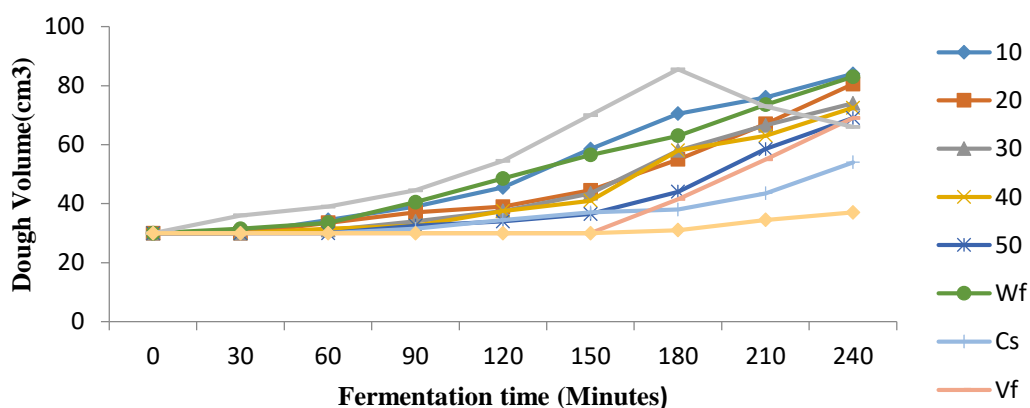


Figure 1: Effect of Varying Proportions of Cassava and Wheat on Dough Leavening

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates), Cs = cassava dough, Vf = virgin flour dough, Positive control = wheat dough, plus commercial yeast, Negative control = Uninoculated dough

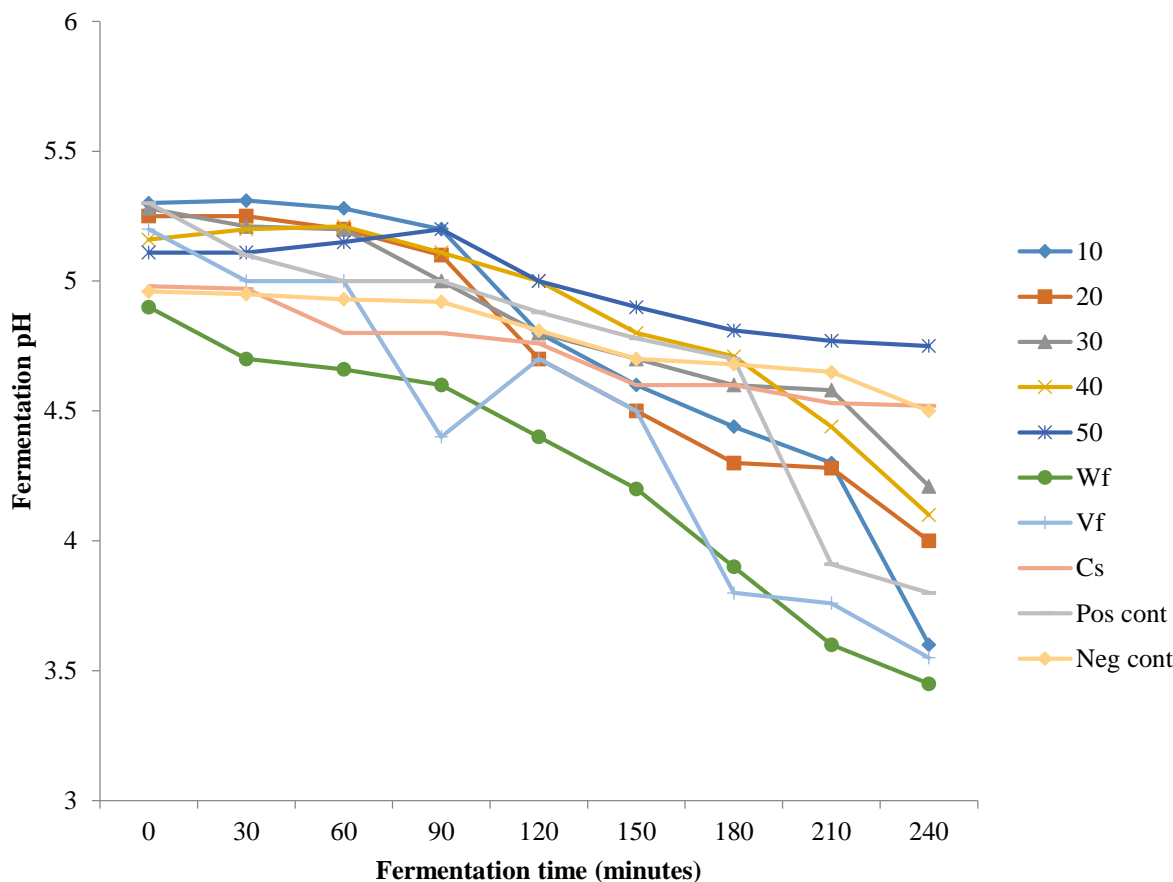


Figure 2: Changes in pH of Cassava and Wheat Dough Fermentation

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf = virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

Table 1: Total titratable acid of fermenting cassava and wheat dough samples

Samples	Ind Mean ± S.E.
10%	6.51±0.65 ^{ab}
20%	5.51±0.66 ^{bc}
30%	5.07±0.56 ^{bc}
40%	4.69±0.55 ^{cd}
50%	4.59±0.57 ^{cd}
Wf	6.66±0.90 ^{ab}
Vf	4.07±0.32 ^{cd}
Cs	3.02±0.23 ^d
Post cont.	7.49±0.84 ^a
Neg. cont.	3.73±0.26 ^d
Total	5.13±0.23
F-value	5.652
p-value	0.000*

Means in the same column with different superscripts are significantly different by ($p < 0.05$) Duncan multiple range test (DMRT)

Key: 10,20,30,40, and 50% = different proportions of cassava and wheat flour in dough. Wf=, dough started with isolates, Vf= virgin flour dough, Cs= cassava dough, Positive control= dough started with commercial yeast, Negative control = uninoculated dough, S.E= Standard Error of Mean, Ind= industrial, Mkt=market

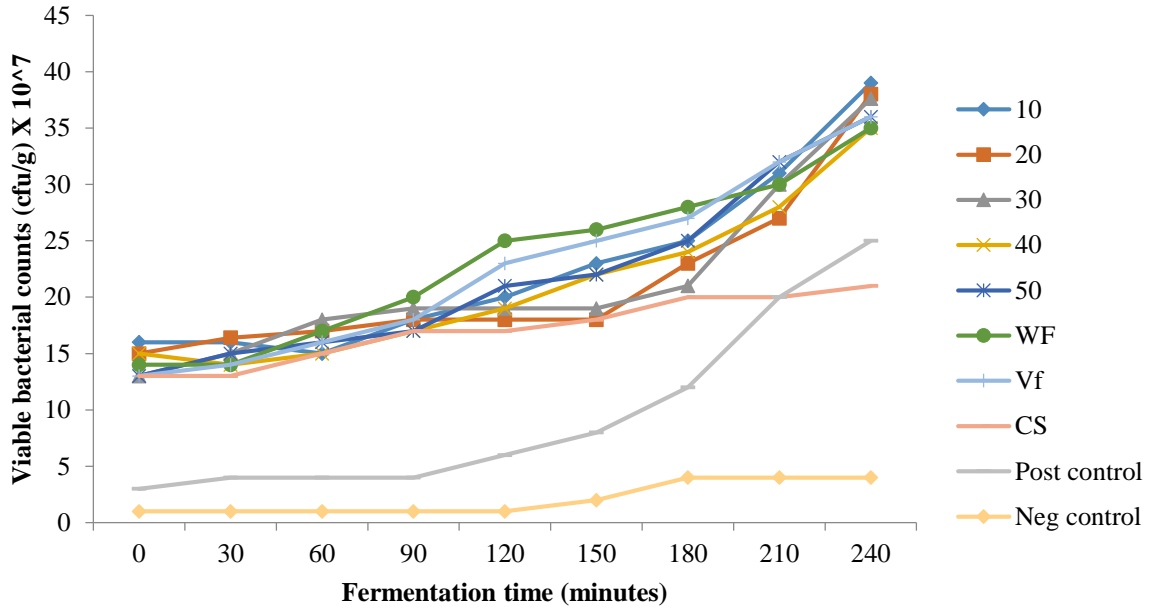


Figure 3: Growth of ALAB during Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

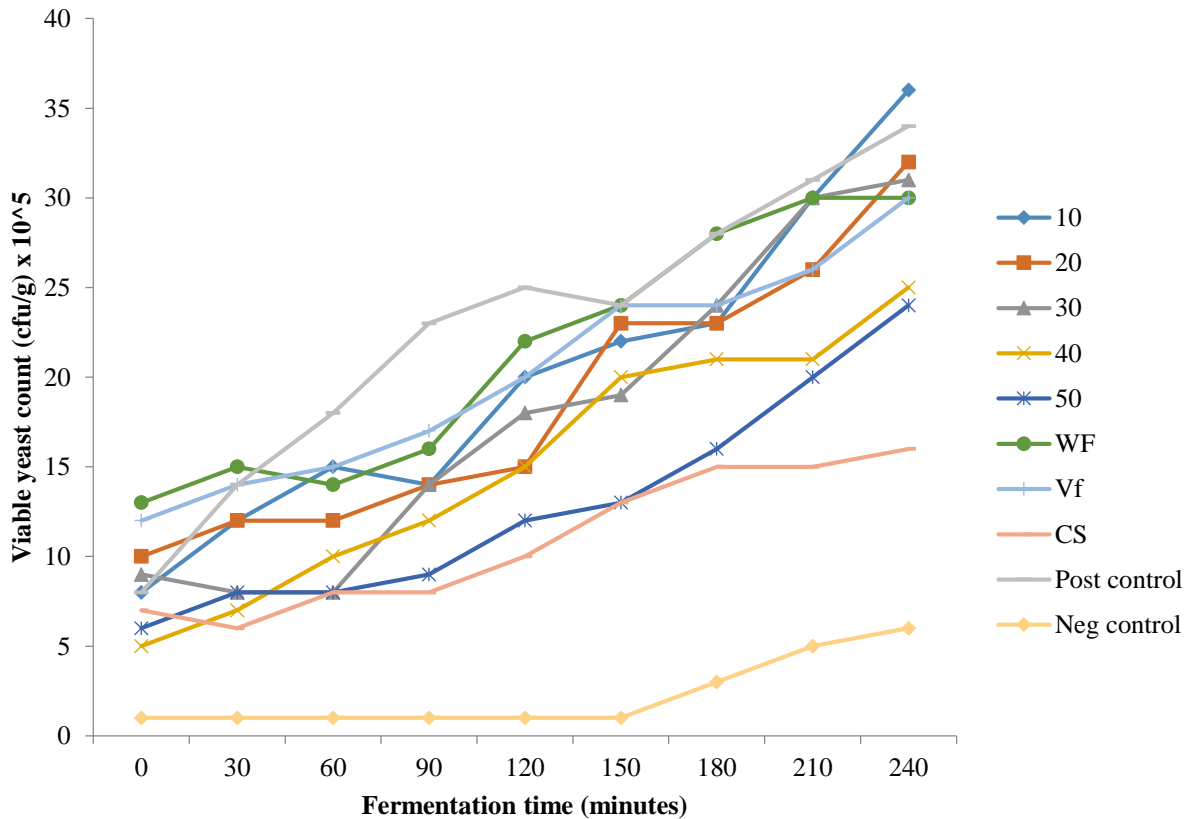


Figure 4: Growth of Yeast during Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough