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Isolation, Screening, Optimization, Purification and Application of an α-Galactosidase-Producing Lactic Acid Bacterium from Nigerian Fermented Dairy Products

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Abstract
Lactic acid bacteria (LAB) are a food-grade source of α-galactosidase (α-Gal; EC 3.2.1.22), an enzyme relevant both to reducing raffinose-family-oligosaccharide-associated flatulence in legume foods and to enzymatic clarification of fruit juices, yet α-Gal from West African fermented-dairy LAB remains largely uncharacterized.This study aimed to isolate and identify α-Gal-producing LAB from Nigerian yogurt, *nunu*, and cheese; to optimize and characterize the enzyme from the best-performing isolate; and to evaluate its effect on fruit-juice quality.LAB were isolated on MRS agar, screened for α-Gal production using a Congo red/locust-bean-gum plate assay and a quantitative 3,5-dinitrosalicylic acid (DNS) assay, and the highest-yielding isolate was identified by 16S rRNA gene sequencing. Fermentation conditions (pH, temperature, substrate concentration) were optimized; the enzyme was partially purified by ammonium sulfate precipitation and dialysis; and its molecular weight, pH/temperature profile, and apparent kinetic parameters were determined. The resulting preparation was applied to orange, apple, pineapple, and watermelon juices.Of 24 isolates, isolate NN3 (*Lactobacillus plantarum*) from *nunu* showed the highest activity (0.257 ± 0.011 U/mL; specific activity 0.139 ± 0.006 U/mg protein). Optimized conditions (pH 6.5, 40 °C, 1.5% substrate) raised activity to 0.398 U/mL, a 54.9% increase over the unoptimized baseline. Purification yielded a 62 kDa enzyme with 5.38-fold purification and 53.8% activity recovery. Juice treatment increased light transmittance (clarity) by 47–55%, reduced viscosity by 33–36%, and increased yield by 25–30% across all four juices ($p < 0.001$).Nigerian fermented dairy products harbor LAB capable of producing an industrially relevant α-galactosidase, supporting further development of indigenous bacterial enzymes for food-processing applications, pending confirmatory kinetic and mechanistic studies.

Keywords: Enzyme kinetics; Food-grade biocatalyst; Fruit juice clarification; *Lactobacillus plantarum*; Raffinose-family oligosaccharides

I. Introduction

Lactic acid bacteria (LAB) constitute one of the most economically important groups of microorganisms in food biotechnology, valued both for their central role in the fermentation of dairy, cereal, and vegetable products and for the diverse portfolio of hydrolytic enzymes they secrete during growth. Among these, glycoside hydrolases capable of cleaving non-digestible plant-derived oligosaccharides have attracted sustained industrial interest because they combine food-grade safety status with catalytic versatility. The global industrial enzyme market, valued at approximately US$7.7 billion in 2024 and projected to exceed US$11 billion by 2030, continues to expand rapidly, with carbohydrate-active enzymes for the food and beverage sector representing one of its fastest-growing application areas (ResearchAndMarkets.com, 2025). Within this landscape, α-galactosidase (α-Gal; EC 3.2.1.22), an exo-acting glycosidase that removes terminal α-1,6-linked galactose residues from raffinose-family oligosaccharides (RFOs) and related galactomannans, occupies a niche of particular nutritional and industrial relevance.

A significant and contemporary problem motivating this line of research is the widespread presence of RFOs principallyraffinose and stachyose in legumes, cereals, and several fruits and vegetables. Because the mammalian gastrointestinal tract lacks endogenous α-Gal activity, these sugars pass undigested into the colon, where microbial fermentation generates carbon dioxide, hydrogen, and methane, producing flatulence, bloating, and abdominal discomfort (Elango et al., 2022). This antinutritional burden is widely regarded as one of the principal barriers to greater dietary utilization of

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legumes and legume-derived foods. In parallel, the fruit-juice processing industry faces a related but mechanistically distinct challenge: structural cell-wall polysaccharides and associated haze-forming compounds limit juice clarity, elevate viscosity, and constrain extraction yield, all of which affect processing efficiency and consumer acceptability (Pui&Saleena, 2023). Enzymatic treatment has become the industry standard for addressing both problems, but the enzymes most commonly deployed industrially are of fungal origin, leaving considerable scope for food-grade, GRAS (generally recognized as safe) bacterial alternatives.

Screening of LAB for α-Gal production has repeatedly shown that the trait is strain- and species-dependent rather than a universal property of the group. *Bacillus coagulans* MA-13, a spore-forming lactic acid bacterium isolated from canned-bean manufacturing, has been shown to possess both a characterized GH42 β-galactosidase and a distinct GH36 α-galactosidase, the latter localized extracellularly (Aulitto et al., 2021). More recently, Bamgbose et al. (2022) isolated α-Gal-producing LAB including*Limosilactobacillusfermentum*, *Levilactobacillus brevis*, and *Leuconostocmesenteroides* from traditionally fermented African and Latin American foods, finding that only about one-quarter of tested isolates expressed the trait. Optimization of fermentation conditions for related galactosidase-producing LAB has also advanced: Zhang (2025) recently used single-factor experiments followed by response-surface methodology to raise β-galactosidase yield more than four-fold in a *Lactobacillus plantarum* strain isolated from fermented yak milk. Collectively, these studies establish workable pipelines for primary screening, quantitative assay, and fermentation optimization, but they also demonstrate that yield, optimum pH and temperature, and downstream applicability differ markedly according to strain and substrate source.

Despite this expanding body of work, several gaps persist. First, the majority of α-Gal-producing LAB reported to date have been isolated from Asian, European, or soy-based fermented matrices; comparatively little is known about strains native to West African fermented dairy products such as *nunu*, whose LAB microbiota has been characterized taxonomically and technologically (Akabanda et al., 2014; Saliu et al., 2024) but has rarely been screened specifically for α-Gal activity. Second, full purification and kinetic characterization (molecular weight, K_m, V_{max}, pH/thermal stability) of LAB-derived α-galactosidases remain uncommon outside a small number of reference strains, limiting comparative benchmarking against the fungal enzymes already used industrially. Third, and most critically, the application of an LAB-derived α-galactosidase specifically to fruit-juice quality improvement has essentially no precedent in the literature reviewed for this study: published enzymatic juice-clarification work overwhelmingly employs pectinases and cellulases, which act on pectin and hemicellulose backbones (Pui&Saleena, 2023), whereas α-galactosidase acts only on terminal galactosyl side-chains and has no established pectinolytic activity of its own. Any observed effect of an α-Gal preparation on juice clarity, viscosity, or yield therefore requires either a plausible enzymatic mechanism or an explicit acknowledgement that the preparation used was not fully pure.

Accordingly, the specific gap addressed in the present study is the absence of an integrated, source-to-application investigation that (i) isolates and identifies LAB from indigenous Nigerian fermented dairy products, (ii) screens and quantitatively ranks isolates for α-Gal production, (iii) optimizes fermentation conditions for the best-performing isolate, (iv) purifies and biochemically characterizes the resulting enzyme, and (v) evaluates its practical utility in a downstream food-processing application.

Addressing this gap is scientifically and industrially useful for two complementary reasons. Scientifically, it extends the still-limited catalogue of biochemically characterized LAB α-galactosidases to a new, food-grade, regionally relevant isolate, permitting comparison of its kinetic and stability parameters with those already reported for other bacterial and fungal α-galactosidases (Aulitto et al., 2021; Bamgbose et al., 2022). Industrially, a GRAS-status, indigenous LAB source of α-Gal would offer food processors an alternative to imported fungal enzyme preparations, potentially carrying the additional co-benefit of probiotic or antimicrobial properties inherent to many LAB strains (Saliu et al., 2024).

The present study therefore aimed to isolate and identify lactic acid bacteria from Nigerian fermented dairy products (yogurt, *nunu*, and cheese), to screen the isolates qualitatively and quantitatively for α-galactosidase production, to optimize fermentation conditions for the highest-yielding isolate, to purify and biochemically characterize the resulting enzyme, and to evaluate its effect on the clarity, viscosity, and yield of selected fruit juices. The specific objectives were to: (1) isolate and enumerate LAB from yogurt, *nunu*, and cheese, and confirm the identity of the highest-

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yielding isolates by 16S rRNA gene sequencing; (2) screen isolates for α-Gal production using qualitative plate-based and quantitative colorimetric assays; (3) determine the optimum pH, temperature, and substrate concentration for enzyme production by the best-performing isolate; (4) purify the enzyme and determine its molecular weight, pH/thermal stability, and apparent kinetic constants; and (5) assess the effect of enzyme treatment on the clarity, viscosity, and yield of orange, apple, pineapple, and watermelon juices.

II. Materials and Methods

2.1 Study Design

This study employed a sequential experimental design comprising five phases: (i) isolation and identification of lactic acid bacteria (LAB) from fermented dairy products; (ii) qualitative and quantitative screening of isolates for α-galactosidase (α-Gal; EC 3.2.1.22) production; (iii) optimization of fermentation conditions for the highest-yielding isolate; (iv) purification and biochemical characterization of the resulting enzyme; and (v) evaluation of the enzyme preparation in a fruit-juice quality-improvement application.

2.2 Sample Collection

Fermented dairy samples yogurt (YG), *nunu* (NN), and cheese (CH) were obtained from random vendors in Owode market Offa in Kwara state, Nigeria ($8^0$08' N, $4^0$43' E). All samples were collected in sterile, leak-proof containers, labeled appropriately, and immediately placed in an insulated icebox containing ice packs (4^0C) to prevent microbial proliferation during transport. The samples were transported to the laboratory within2 hoursof collection and processed immediately for microbiological and physicochemical analyses

2.3 Isolation and Enumeration of LAB

Ten-fold serial dilutions of each sample were prepared, and the 10^{-6} dilution was plated in triplicate on de Man, Rogosa and Sharpe (MRS) agar (de Man et al., 1960), a medium selective for lactobacilli that also supports the growth of other LAB genera such as *Leuconostoc* and *Pediococcus*. Plates were incubated at 37 °C for 48 h, after which colonies were counted and expressed as colony-forming units per gram (CFU/g). A total of 24 morphologically distinct isolates (8 from yogurt, 10 from *nunu*, 6 from cheese) were selected for further characterization on the basis of differences in colony morphology.

2.4 Morphological and Biochemical Characterization

Colony morphology (size, color, texture, margin), Gram reaction, cell shape, and catalase activity were recorded for each isolate using standard microbiological procedures. The specific reference protocol followed for Gram staining and catalase testing. Gram-positive, catalase-negative rods and cocci were presumptively assigned to LAB genera (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*) on the basis of cell morphology and arrangement.

2.5 Molecular Identification

The three isolates with the highest quantitative α-Gal activity (NN3, YG1, CH2; see Section 2.7) were selected for molecular confirmation. Genomic DNA was extracted and the 16S rRNA gene was amplified using the universal bacterial primer set of Weisburg et al. (1991). Amplicons were sequenced and compared against the GenBank database by BLAST, with isolates assigned to the species of their closest match.

2.6 Primary (Qualitative) Screening for α-Gal Production

Isolates were inoculated onto locust bean gum (LBG)-containing agar and incubated under the specified conditions. Following incubation, plates were subjected to Congo red staining, and zones of substrate hydrolysis were measured as an indication of extracellular galactomannan-degrading activity. Because Congo red clearing primarily reflects degradation of the polysaccharide backbone and does not specifically distinguish α-galactosidase activity, isolates identified during this preliminary screening were subsequently subjected to a substrate-specific α-galactosidase assay using PNP-α-gal. (Aulitto et al., 2021)

2.7 Secondary (Quantitative) Screening — DNS Assay

α-Gal activity was quantified in cell-free culture supernatants by measuring the reducing sugar (mannose-equivalent) released from LBG, using the 3,5-dinitrosalicylic acid (DNS) method of Miller (1959). Absorbance was read at 540 nm against a glucose/mannose standard curve, and one unit (U) of enzyme activity was defined as the amount of enzyme releasing 1 µmol of reducing sugar per minute under the stated assay conditions.

2.8 Protein Determination

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Total protein concentration in crude and purified enzyme fractions was determined by the dye-binding method of Bradford (1976), using bovine serum albumin as the calibration standard and monitoring absorbance at 595 nm. Specific activity was calculated as enzyme activity (U/mL) divided by protein concentration (mg/mL).

2.9 Optimization of Fermentation Conditions

The effects of pH (4.0–8.0), incubation temperature (25–50 °C), and LBG substrate concentration (0.5–2.5%, w/v) on α-Gal production by the top-ranked isolate (NN3) were evaluated sequentially, with each variable optimized while holding the other two at their previously determined optimum values — a classical one-factor-at-a-time strategy comparable to the single-factor screening step used by Zhang (2025) prior to response-surface optimization (Tables 4.7–4.9). The combination of conditions yielding maximal activity was adopted as optimal for subsequent enzyme production.

The extracellular α-galactosidase produced by isolate NN3 under the optimized fermentation conditions established in Section 2.9was subjected to partial purification. Following incubation under the optimized production conditions, the culture broth was harvested and centrifuged to remove the bacterial cells. The resulting cell-free culture supernatant, containing the extracellular enzyme, was collected and used as the crude enzyme preparation.

The crude enzyme preparation was subjected to fractional ammonium sulfate precipitation to concentrate the enzyme and remove a proportion of the contaminating proteins. The appropriate ammonium sulfate fraction obtained from the precipitation step was recovered by centrifugation, and the resulting protein precipitate was carefully resuspended in an appropriate buffer. The resuspended preparation was subsequently subjected to dialysis to remove residual ammonium sulfate and other low-molecular-weight contaminants. The dialyzed preparation constituted the partially purified α-galactosidase enzyme and was retained under appropriate conditions for subsequent characterization.

Protein concentration and α-galactosidase activity were determined for the crude and partially purified enzyme preparations using the respective analytical procedures described in the preceding sections. The purification profile was evaluated in terms of total enzyme activity, total protein content, specific activity, purification fold, and percentage enzyme yield. Purification fold was calculated as the ratio of the specific activity of the partially purified enzyme to that of the crude enzyme, whereas percentage yield was calculated relative to the total enzyme activity recovered in the crude preparation.

2.11 Molecular Weight Determination

The apparent molecular weight of the purified enzyme was estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) following the discontinuous buffer system of Laemmli (1970), with protein bands visualized by staining and molecular weight estimated by comparison with standard markers.

2.12 Biochemical Characterization of the Partially Purified α-Galactosidase

The biochemical properties of the partially purified α-galactosidase obtained from isolate NN3 were evaluated by determining its optimum pH, optimum temperature, pH stability, and thermal stability. Enzyme activity was assayed under the experimental conditions described for the α-galactosidase activity assay, while individual physicochemical parameters were varied systematically.

2.12.1 Determination of Optimum pH

The effect of pH on α-galactosidase activity was determined by assaying the enzyme at different pH values within the selected experimental range. Appropriate buffer systems were used to maintain the required pH conditions. Equal amounts of the partially purified enzyme preparation were incubated with the substrate under otherwise identical assay conditions. Enzyme activity was determined spectrophotometrically, and the activity obtained at each pH was expressed relative to the maximum activity, which was designated as 100%. The pH corresponding to the highest enzyme activity was considered the optimum pH**.**

2.12.2 Determination of Optimum Temperature

The effect of temperature on enzyme activity was determined by conducting the α-galactosidase assay at different temperatures within the predetermined experimental range. All other reaction conditions, including enzyme concentration, substrate concentration, reaction time, and pH, were maintained constant. Following incubation, enzyme activity was determined using the established assay procedure. The temperature at which the enzyme exhibited its highest activity was recorded as the optimum temperature**.**

2.12.3 Determination of pH Stability

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The pH stability of the partially purified α-galactosidase was evaluated by pre-incubating aliquots of the enzyme preparation at different pH values for a predetermined period under controlled conditions. Following pre-incubation, the enzyme preparations were returned to the standard assay conditions and the residual α-galactosidase activity was determined. Activity obtained without prior pH exposure was used as the reference activity (100%). Residual activity at each pH was expressed as a percentage of the reference activity. The pH range within which the enzyme retained substantial residual activity was considered its pH-stability range.

2.12.4 Determination of Thermal Stability

Thermal stability was evaluated by incubating aliquots of the partially purified enzyme at selected temperatures for predetermined periods. After the thermal treatment, the enzyme preparations were cooled under controlled conditions and assayed for residual α-galactosidase activity using the standard assay procedure. Enzyme activity measured without prior thermal treatment was considered the control activity (100%). Residual activity following heat treatment was expressed as a percentage of the control activity, allowing the thermal stability profile of the enzyme to be determined.

2.12.5 Determination of Kinetic Parameters

The kinetic properties of the partially purified α-galactosidase were determined by measuring the initial reaction velocity at different substrate concentrations. A series of substrate concentrations was prepared, and the enzyme reaction was performed under conditions corresponding to the previously established optimum pH and temperature. The amount of product released during the initial linear phase of the reaction was determined for each substrate concentration.

2.13 Application of α-Gal in Fruit-Juice Processing

An enzyme preparation was applied to freshly extracted orange (*Citrus sinensis*), apple (*Malusdomestica*), pineapple (*Ananascomosus*), and watermelon (*Citrulluslanatus*) juices. Treated and untreated (control) juices were compared for clarity (percentage light transmittance at 660 nm), viscosity (rotational viscometry), and juice yield (percentage v/w recovery relative to fresh fruit mass), following an experimental framework broadly comparable to that used in enzymatic juice-clarification studies employing pectinolytic and cellulolytic enzyme preparations (Pui&Saleena, 2023)

2.14 Statistical Analysis

Data are presented as mean ± standard deviation of triplicate determinations (n = 3) unless otherwise stated. Differences among more than two groups (isolates, pH levels, temperatures, substrate concentrations) were assessed by one-way analysis of variance (ANOVA), with means differing at $p < 0.05$ as indicated by differing superscript letters.

IV. Results

Table 4.1
Colony Counts and Recovery of Bacterial Isolates from Fermented Dairy Samples.

Sample Source	Sample Code	Dilution Factor	Colony Count (CFU/g)	No. of Distinct Isolates Selected
Yogurt	YG	10^{-6}	4.20×10^{7}	8
Nunu	NN	10^{-6}	6.50×10^{7}	10
Cheese	CH	10^{-6}	2.80×10^{7}	6

values are means of triplicate plate counts (n = 3) on MRS agar after 48 h incubation at 37 °C. A total of 24 isolates were selected across the three sample sources.

Table 4.2
Morphological, Cultural and Biochemical Characteristics of Bacterial Isolates.

Isolate Code	Source	Colony Morphology	Gram Reaction	Cell Shape	Catalase Test	Genus
NN1	Nunu	Small, creamy, circular, convex	+	Rod (short)	−	Lactobacillus
NN3	Nunu	Small, white, circular, entire margin	+	Rod	−	Lactobacillus
YG1	Yogurt	Creamy, circular, smooth	+	Coccus (chains)	−	Lactococcus

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YG4	Yogurt	Off-white, circular, mucoid	+	Coccus	−	Leuconostoc
CH2	Cheese	Small, opaque, round	+	Coccus (tetrads)	−	Pediococcus
CH5	Cheese	Creamy, circular, raised	+	Rod	−	Lactobacillus

"+" = Gram-positive; "−" = catalase-negative. Representative isolates shown.

Table 4.3
Molecular Identification of Selected LAB Isolates by 16S rRNA Gene Sequencing

Isolate Code	Closest BLAST Match (Species)	% Sequence Identity	GenBank Accession No.
NN3	*Lactobacillus plantarum*	99.1	OQ458213.1
YG1	*Lactococcuslactis*	98.7	OQ458214.1
CH2	*Pediococcuspentosaceus*	99.4	OQ458215.1

Table 4.4
Primary (Qualitative) Screening of LAB Isolates for α-Galactosidase Production on Locust Bean Gum Agar (Congo Red Assay)

Isolate Code	Zone of Clearance (mm)	Qualitative Result	Remark
NN3	22.5 ± 0.6[a]	+	Strong producer
YG1	18.3 ± 0.5[b]	+	Strong producer
CH2	16.7 ± 0.5[b]	+	Moderate producer
NN1	14.2 ± 0.4[c]	+	Moderate producer
CH1	13.4 ± 0.4[c]	+	Moderate producer
YG2	11.6 ± 0.4[d]	+	Weak producer
NN4	9.8 ± 0.3[e]	+	Weak producer
NN2	0.0	−	Non-producer
YG3	0.0	−	Non-producer
NN5	0.0	−	Non-producer

Values are mean ± SD of triplicate plates (n = 3). Means with different superscript letters differ significantly at $p < 0.05$ (one-way ANOVA, $F(6,14) = 48.6$, $p < 0.001$, among positive isolates). "+" = visible clear zone; "−" = no clear zone.

Table 4.5
Secondary (Quantitative) Screening of LAB Isolates for α-Galactosidase Activity by DNS Assay

Isolate Code	Absorbance (540 nm)	Reducing Sugar Released (µmol/mL)	Enzyme Activity (U/mL)	Rank
NN3	0.842	7.71	0.257 ± 0.011[a]	1
YG1	0.715	6.54	0.218 ± 0.009[b]	2
CH2	0.658	6.03	0.201 ± 0.008[c]	3
NN1	0.512	4.68	0.156 ± 0.007[d]	4
YG2	0.487	4.44	0.148 ± 0.006[e]	5
CH1	0.401	3.66	0.122 ± 0.005[f]	6
NN4	0.322	2.94	0.098 ± 0.004[g]	7

One unit (U) of activity releases 1 µmol of mannose-equivalent reducing sugar per minute; reducing sugar values correspond to a 30-minute assay (Activity = reducing sugar released ÷ 30). Values are mean ± SD (n = 3). Superscript letters differ significantly at $p < 0.05$ (one-way ANOVA, $F(6,14) = 112.3$, $p < 0.001$).

Table 4.6
Crude Protein Concentration (Bradford Assay) and Specific α-Galactosidase Activity of Top-Ranked Isolates

Isolate Code	Protein Concentration (mg/mL)	Enzyme Activity (U/mL)	Specific Activity (U/mg protein)	p-value
NN3	1.85 ± 0.06	0.257 ± 0.011	0.139 ± 0.006[a]	< 0.001
CH2	1.72 ± 0.05	0.201 ± 0.008	0.117 ± 0.005[b]	< 0.001

YG1	2.10 ± 0.07	0.218 ± 0.009	0.104 ± 0.004[b]	< 0.001
YG2	2.02 ± 0.06	0.148 ± 0.006	0.073 ± 0.003[c]	< 0.001
NN1	2.35 ± 0.08	0.156 ± 0.007	0.066 ± 0.003[c]	< 0.001

Protein determined by the Bradford method (BSA standard, 595 nm). Specific activity = enzyme activity ÷ protein concentration. Values are mean ± SD (n = 3); superscripts differ at p < 0.05 (one-way ANOVA, F(4,10) = 38.4, p < 0.001).

Table 4.7

Effect of pH on α-Galactosidase Production by Isolate NN3 (Lactobacillus plantarum)

pH Level	Enzyme Activity (U/mL)	% Relative Activity	p-value
4.0	0.090 ± 0.006	29.0	< 0.001
5.0	0.148 ± 0.009	47.7	< 0.001
6.0	0.221 ± 0.011	71.3	< 0.001
6.5	0.310 ± 0.014	100.0	reference (peak)
7.0	0.257 ± 0.011	82.9	< 0.001
8.0	0.136 ± 0.008	43.9	< 0.001

Values are mean ± SD (n = 3) at fixed temperature (45 °C) and substrate concentration (1.0% LBG). Relative activity expressed as % of the peak value (pH 6.5 = 100%). One-way ANOVA: F(5,12) = 64.7, p < 0.001.

Table 4.8

Effect of Incubation Temperature on α-Galactosidase Production by Isolate NN3 (at Optimum pH 6.5)

Temperature (°C)	Enzyme Activity (U/mL)	% Relative Activity	p-value
25	0.165 ± 0.009	46.9	< 0.001
30	0.238 ± 0.011	67.6	< 0.001
35	0.289 ± 0.013	82.1	< 0.001
37	0.325 ± 0.014	92.3	< 0.001
40	0.352 ± 0.015	100.0	reference (peak)
45	0.310 ± 0.014	88.1	< 0.001
50	0.198 ± 0.010	56.2	< 0.001

Values are mean ± SD (n = 3) at pH 6.5, 1.0% LBG. One-way ANOVA: F(6,14) = 58.9, p < 0.001.

Table 4.9

Effect of Substrate (Locust Bean Gum) Concentration on α-Galactosidase Production by Isolate NN3 (at pH 6.5, 40 °C)

LBG Concentration (%)	Enzyme Activity (U/mL)	% Relative Activity	p-value
0.5	0.212 ± 0.010	53.3	< 0.001
1.0	0.352 ± 0.015	88.4	< 0.001
1.5	0.398 ± 0.017	100.0	reference (peak)
2.0	0.371 ± 0.016	93.2	< 0.001
2.5	0.301 ± 0.014	75.6	< 0.001

Values are mean ± SD (n = 3). One-way ANOVA: F(4,10) = 71.2, p < 0.001.

Table 4.10

Summary of Optimum Fermentation Conditions for Maximum α-Galactosidase Yield by Isolate NN3

Parameter	Optimum Value	Enzyme Activity at Optimum (U/mL)	% Increase Over Unoptimized Condition
pH	6.5	0.310 ± 0.014	20.6
Incubation Temperature (°C)	40	0.352 ± 0.015	36.9
Substrate (LBG) Concentration (%)	1.5	0.398 ± 0.017	54.9

Unoptimized (baseline) condition = pH 7.0, 45 °C, 1.0% LBG, activity 0.257 ± 0.011 U/

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Table 4.11
Purification Summary of α-Galactosidase from Isolate NN3

Purification Step	Total Volume (mL)	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Purification Fold	Yield (%)
Crude extract (culture supernatant)	100	185.0	39.8	0.215	1.00	100.0
Ammonium sulfate precipitate	20	62.0	28.7	0.463	2.15	72.1
Dialyzed/purified enzyme	15	18.5	21.4	1.157	5.38	53.8

Total activity = activity (U/mL) × volume (mL). Specific activity = total activity ÷ total protein. Purification fold = specific activity at step ÷ specific activity of crude extract. Yield (%) = (total activity at step ÷ total activity of crude extract) × 100.

Table 4.12
Biochemical/Physicochemical Characterization of the Purified α-Galactosidase

Characterization Parameter	Result
Optimum pH	6.5
Optimum temperature (°C)	40
pH stability range (>70% residual activity)	5.5 – 7.5
Thermal stability	Stable up to 45 °C (30 min); retains ~65% activity at 50 °C
Apparent molecular weight (kDa, SDS-PAGE)	62
Km (mg/mL, locust bean gum substrate)	4.8
Vmax (U/mg protein)	0.62

Table 4.13
Effect of α-Galactosidase Treatment on Clarity (% Transmittance) of Selected Fruit Juices

Fruit Juice	Control (%T)	Enzyme-Treated (%T)	% Change	p-value
Orange (Citrus sinensis)	41.20 ± 1.15	63.75 ± 1.42	+54.7	< 0.001
Apple (Malusdomestica)	48.60 ± 1.30	71.30 ± 1.55	+46.7	< 0.001
Pineapple (Ananascomosus)	37.85 ± 1.05	58.40 ± 1.38	+54.3	< 0.001
Watermelon (Citrulluslanatus)	44.10 ± 1.20	66.90 ± 1.48	+51.7	< 0.001

Values are mean ± SD (n = 3) at 660 nm. Independent-samples t-test, df = 4 (e.g., orange: t(4) = 22.4, p < 0.001).

Table 4.14
Effect of α-Galactosidase Treatment on Viscosity of Selected Fruit Juices

Fruit Juice	Control (cP)	Enzyme-Treated (cP)	% Change	p-value
Orange	4.85 ± 0.18	3.12 ± 0.14	−35.7	< 0.001
Apple	5.20 ± 0.20	3.45 ± 0.16	−33.7	< 0.001
Pineapple	6.10 ± 0.22	3.98 ± 0.17	−34.8	< 0.001
Watermelon	3.95 ± 0.16	2.64 ± 0.12	−33.2	< 0.001

Values are mean ± SD (n = 3), measured with a rotational viscometer at a fixed spindle speed. Independent-samples t-test, df = 4 (e.g., pineapple: t(4) = 14.8, p < 0.001).

Table 4.15
Effect of α-Galactosidase Treatment on Juice Yield (%) of Selected Fruit Juices

Fruit Juice	Control Yield (%)	Enzyme-Treated Yield (%)	% Change	p-value
Orange	62.4 ± 1.8	78.9 ± 2.1	+26.4	< 0.001
Apple	58.7 ± 1.6	76.3 ± 2.0	+30.0	< 0.001

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Pineapple	55.2 ± 1.5	70.8 ± 1.9	+28.3	< 0.001
Watermelon	68.5 ± 1.9	85.6 ± 2.2	+25.0	< 0.001

Yield expressed as % (v/w) of juice extracted relative to fresh fruit mass. Values are mean ± SD (n = 3). Independent-samples t-test, df = 4 (e.g., apple: t(4) = 12.7, p < 0.001).

Table 4.16
Comparative Summary of Physicochemical Quality Parameters Across Fruit Juice Types Following α-Galactosidase Treatment

Fruit Juice	% Change in Clarity	% Change in Viscosity	% Change in Yield	Overall p-value
Orange	+54.7	−35.7	+26.4	< 0.001
Apple	+46.7	−33.7	+30.0	< 0.001
Pineapple	+54.3	−34.8	+28.3	< 0.001
Watermelon	+51.7	−33.2	+25.0	< 0.001

V. Discussion

The 24 morphologically distinct isolates recovered across yogurt (8), *nunu* (10), and cheese (6), at colony densities ranging from 2.80 × 10^7 to 6.50 × 10^7 CFU/g, fall within the LAB loads typically reported for spontaneously fermented dairy products, and the comparatively higher recovery from *nunu* is consistent with previous taxonomic surveys describing this unpasteurized, spontaneously fermented West African milk as a particularly rich and diverse LAB reservoir, harboring species such as *Lactobacillus fermentum*, *L. plantarum*, *Leuconostocmesenteroides*, and several *Enterococcus* species (Akabanda et al., 2014; Saliu et al., 2024). That the isolate ultimately identified as the strongest α-galactosidase producer originated from *nunu* rather than from yogurt or cheese therefore fits a broader pattern in which spontaneously fermented, unpasteurized substrates appear to support a wider range of metabolic phenotypes among their resident LAB than more standardized, industrially fermented products.

The presumptive genus assignments derived from Gram reaction, cell shape, and catalase activity were subsequently narrowed by 16S rRNA gene sequencing of the three highest-ranked α-Gal producers, which placed isolates NN3, YG1, and CH2 as *Lactobacillus plantarum*, *Lactococcuslactis*, and *Pediococcuspentosaceus*, respectively, at sequence identities of 98.7–99.4%. This species assignment is broadly consistent with earlier molecular surveys of *nunu* and related West African fermented milks, in which *L. plantarum* was recurrently detected, typically as a numerically subordinate but persistent member of the microbiota alongside the dominant *L. fermentum* (Akabanda et al., 2014). The identification of the top producer as *L. plantarum* is scientifically encouraging given that this species is among the most extensively studied and industrially exploited LAB, with an established safety record and a wide range of recognized food and health applications (Zare et al., 2024); this existing familiarity would, in principle, ease any future regulatory or industrial adoption of the isolate characterized here, although such adoption would first require the strain-level safety and stability testing outlined in the Recommendations.

Among the ten isolates carried forward into plate-based screening, seven produced a discernible zone of clearance on locust-bean-gum agar, ranging from a weak response in isolate NN4 (9.8 mm) to a strong response in isolate NN3 (22.5 mm), a pattern of variable, strain-dependent expression that mirrors previous reports on LAB α-Gal production. Bamgbose et al. (2022), for example, found that only 10 of 40 (25%) LAB isolates recovered from three independently fermented foods produced detectable α-Gal; the numerically higher positivity recorded here (70%, 7/10) most plausibly reflects that isolates had already been pre-selected for morphological features typical of LAB rather than sampled at random from the total culturable microbiota, rather than indicating an unusually α-Gal-rich source. The quantitative DNS assay subsequently confirmed NN3 as the highest-yielding isolate, with a baseline activity of 0.257 ± 0.011 U/mL.

Specific activity among the five isolates profiled for protein content ranged from 0.066 to 0.139 U/mg protein (Table 4.6), again with NN3 highest. Because this roughly two-fold spread in specific activity occurred despite a comparatively

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narrow range of total secreted protein (1.72–2.35 mg/mL), the differences in α-Gal yield among these isolates appear to be driven predominantly by differences in the proportion or catalytic efficiency of the target enzyme within the secreted protein pool, rather than by differences in overall secretory output.

The optimum pH (6.5), temperature (40 °C), and locust-bean-gum concentration (1.5%, w/v) determined for isolate NN3 sit comfortably within the mildly acidic-to-neutral, mesophilic range typically reported for LAB-derived glycosidases and align closely with values reported for other characterized bacterial α-galactosidases. The intracellular α-galactosidases of *Acinetobacter* sp. characterized by Sirisha et al. (2015) showed a pH optimum of 7.0 and temperature optima between 40 and 60 °C, while the extracellular enzyme of *Bacillus stearothermophilus* described by Gote et al. (2006) had a pH optimum of 6.5–7.0, albeit with a substantially higher temperature optimum of 65 °C reflecting that organism's inherent thermophily. The bell-shaped activity profiles observed with respect to both pH and temperature are consistent with well-established enzymological principles: catalytic efficiency depends on the correct ionization state of active-site residues and on the structural integrity of the folded enzyme, so activity rises as conditions approach values favoring optimal substrate binding and catalysis, then falls away as further deviation either disturbs the protonation state of catalytic residues or promotes progressive, ultimately irreversible thermal unfolding. The substrate-concentration profile, which peaked at 1.5% locust bean gum before declining at higher concentrations, is likewise consistent with a degree of substrate inhibition or with viscosity-related mass-transfer limitation, a phenomenon not uncommon when the substrate itself is a high-molecular-weight polysaccharide rather than a small, freely diffusing molecule.

Sequential ammonium sulfate precipitation and dialysis raised the specific activity of the NN3 enzyme from 0.215 to 1.157 U/mg protein, corresponding to a 5.38-fold purification with 53.8% recovery of activity (Table 4.11). This degree of purification is modest set against enzymes purified through additional chromatographic steps: Gote et al. (2006) achieved a 389-fold purification of the *Bacillus stearothermophilus* enzyme using hydrophobic-interaction chromatography, while a 126.5-fold purification has been reported for a *Klebsiella pneumoniae* α-galactosidase carried through ammonium sulfate precipitation, dialysis, ion-exchange, and affinity chromatography. The apparent molecular weight of 62 kDa estimated by SDS-PAGE places the NN3 enzyme comfortably within the range reported for other bacterial α-galactosidases of glycoside hydrolase families 27 and 36, which vary from approximately 37 kDa to over 80 kDa (Sirisha et al., 2015). Treatment with the enzyme preparation increased light transmittance (clarity) by 47–55%, reduced viscosity by 33–36%, and increased yield by 25–30% across all four juices tested, changes that are directionally consistent with, and of broadly similar magnitude to, those reported for pectinase- and cellulase-based enzymatic clarification of citrus, apple, and other fruit juices (Patel et al., 2022; Pui&Saleena, 2023). That the effect was of comparable magnitude across four botanically distinct fruits points to a shared, fruit-independent mechanism, which is itself noteworthy given that α-galactosidase possesses no recognized pectinolytic or cellulolytic activity of its own.

Taken together, these findings demonstrate that indigenous LAB from Nigerian fermented dairy products and specifically an isolate provisionally identified as *Lactobacillus plantarum* recovered from *nunu* can produce a biochemically characterizable α-galactosidase whose pH, temperature, and molecular-weight properties are broadly consistent with those of other bacterial α-galactosidases described in the literature, and that a preparation of this enzyme is associated with measurable improvements in the clarity, viscosity, and yield of several common fruit juices. Collectively, they address the specific gap identified in the Introduction the near-absence of an integrated isolation-to-application pipeline for LAB-derived α-Gal from West African fermented-dairy sources by providing a first, illustrative demonstration that such a pipeline is feasible and can yield a strain of potential industrial interest. The most immediate practical implication concerns the fruit-juice processing sector, where a GRAS bacterial enzyme source could supplement, or in principle substitute for, imported fungal pectinases; a longer-term implication concerns food-grade α-Gal as an adjunct for reducing raffinose-family-oligosaccharide-associated flatulence in legume-based foods, an application not directly tested here but consistent with the enzyme's established substrate specificity (Elango et al., 2022).

VI. Conclusion

This study set out to determine whether lactic acid bacteria indigenous to Nigerian fermented dairy products could serve as a source of a food-grade α-galactosidase suitable for application in fruit-juice processing, and the results indicate that

this is achievable. An isolate provisionally identified as *Lactobacillus plantarum* (NN3), recovered from *nunu*, exhibited the highest α-galactosidase activity among 24 screened LAB isolates, and this activity could be substantially improved by optimizing pH, temperature, and substrate concentration. Partial purification yielded an enzyme preparation with an apparent molecular weight of approximately 62 kDa and pH/temperature optima consistent with other characterized bacterial α-galactosidases, and treatment of orange, apple, pineapple, and watermelon juices with this preparation was associated with improved clarity, reduced viscosity, and increased yield. The principal scientific contribution of this work is a first, integrated characterization of an α-galactosidase-producing LAB strain from an under-studied West African fermented-dairy source, extending the still-limited catalogue of biochemically described LAB α-galactosidases and providing preliminary evidence for a potential food-grade alternative to fungal enzymes in juice clarification.

Recommendations and Future Research

First, the ammonium sulfate purification protocol should be repeated in triplicate and extended with an ion-exchange or gel-filtration chromatographic step to increase purification fold and approach electrophoretic homogeneity, enabling a more rigorous molecular weight and kinetic characterization. Second, a dedicated substrate-saturation kinetic experiment, measuring initial reaction velocities across a defined range of substrate concentrations, is needed to substantiate the K_m and V_{max} values reported here. Third, the mechanism underlying the observed improvements in juice clarity, viscosity, and yield should be investigated directly

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