AMYLOLYTIC BACTERIA IN FERMENTED 'OGI' FORTIFIED WITH ALMOND NUTS *(PRUNUS AMYGDALUS L.)* AND AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA L.*)

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DOI10.51459/jostir.2026.2.1.0256

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History
Received: 07-03-2026
Accepted: 15-04-2026
Published: April, 2026

https://www.futa.edu.ng

JOSTIR
JOURNAL OF SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH
https://jostir.futa.edu.ng

ABSTRACT

Amylolytic activity of bacteria has been reported to have functional properties in food processing and production. This study assessed amylolytic bacteria and their contributions to the quality and safety of 'Ogi' fortified with almond nuts and African yam bean. Samples were formulated in the ratios Maize: Almond: African Yam Bean (M: A: AFB). A- 100:0:0, B- 85: 10: 5, C- 85: 5:10, D- 65: 15: 20, E- 65: 20: 15 and commercial custard (F- control). Isolation and identification of lactic acid bacteria were carried out using standard microbiological methods. The amylase producers were determined using starch hydrolysis techniques. The lactic acid bacteria isolated and screened for amylase production were *Lactobacillus plantarum* strain pmm095_25282; Lactococcus lactis strain IFM 63512; *Lactobacillus acidophilus* strain MK_S10; Pediococcus cellicola strain Pmm 25, and *Leuconostoc mesenteroides* strain M6. *Lacobacillus plantarum* strain pmm095_25282. They exhibited the highest clear zones, amylolytic index, and amylolytic activity of 12.24 ± 0.13a mm; 1.96 ± 0.01a and 0.68 ±0.01 c U/ml respectively. While the lowest clear zone, amylolytic index, and amylolytic activity were recorded for *Pediococcus cellicola* strain Pmm-25 with values 5.23 ±0.21b mm; 1.22 ± 0.01 c and 0.21 ± 0.02 c respectively. The control (Sample F) did not exhibit any amylase producers compared to the formulated samples. Findings from this study showed that amylolytic bacteria can be obtained from fermented 'Ogi' fortified with almond nuts and African yam bean.

Keywords: Almond, Amylase activity. Fermentation, Lactic acid bacteria

1. | Introduction

Fermented foods are widely regarded as functional foods because of their numerous health benefits. For centuries, they have supported human survival while enhancing nutritional value and improving sensory characteristics such as taste and aroma. Fermented cereal-based products continue to play a significant role in daily diets and household food practices across many regions of the world. Lactic acid bacteria (LAB) are recognized as safe microorganisms and are the dominant agents in the fermentation process. They are especially important in the production of traditional foods like gari, fufu, ogi, and similar products, where they contribute to preservation, flavor development, and improved digestibility. These attributes make LAB indispensable in traditional food systems globally. In recent years, growing research attention has focused on the potential application of lactic acid bacteria in the production of industrially important enzymes, particularly amylases, due to their efficiency in breaking down starch-rich substrates. (Hmidet *et al.,* 2009), which covers over 30% of the global market (Gupta *et al.,* 2003). Therefore, many food, beverage, and textile industries widely adopt the application of amylases produced by lactic acid bacteria to enhance their products (Sundarram *et al.,* 2014). Adults and children considered 'Ogi' a stable food for their breakfast, and weaning foods for infants after six months of excluding breastfeeding (United Nations Children's Fund, 2023**)**. 'Ogi' undergoes spontaneous fermentation, and many nutrients are lost during the production of 'Ogi' (Adebo, 2020). To restore the nutrient loss, there is a need for fortification with plant proteins, such as almond and African yam bean, that are easily accessible (Adebo *et al.,* 2022; Oyeyinka *et al.,* 2024).

Almonds are under-exploited nuts with abundant phytochemicals and proteins that can mitigate the health challenges of consumers. Almond is rich in all essential amino acids and has a very small amount of antinutritional factors compared to African yam bean (Kendall *et al.* 2010; Yada *et al.* 2013). The United States Food and Drug Administration (US-FDA) rated almonds as nutrient-rich plant proteins (Chen *et al.,* 2006). Almonds are neglected plants that contain a lot of minerals (magnesium, zinc), vitamin E, which is >20% of the daily value (FDA, 2013). Almonds harbour a lot of nutrients like proteins, fibre, phosphorus, amino acids, and riboflavin that are needed by the body (Milbury *et al.* 2006).

African yam bean (AYB) is an underutilized legume and is unexploited (Uchegbu, 2015). The plant is regarded as a plant-rich protein whose values range between 19 and 30% (Klu *et al.,* 2000; Nwosu, 2013). In addition to the protein content, AYB has been reported to possess bioactive compounds and be rich in micronutrients to mitigate the health challenges of individuals (Oyeyinka and Oyeyinka, 2018; Nwadi *et al.,* 2020). The seed herbours antinutritional factors such as tannin, trypsin inhibitors, and phytate (Taofeek *et al.,* 2020), which can be reduced to a lower level by fermentation. AYB is reported to be low in fibre compared to almonds (Obilana *et al.,* 2020). Combining the two plants will complement each other in the production of amylases. Therefore, this work aimed at assessing amylolytic bacteria in fermented 'Ogi' fortified with almond nuts (*Prunus amygdalus* L.) and African yam bean (*Sphenostylis stenocarpa* L.).

2. | Materials and Methods

2.1 | Sample Collection

Maize (1.0 kg) (*Zea mays* L.) grains and 1.0 kg of African yam bean (*Sphenostylis stenocarpa* L.) seeds were obtained from a local vendor at Oja-Oba market in Akure, Nigeria. The almond (*Prunus amygdalus* L.) nuts were obtained from Idah town, Nigeria. The samples were taken to the laboratory for further processing.

Processing of 'Ogi' Fortified with Almond Nuts and African Yam Bean

The pretreatment was given to the African yam bean to reduce its antinutritional factors. This was done by following the method described by Kok and Sze (2018). All the stones and unwanted materials were removed while leaving the good ones for further processing. The seeds of AYB were washed and soaked in water at room temperature (28±2°C) for 24 hours. Then, dried at 60°C. One kilogram (1.0 kg) of almond nuts was sun-dried. The pericarp of the almond was broken with a hammer to remove the nut and sun-dried again. The maize was sorted to remove stones and unwanted material and packaged in a sterile polyethylene bag. The Table of formulation is presented in Table 1.

Fermentation of 'Ogi' Fortified with Almond Nuts and African Yam Bean

The maize, almond, and African yam bean were fermented for 96 hours in sterile containers at an ambient temperature of 28±2^{o}C.

Table 1 | Formulation of 'Ogi' Fortified with Almond Nuts and African Yam Bean.

Sample Code	Maize (%)	Almond (%)	African Yam Bean (%)
A	100	0	0
B	85	10	5
C	85	5	10
D	65	15	20
E	65	20	15
F	Control		

Maize: Almond: African Yam Bean (M: A: AFB). A- 100:0:0, B- 85: 10: 5, C- 85: 5:10, D- 65: 15: 20, E- 65: 20: 15 and commercial custard (F- control)

Lactic Acid Bacteria Isolation from 'Ogi' Fortified with Almond Nuts and African Yam Bean

The isolation of lactic acid bacteria from almond nut and African yam bean–fortified 'ogi' was performed following the procedure described by Bhattacharya and Das (2022), with minor adjustments. Initially, a stock suspension of the sample was prepared by aseptically weighing 10 g of the fortified ogi and homogenizing it in 90 mL of sterile distilled water to ensure uniform distribution of microorganisms. Subsequently, a serial dilution process was carried out. Four sterile test tubes, each containing 9 mL of distilled water, were arranged under aseptic conditions. One milliliter of the prepared stock solution was transferred into the first test tube to obtain a 10^{-1} dilution. From this dilution, 1 mL was further transferred into the next tube to achieve a 10^{-2} dilution. This stepwise transfer continued sequentially until the 10^{-4} dilution level was obtained. The serial dilution technique was used to reduce microbial concentration to a manageable level, enabling the isolation of distinct lactic acid bacteria colonies for further microbiological analysis.

De Man, Rogosa, and Sharpe (MRS) agar was prepared according to the manufacturer's instructions and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45°C to prevent damage to microbial cells. One milliliter of the selected dilution was aseptically inoculated into two sterile Petri dishes. Subsequently, about 20 mL of molten MRS agar was poured into each plate. The plates were gently swirled to ensure even distribution of the sample within the medium before solidification for further incubation and analysis. The plates were swirled to thoroughly mix the inoculum and the medium. The plates were allowed to solidify and incubated anaerobically at 30°C for 48 hours. Following incubation, single, isolated and distinct colonies were picked and purified by repeated subculturing onto fresh MRS agar plates, with anaerobic incubation at 37°C for an additional 48 hours. The isolates were then Gram stained and biochemically characterized to confirm and identify the lactic acid bacteria. The isolates were transferred into a slant medium of MRS and preserved in a refrigerator at 4°C till further use. Identification of Lactic Acid Bacteria Isolated from 'Ogi' Fortified with Almond Nuts and African Yam Bean.

The characterization and identification of the lactic acid bacteria (LAB) were done based on their cultural, morphological, biochemical, and molecular identification.

Screening for Amylolytic Lactic Acid Bacteria from 'Ogi' Fortified with Almond Nuts and African Yam Bean

Following the method described by Sun *et al.* (2010), the starch hydrolysis was adopted for lactic acid bacteria to identify their amylolytic abilities on the starch-MRS medium flooded with Gram's iodine and observed for blue coloration with clear zones around the colonies. The isolates with the formation of the blue coluor on the plates were sub-cultured on MRS agar and kept for further use.

Extraction of Crude Enzymes and Fermentation of Enzymes.

The isolates with the amylolytic activity were grown in a 75ml basal medium containing 1% soluble starch (w/v) and 0.5% (w/v) yeast extract and were incubated in an incubator shaker at 37°C for 96 hours. The sterile test tubes were filled with 10 ml of the broth culture every 24 hours and centrifuged at 15000 rpm for 60 minutes. A sterile No1 Whatman filter paper was used to filter the culture to get crude amylase extracts (Amapu *et al.,* 2016).

Evaluation of Enzyme Activity and Reducing Sugar Concentration

Reducing sugars and enzyme activity were measured following a modified version of Abu et al. with minor modifications, using the 3,5-dinitrosalicylic acid (DNS) reagent. This involved the addition of 2 mL of soluble starch solution to 5 mL of crude enzyme extract in sterile tubes and vortexed well. The reaction tubes were then incubated at 50°C in a water bath for 30 minutes to allow the amylase enzyme to break down the starch into reducing sugars. Then the reaction was stopped and the absorbance was read at 600 nm on a spectrophotometer to determine the reducing sugars formed. An enzyme unit (U) was defined as the amount of enzyme needed to convert 1 gram of soluble starch in 60 minutes under the given conditions. This approach gave a good indication of enzyme activity.

3. | Statistical Analysis

The results were subjected to statistical analysis (one-way analysis of variance (ANOVA) using SPSS software to determine the significance of the differences among the treatments. Duncan's Multiple Range Test at 95% probability level was used for mean separation to determine significant differences.

4. | Results

The cultural, morphological, and biochemical characteristics of lactic acid bacteria isolated from 'Ogi' fortified with almond and African yam bean are shown in Table 2. All isolates exhibited Gram-positive, catalase-negative, and fermented a range of sugars (glucose, lactose, and raffinose). The lactic acid bacteria isolated from 'Ogi' fortified with almond nuts and African yam bean were *Lactobacillus plantarum, Lactococcus lactis*, *Lactobacillus acidophilus*, *Pediococcus* species, and *Leuconostoc* species.

The amylolytic lactic acid bacteria from 'Ogi' fortified with almond nuts and African yam bean blends are as shown in Table 3. The resulting clear zone ranged from 5.23 mm to 12.24 mm while the amylolytic index ranged from 1.22 ± 0.01 ^{c} to 1.96 ± 0.01 ^{a}. The amylolytic activity ranged from 0.21 ± 0.02 ^{c} to 0.68 ±0.01 ^{c} U/ml. The *Lactobacillus plantarum* strain pmm095_25282 from May 1 showed the highest clear zones, amylolytic index, and amylolytic activity of 12.24 ± 0.13^{a} mm; 1.96 ± 0.01^{a} and 0.68 ±0.01 ^{c} U/ml respectively. While the lowest clear zone, amylolytic index, and amylolytic activity were recorded for *Pediococcus cellicola strain* Pmm-25 from MAY 4, with values 5.23 ±0.21^{b} mm; 1.22 ± 0.01 ^{c} and 0.21 ± 0.02 ^{c} respectively.

Table 2 | Cultural, Morphological, and Biochemical Characteristics of Lactic Acid Bacteria Isolated from 'Ogi' Fortified with Almond and African Yam Bean

Isolate code	Cell shape	Gram reaction	Catalase	Coagulase	Motility	Indole	Gas from CO_2	Lactose	Glucose	Arabinose	Fructose	Sorbitol	Raffinose	Mannose	Probable organisms
M1	Rod	+	-	-	-	-	+	+	+	+	-	+	+	-	*Lb. plantarum*
M2	Cocci	+	-	-	+	-	+	+	+	+	-	-	+	+	*Lactococcus lactis*
M3	Rod	-	-	-	-	-	-	+	+	-	-	+	+	-	*Lactobacillus acidophilus*
M4	Rod	-	-	-	-	-	-	+	+	-	-	-	+	-	*Pediococcus species*
M5	Rod	-	-	-	+	-	+	+	+	+	-	-	+	-	*Leuconostoc species*

Key | + Positive - Negative

Table 3 | Amylolytic Lactic Acid Bacteria from 'Ogi' Fortified with Almond Nuts and African Yam Bean Blends

Isolate code	**Isolate**	**Diameter of clearance zone (mm)**	**Amylolytic index**	**Amylolytic activity U/ml**
MAY1	*Lactobacillus plantarum* strain pmm095_25282	12.24 ± 0.13^{a}	1.96 ± 0.01^{a}	0.68 ± 0.01^{c}
MAY2	*Lactococcus lactis* strain IFM 63512	6.32 ± 0.11^{c}	1.43 ± 0.05^{c}	0.35 ± 0.02^{b}
MAY3	*Lactobacillus acidophilus* strain MK_S10	7.14 ± 0.22^{c}	1.54 ± 0.04^{a}	0.51 ± 0.03^{c}
MAY4	*Pediococcus cellicola*P-mm-25	5.23 ± 0.21^{b}	1.22 ± 0.01^{c}	0.21 ± 0.02^{c}
MAY5	*Leuconostoc mesenteroides* strain M6	5.82 ± 0.14^{a}	1.36 ± 0.06^{c}	0.26 ± 0.01^{b}

Values are means ± standard deviation of three determinations; Values not followed by the same superscript along column are not significantly different; MAY: Maize-Almond - African Yam Bean

5. | Discussion

Lactic acid bacteria (LAB) play a significant role in the fermentation of foods. There has been much focus on the potential industrial applications of amylolytic LAB from fermented starchy foods (Hmidet *et al.*2009). Amylolytic activities were found in 'ogi' fortified with almond nuts and African yam bean in this study. The LAB isolates were characterised as belonging to the genera Lactobacillus, Pediococcus and Leuconostoc, which is in agreement with previous studies that identified these as the major LAB in sourdough fermentation (Amapu *et al*., 2016). LAB are known for their production of amylase enzymes, especially α-amylase, allowing them to hydrolyse starch in fermented products (Sundarram *et al*., 2014). But the amylolytic activity of these isolates is different from LAB from traditional fermented foods in Benin, which could be due to variations in the substrate or environmental conditions in which the enzyme is produced (Tchekessi *et al.,* 2014). This could be due to the supplementation with almond nuts and African yam bean, which supply essential nutrients (proteins and vitamins) that enhance microbial growth, enzyme synthesis and acid development (Tatsinkou and Tavea, 2013). The amylolytic potential of the LAB isolates in this study indicates their potential as starter cultures, especially in the food industry where hydrolysis of starch is required to enhance quality and functionality of food products.

5. | Conclusion

This study confirms that 'Ogi' fortified with almond nuts and African yam bean is an inducer of amylolytic lactic acid bacteria. The predominant lactic acid bacteria strains obtained in this study were *Lactobacillus plantarum* strain pmm095_25282, *Lactococcus lactis* strain IFM 63512, *Lactobacillus acidophilus* strain MK_S10, *Pediococcus cellicola Pmm-25,* and *Leuconostoc mesenteroides* strain M6. The *Lactobacillus plantarum* strain pmm095_25282 possessed the highest amylolytic activity, amylolytic index, and starch hydrolysis among other isolates. The application of these isolates could be maximized in bread-making and food fermentation processes as a starter culture.

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