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Research Article

Influence of Back Slopping on the Safety and Nutrition of African Almond Seeds Condiment (Ogiri)

Olubode T. P.[1,2], Fatoki O. A.[3], Akande O. O.[1], Adaramola K. A.[1], Adebisi T. T.[4] and Bada V. T.[4]

[1]Department of Science Laboratory Technology, Faculty of Applied Sciences, Oyo State College of Agriculture and Technology, Igboora, Nigeria
[2]Department of Biological Sciences, Lead City University, Ibadan, Nigeria
[3]Department of Biology of the Polytechnic Ibadan, Ibadan, Nigeria
[4]Department of Agriculture and Bioenvironmental Engineering, Faculty of Engineering, College of Agriculture and Technology, Igboora, Oyo State, Nigeria
***Corresponding Author's email*: tolu4real725@gmail.com**

ABSTRACT
Fermented condiments play a vital role in human diets and contribute significantly to nutritional intake. However, traditional fermentation methods often lack standardized safety controls. This study developed a novel condiment from African almond seeds (ASC) and evaluated its safety and nutritional quality using a back-slopping fermentation approach. The condiment was produced through spontaneous fermentation, while aflatoxins, fumonisins, and biogenic amines were analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). Repeated back-slopping was employed to obtain 10th and 20th-generation cultures. Metabolites from the 10th culture were assessed, and bacterial isolates from the 20th culture were screened for safety before use. Results showed that back-slopping significantly enhanced product safety and quality through significant reduction of the initial high levels of mycotoxins-AFB1 (9.98 mg/kg), AFB2 (29.46 mg/kg), FB1 (24.46 mg/kg), and FB2 (37.42 mg/kg) to 0.34 µg/kg, 0.73 µg/kg, 0.51 µg/kg, and 0.07 µg/kg, respectively (ANOVA; $P<0.05$), while AFG1, AFG2, and FB3 became undetectable, biogenic amines, including cadaverine, tyramine, and spermidine, were reduced to negligible levels. Five bacteria were isolated from the 10th mother culture, two of which were *Bacillus subtilis* of different strains, while the remaining were *Lysinibacillus capsici*, *L. macroides* and *Enterococcus faecium*. Selected non-pathogenic strains used as starters (*Bacillus subtilis* and *Lysinibacillus capsici*) enhanced protein (31.67-33.47%) and carbohydrate (24.05–29.86%) contents compared to spontaneous fermentation (25.73% and 19.76%). These findings demonstrate that controlled fermentation improves safety and nutritional quality, supporting almond seeds as a viable substrate for condiment production.

Keywords: Back-slopping; Concentrations; Culture; Fermentation; Isolates

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INTRODUCTION

Fermented seed-based condiments constitute an important component of West African food systems, serving both as flavour enhancers and as sources of dietary protein and micronutrients. Well-known examples include *iru*, *dawadawa*, *ogiri*, and *ugba*, which are traditionally produced through the natural fermentation of legumes and oil seeds such as African locust bean (*Parkia biglobosa*), melon seed (*Citrullus colocynthis*), and African oil bean (*Pentaclethra macrophylla*). These products are commonly prepared at household or artisanal scales and contribute significantly to improving the sensory attributes and nutritional value of staple foods

(Obafemi *et al.,* 2022; Omotayo *et al.,* 2024; Golly *et al.,* 2025).

In West Africa, seed fermentation largely occurs through spontaneous processes driven by indigenous microorganisms that mediate complex biochemical transformations. During fermentation, proteins are hydrolysed, lipids are modified, anti-nutritional compounds are reduced, and characteristic flavours and aromas develop (Asiedu *et al.*, 2026). Despite these benefits, spontaneous fermentation often results in substantial variability in product quality and may pose challenges related to nutritional consistency and microbiological safety. These limitations are largely attributed to the absence of standardised starter cultures and controlled fermentation conditions (Senanayake *et al.,* 2023; Obafemi *et al.,* 2024; Cirunaya *et al.,* 2025).

African almond (*Terminalia catappa* L.) seeds remain under-utilised in West Africa despite their favourable nutritional composition, which includes appreciable amounts of proteins, unsaturated fatty acids, dietary fibre, and essential minerals (Iyekowa *et al.,* 2023; Ramanan *et al.,* 2024; Zannou *et al.,* 2026). Although the seeds are commonly consumed in roasted or raw forms, their potential application as substrates for fermented condiments has received limited attention. Considering the success of fermentation in improving the nutritional and sensory properties of other traditional seed condiments, African almond seeds represent a promising raw material for developing novel fermented food products with potential functional and nutritional benefits (Agunwah *et al.*, 2024; Okoth *et al.,* 2025).

Back-slopping is a traditional fermentation practice widely employed in West African food processing, whereby a portion of a previously fermented batch is introduced into a fresh substrate to initiate fermentation. This technique introduces a microbial consortium that is already adapted to the fermentation environment, thereby enhancing fermentation efficiency, stabilising microbial succession, and inhibiting the growth of spoilage and pathogenic microorganisms (Liao *et al*., 2024; Yee *et al.,* 2025). Previous studies on fermented foods have shown that back-slopping can improve protein digestibility, enhance nutrient bioavailability, and contribute to product safety through microbial competition and metabolic inhibition of undesirable microorganisms (Obafemi *et al.,* 2022; Bouakkaz *et al.,* 2024; Santa *et al.,* 2025; Dhiman *et al.,* 2025).

Despite extensive documentation of back-slopping in established West African fermented condiments, its application in the fermentation of emerging substrates such as African almond seeds have not been adequately investigated. Examining this technique in the context of African almond seed fermentation is therefore important for understanding its potential effects on nutritional quality and microbiological safety, particularly when adapting traditional fermentation practices to novel food materials (Uchenna *et al.,* 2023; Agunwah *et al.*, 2024; Ladeji & Ogidi, 2025).

Therefore, this study evaluates the influence of back-slopping on the nutritional composition and safety of a novel fermented condiment produced from African almond seeds within the context of West African fermentation practices. The findings are expected to provide scientific insights for the development of new fermented condiments, promote the sustainable utilization of underexploited plant resources, and contribute to improving traditional fermentation technologies in the region.

MATERIALS AND METHODS

Collection of the raw materials

The hard shells of African almond seeds were collected from Igboora, Ibarapa Central Local Government where it was cultivated into a dried and sterile nylon. These were transported into the laboratory where it was sorted to remove those that been contaminated by moulds as well as insects damage. The shells were manually broken to release the seeds which were washed with sterile water and air dried. The banana leaves used during fermentation and production of the condiment (ogiri) were obtained from the same location and were thoroughly cleaned before use. Five grams (500 g) of the seeds were weighed, wrapped in banana leaf and boiled for two hours in order to soften the seeds. The seed coats were removed after boiling, mashed in mortar with pestle, so that the pastes thus obtained were wrapped in another clean banana leaf and left to ferment at 28^0C for 5 days (Voidarou *et al.,* 2020). The condiment thus formed was used as starter in the next preparation by adding 2 g of starter to newly prepare and pasteurized almond pastes in a cleaned banana leaf. The mixture was fermented for 5 days at room temperature. The procedure was repeated (back-slopping) until the 10th and 20th mother culture were obtained.

GC-MS Analysis of Mycotoxins in the 10th mother culture of Almond Condiment

Chemical Standard

Aflatoxins (AFs) were obtained from Makor Chemical Co. (Lagos, Nigeria), citrinin (CA) and kojic acid (KA) from Sigma Chem. Co. (MO, USA), and cholestanol

(internal standard) from Tokyo Kasei Co. (Tokyo, Japan). All solvents and chemicals used were reagent grade or higher. AFs and CA were dissolved in benzene-acetonitrile (98:2), while KA was dissolved in methanol. The mycotoxins were mixed and diluted before use. Samples were ground through a 16-mesh sieve and stored in double plastic bags at –18°C until analysis

Extraction and Clean-up

A 25 g of the 10th mother culture ground sample was extracted with 100 ml acetonitrile-water (84:16 v/v) for 5 min using a high-speed blender. An 8 ml aliquot of the extract was purified by passing it through a Mycosep 227 column. The cleaned extract was collected and 4 ml was transferred to a vial and evaporated to dryness under a stream of nitrogen at 60^{o}C (Tamang and Jyoti, 2020).

Derivatization Procedure for Mycotoxins Analysis

Derivatization was performed after adding α-chloralose (internal standard) to the extract, followed by evaporation. The residue was dissolved in 1 ml of methanol, sonicated for 1 minute, and evaporated at 60°C under nitrogen. To the dried residue, 200 µl of TFAA and 10–30 mg of sodium bicarbonate (NaHCO3) was added, and the vial was sealed with a Teflon-lined screw-cap and heated at 80°C for 30 minutes. After cooling, the mixture was evaporated to dryness under nitrogen. The residue was re-dissolved in 100 µl of toluene, sonicated for 1 minute, and then manually mixed. To remove excess reagents, 500 µl of water was added and mixed gently. The layers were allowed to separate, and the toluene phase was transferred to a cleaned 4 ml vial. Anhydrous Na2SO4 was added, mixed briefly, and the toluene layer was transferred to a 600 µl vial with a Teflon-faced septum. A 1 µl sample was injected into the GC-MS (Marco and Maria, 2020; Amumudu *et al.,* 2024).

GC/MS Conditions

Target compound concentrations were determined using a Varian 3800/4000 gas chromatograph-mass spectrometer with an Agilent Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, 100% Dimethylpolysiloxane). The system operated in electron impact mode at 70 eV, with a scan interval of 0.5 s and fragment range of 40-800 Da. The injection volume was 1 µl, and the conditions included: injection port at 260°C with a split less injection for 1 minute, oven temperature starting at 80°C for 0.2 minutes, then increasing at 22°C/min to 205°C, holding for 0.25 minutes, followed by a 2°C/min increase to 270°C. Nitrogen flow was set at 1.0 mL/min. The transfer line and ion source temperatures were 275°C and 200°C, respectively. Data were collected using Chem Station software, with full scan data and multiple ion chromatograms built for quantification based on characteristic ions: m/z 81, 109, 223, 251, and 319 for α-chloralose; m/z 117, 145, 231, 259, and 584 for DON; m/z 229, 257, 359, 371, and 696 for NIV; m/z 91, 124, 229, 329, and 402 for DAS; m/z 121, 138, 180, 341, and 455 for HT2; and m/z 121, 138, 180, 227, 327, and 401 for T2. Compound identity was confirmed by retention time, mass spectra, and characteristic fragment ratios. For sample preparation, 1.0 g of the sample was defatted using a chloroform/methanol mixture (2:1) and extracted for 1 hour in a Soxhlet extractor. The Calibration curves were obtained by plotting peak area versus concentration of mycotoxin standards. Linearity was evaluated using R^2. LOD and LOQ were defined at signal-to-noise ratios of 3:1 and 10:1, respectively.

Isolation of Bacteria and selection of starter for improvement of the Condiment

The 10th and 20th mother culture of the almond seed's condiment was subjected to isolation by weighing 1 g of the condiment into 9 ml of sterile distilled water in a tube. The mixture was shaken and serial dilution was carried on it through pipetting of 1 ml of the homogenate in to 9 ml of sterile distilled water (diluents) to obtain first serial dilution (10^{-1}). The procedure was repeated by pipetting 1 ml of the solution of the first serial dilution into the second tube containing sterile diluents until serial dilution of 10^{-5} was obtained. Sterile molten deManRogosa Sharpe Agar (MRSA) and Nutrient Agar (NA) were prepared and poured on plates and 0.1 ml each of the last sample's dilution (10^{-5}) were inoculated on the plates of each agar in duplicates. Inoculated MRSA plates were incubated at 30^{0}C for 48 h while NA plates were incubated at the same temperature for 24 h. Pure isolates were obtained via sub-culturing and stored on agar slants for use.

Extraction of DNA from bacterial cells

Two milliliters (2 ml) of bacterial cell broth and 750 µl of lytic solution were added to a ZR BashingTM Lysis Tube, secured in a bead mill assembly, and processed at maximum speed for over 5 minutes. The mixture was centrifuged at 10,000 x g for 1 minute, and 400 µl of the supernatant was transferred to a Zymo-SpinTM IV spin filter (orange top) in a collection tube. The filter was centrifuged at 7,000 x g for 1 minute. 1,200 µl of DNA Binding Buffer was added to the filtrate, and 800 µl was transferred to a Zymo-SpinTM IIC column. The column was centrifuged at 10,000 x g for 1 minute, and the flow-through discarded. The process was repeated, followed by adding 200 µl of

DNA pre-wash buffer to the column. The column was transferred to a clean 1.5 ml micro-centrifuge tube, and 100 µl (min. 35 µl) of DNA elution buffer was added. Centrifugation at 10,000 x g for 30 seconds eluted the DNA (Wright *et al.,* 2017).

Purification of DNA

One gram (1 g) of agarose for DNA and 2 g for PCR were each mixed with 100 ml of 1xTAE in a microwavable flask. The mixture was microwaved for 1-3 minutes until fully dissolved, then cooled to ~50°C for 5 minutes. 10µl of EZ Vision DNA stain was added for DNA visualization under UV light. The agarose solution was poured into a gel tray with a comb in place and left to solidify (IIamperuma, 2020). The loading buffer was added to the DNA samples, and the agarose gel was placed in the electrophoresis unit, covered with 1xTAE (or TBE). A molecular weight ladder was loaded into the first lane, followed by the DNA samples into the remaining wells. The gel was run at 80-150 V for 1-1.5 hours, and removed after turning off the power and disconnecting the electrodes. DNA fragments or PCR products were visualized under a UV transilluminator (Sowersby and Lewis, 2024).

Amplification of Genes

The Polymerase Chain Reaction mix contained 12.5 µl of Taq 2X Master Mix (New England Biolabs, M0270), 1 µl each of 10 µM forward (27F: AGAGTTTGATCMTGGCTCAG) and reverse (1525R: AAGGAGGTGWTCCARCCGCA) primers, 2 µl of DNA template, and 8.5 µl of nuclease-free water. The DNA was denatured at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 45 seconds. A final elongation step was performed at 72°C for 7 minutes, and the temperature was held at 10°C indefinitely (Khehra *et al.,* 2025).

Sequencing of the Bacterial DNA

The 16SrRNA amplicons of the bacteria from the 10th and 20th mother cultures were commercially sequenced and the gene sequence thus obtained were copied into local alignment search tool (BLAST) algorithm on National Centre for Biotechnology Information (NCBI) GenBank so that the closely related isolates after gene sequence submission with the allotted accession numbers were recorded.

Safety Test on the Isolates

Haemolytic test of the Isolates

The bacterial isolates were inoculated on to sterile blood agar plates prepared by weighing 28 g into 1000 ml of distilled water in a conical flask and autoclaving it at 121^{o}C for 15 min, cooled to 45^{o}C and sterile 5% v/v defibrinated blood warmed to 25^{0}C was added. The catalase negative bacterial cells from the MRSA and catalase positive cells from NA were inoculated on the blood agar and incubated at 30^{o}C for 48 h and at the same temperature for 24 h respectively. The plates were examined for haemolysis and plates without lysis of blood cells were selected as safe and are subjected to other phenotypic virulent tests (Mushtaq and Ayesha, 2022).

Production of DNase by Isolates

Sterile DNase agar plates were prepared and the bacterial isolates which were not haemolytic were tested for the ability to produce DNase by inoculating them on the agar. Plates were incubated at 37^{o}C for 24 h. After 24 h methyl green dye was added to each plate and plates without clear zone around the colonies which indicated that they lack the ability to produce DNA were selected as safe as starter and were stored on agar slant for further analysis (Singh *et al*., 2022)

Production of Gelatinase by the Isolates

A modified method of Balta and Mehmet-Fikret (2022) was used. Gelatin agar was prepared in 250 ml of distilled water by adding; 1 g of peptone, 0.25 g of yeast extract, 3.75 g of gelatin and 3.75 g of agar-agar and were gently mixed together with application of gentle heat to obtain homogenous mixture. This was sterilized at 121^{o}C for 15 min and cooled to molten condition, before pouring on sterile plates. Plates were allowed to solidify. The bacterial isolates were inoculated each on the solidified gelatin agar medium, well labelled and in duplicates. The inoculated plates were incubated at 30^{o}C for 48 h. After incubation the inoculated medium was observed for liquefaction by flooding it with ammonium sulphate. A clear zone around the culture on plate was taken as positive for production of gelatinase by the isolates while no zone was taken as negative.

Production of Protease by the Isolates

Sachet sterile skimmed milk of 250 g was purchased from a supermarket and 2.5% (w/v) of it was prepared aseptically by weighing 2.5 g in to sterile distilled water. 1.0% (w/v) peptone, 1.5% (w/v) NaCl and 1.5% agar-agar were prepared, mixed together and sterilized at 121^{o}C for 15 min. The mixture was cooled, added to the skimmed milk, gently mixed together and poured on plates to solidify. The plates were inoculated with the isolates and incubated at 30^{o}C for 24 h. Zone of clearance around the colonies on plates was taken as positive for protease production by the isolate while absence of zone of

clearance around the colonies was recorded as negative and no protease production (Balta and Mehmet-Fikret, 2022).

Production of Controlled and Spontaneously Fermented Almond Seeds Condiment

One hundred grams (100 g) of almond seeds were weighed, washed with sterile distilled water, and wrapped in sterile banana leaves. The seeds were boiled for 3 hours to soften their coats, then crushed into a paste in a sterile mortar and divided into four portions, each wrapped in a sterile banana leaf. The first portion was left to ferment at 28^0C for five days (Voidarou *et al.,* 2020) while the other three were frozen for 48 hours to eliminate any remaining microbes. Isolates ASC1 and ASC2 were sub-cultured on sterile nutrient agar, incubated at 30°C for 24 hours, and serially diluted to 10^{-7}. A 0.1 mL aliquot from the final dilution was plated in duplicate using the spread plate method. Plates were incubated at 30°C for 24 hours, and colonies were counted to achieve a concentration of $3x10^8$ CFU/g for each isolate. The frozen almond pastes were inoculated with 1 ml of the $3x10^8$ CFU/g of each isolate (ASC1, ASC2, and ASC1+ASC2) after thawing. Inoculated pastes were incubated at 28^0C for five days.

Proximate Compositions of Controlled and Spontaneously Fermented Almond Seeds Condiments

Determination of Moisture Content

One gram (1 g) of each sample was placed in an oven and dried at a constant temperature of 105^0C until it reached a constant weight for 12 to 24 h. The final weight was recorded and the moisture content was calculated based on the weight loss as thus; % Moisture content = (w1-w2/w1 x100) (Masihuzzaman *et al*., 2020).

Determination of Ash Content

The dried samples (1 g) in dry and clean crucible from the moisture test were incinerated in a muffle furnace at a high temperature of 600^0C for 4 h, cooled to 300^0C in the muffle furnace and transferred in to desiccators. The residues after all the organic matter have burned off, was taken as the ash content which was a measure of inorganic minerals (Balta and Mehmet-Fikret, 2022) calculated thus;

$$\% \text{ Ash content} = W2 - \frac{W1X100}{W1}$$

Determination of Crude Fibre

The fibre contents were determined by counting the numbers of dried lipid-free residues that were unable to ignite after being digested for 4 h with 200 ml of each 1.25 N H_2SO_4 and 1.25 N NaOH before rinsing at 600^0C while the crude was calculated for each sample as (Esfahlan *et al*., 2021).:

$$\% \text{ Crude fibre} = W2 - \frac{W1X100}{W1}$$

Determination of Crude Protein

Crude protein was determined using the micro Kjeldahl method. One gram of sample was dissolved in 10 ml of concentrated H_2SO_4 in a heating tube, with 1 selenium tablet as a catalyst. The mixture was heated in a fume cupboard, then transferred to a 100 ml flask and diluted with distilled water. Ten ml of the mixture was combined with an equal volume of 45% NaOH and transferred to the Kjeldahl apparatus. The distillate was mixed with 4% H_3BO_3, and three drops of methyl red were added as an indicator, then titrated (50 ml distillate). The procedure was repeated three times, and the average value was calculated. Nitrogen content was determined and multiplied by 6.25 to obtain crude protein (Alasalvar *et al*., 2020).

$$CP\ (\%) = \frac{(T-B)*N*14*100*6.25)}{W_S*100}$$

Determination of Crude Fat

One gram (1 g) of each sample was wrapped in filter paper and placed in a thimble fixed to a cleaned, dried, and weighed round-bottom flask. The flask was filled with 120 ml of petroleum ether, boiled using a heating mantle, and refluxed for 5 hours. Afterward, the heat was turned off, and the thimbles with the extracted samples were left to cool before weighing. The weight difference was used to determine the fat content, and the percentage of crude fat was calculated (Alasalvar *et al*., 2020) thus;

$$\%\text{Crude fat} = \frac{W2 - W1X100}{W1}$$

Determination of Carbohydrate Content

The percentages of carbohydrate contents were determined by nitrogen-free approach. It was calculated by subtracting the total sum of other proximate contents using the formula (Alasalvar *et al*., 2020). %Carbohydrate= 100-(%Ash+%Moisture+%Fats+%+%Fibres+%Protein).

Data Analysis

The results were analysed with Statistical Packages for Social Sciences (SPSS) version 20.0. Analysis of Variance (ANOVA) was used to compare the mean differences among the groups with significant effects observed while post hoc test of comparisons was performed using Duncan's test

RESULTS

Back slopping during fermentation was associated with substantial reductions in mycotoxin concentrations in almond seed condiments. Fumonisin B1 declined from 5.86 mg/kg in ASC to 0.51 µg//kg in ASC1. Aflatoxin B1 decreased from 3.64 mg/kg to 0.34 / µg kg, while fumonisin B2 reduced from 3.63 µg/kg to 0.07 µg /kg. Aflatoxins G1, G2, and B3, which were detected in ASC, were not detected in ASC1. Similarly, aflatoxin B2 decreased from 4.83 mg/kg to 0.73 µg/kg.

In the assessment of biogenic amines, six compounds were identified, with cadaverine exhibiting the highest concentration (6.64 mg/kg), followed by tyramine (5.02 mg/kg). Putrescine and histamine occurred at comparable levels, while spermidine showed the lowest concentration. Following back slopping, pronounced reductions were observed across all biogenic amines. Cadaverine declined to 0.00138 mg/kg, tyramine to 0.00229 mg/kg, histamine to 0.00121 mg/kg, and spermidine to 0.00039 mg/kg. The 10th mother culture was characterized by markedly low concentrations of all quantified biogenic amines.

Five bacterial isolates (ASC1–ASC5) obtained from the 10th mother culture were identified as *Bacillus subtilis, Lysinibacillus capsici*, *Enterococcus faecium, Bacillus subtilis* subsp. *subtilis*, and *Lysinibacillus macroides*, each showing 100% sequence similarity with reference strains. These isolates are implicated in the reduction of mycotoxins and biogenic amines in the almond seed culture.

Phenotypic characterization of isolates from the 20th mother culture indicated that ASC1 and ASC2 were non-haemolytic, DNase-negative, and lacked gelatinase activity, whereas ASC3 exhibited haemolytic activity, DNase production, and gelatin hydrolysis. All isolates demonstrated proteolytic activity.

Proximate analysis showed that controlled fermentation enhanced nutritional composition. Protein content was higher in ASC1 (31.67%) and ASC1+ASC2 (33.47%) compared to spontaneously fermented samples (25.73%). Carbohydrate content was highest in ASC2 (29.86%), while fat content remained relatively constant across samples (31.55–31.98%). Crude fibre and ash contents were higher in spontaneously fermented samples, whereas moisture content was also greatest in these samples. Controlled fermentations (ASC1 and ASC1+ASC2) exhibited lower moisture levels.

Molecular identification based on 16S rRNA gene sequencing confirmed ASC1 as *Bacillus subtilis* and ASC2 as *Lysinibacillus capsici,* both showing 100% similarity with NCBI reference strains.

Table1: Mycotoxins Contents of African Almond Condiments and Almond 10th Mother Culture

Mycotoxins	RT	Peak area (%)	ASC (mg/kg)	RT	Peak Area (%)	ASC_1 (µg/kg)
Aflatoxin B_1	9.98	7.78	3.64^c	9.98	2.20	0.34
B_2	29.46	36.47	4.83^b	29.46	31.65	0.73
G_1	15.24	4.50	1.21^e	15.24	8.61	0.00
G_2	19.25	9.40	2.28^d	7.70	4.83	0.00
Fumonisin B_1	24.46	22.70	5.86^a	24.46	5.60	0.51
B_2	37.42	10.82	3.63^c	37.42	3.55	0.07
B_3	39.23	2.98	1.02^f	19.25	6.87	0.00

Mean values within the columns are significantly different ($p<0.05$)

Key: ASC; Almond seeds condiment, ASC1; Almond seeds condiment 10th mother culture RT; Retention time

Table 2: Biogenic Amines Content of African Almond and the 10th Mother culture

Isolates codes	Gene bank isolates	Percentage similarities (%)	Accession number
ASC1	*Bacillus subtilis* strain HBUAS69294	100	OP420653.1
ASC2	*Lysinibacillus capsici* strain TSLBM	100	CP122283.1
ASC3	*Enterococcus faecium* strain HBUAS62676	100	ON013218.1
ASC4	Mutant *Bacillus subtilis* sub sp. Subtilis strain MGP060	100	CP116830.1
ASC5	*Lysinibacillus macroides* strain TE1	100	OP558974.1

Mean values within the columns significantly different ($p<0.05$)

Key: BAASC; Biogenic Amines of Almond seeds condiment, RT; Retention time, TMS; Tri-methyl silyl BAASC1

Table 3: Identity of bacterial isolates from 10th mother Culture Almond Seeds Condiment

Isolates codes	Gene bank isolates	Percentage similarities (%)	Accession number
ASC1	*Bacillus subtilis* strain NOK 44	100	ON287077.1
ASC2	*Lysinibacillus capsici* strain NEB 659	100	CP154860.1

Key: ASC-Almond seeds condiment

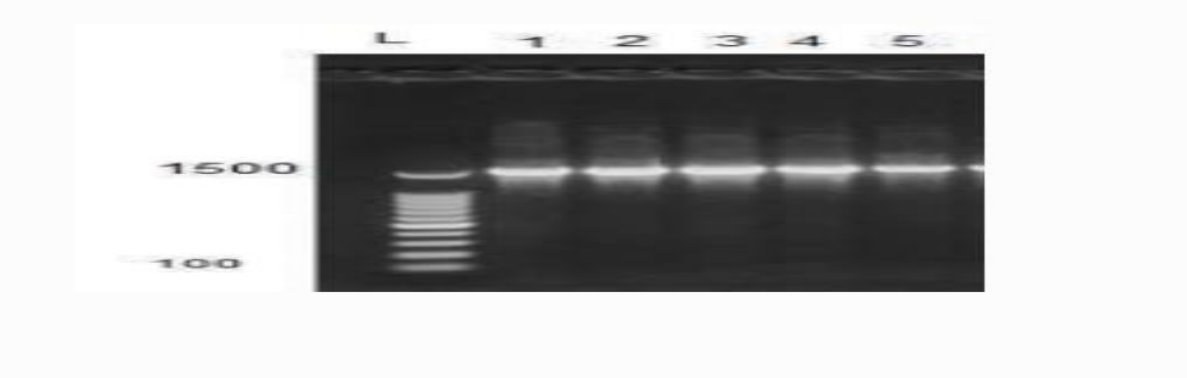

Fig 1: Gel image of Amplification of 16SrRNA Gene

Agarose gel electrophoresis of 16SrRNA (1500bp) gene of bacterial isolates from the 10th mother culture of almond seeds condiment, L is the molecular ladder; 100bp DNA. Lane 1; Isolate ASC1, Lane2; ASC2, Lane 3; ASC3, Lane 4; ASC4 and Lane 5; ASC5

Table 4: Phenotypic Pathogenicity Test of Almonds Seeds Condiment 20th mother culture Isolates

Isolates codes	Haemolysis	DNase	Gelatin	Proteolysis
ASC1	No haemolysis	Negative	Negative	Positive
ASC2	No heamolysis	Negative	Negative	Positive
ASC3	Haemolysis	Positive	Positive	Positive

Key: ASC: Almond seeds condiment

Table 5: Proximate Composition of Controlled and Spontaneously Fermented Almond Seeds Condiment (%)

Proximate parameters	Controlled -fermented almond seeds condiment ASC1	ASC2	ASC1+ASC2	Spontaneously fermented almond seeds condiment
Protein	31.67^{a}	25.86^{b}	33.47^{a}	25.73^{b}
Carbohydrate	23.68^{b}	29.86^{a}	24.05^{b}	19.76^{c}
Crude Fat	31.68^{a}	31.96^{a}	31.55^{a}	31.98^{a}
Crude fibre	1.10^{d}	1.93^{b}	1.24^{c}	2.85^{a}
Ash	4.70^{ab}	3.53^{c}	4.20^{bc}	5.03^{a}
Moisture	6.20^{b}	6.88^{bc}	5.51^{c}	14.65^{a}

Key: ASC-Almond seeds condiment

Table 6: Identity of the Bacterial Isolates from 20th ASC Mother Culture

Isolates codes	Gene bank isolates	Percentage similarities (%)	Accession number
ASC1	*Bacillus subtilis* strain NOK 44	100	ON287077.1
ASC2	*Lysinibacillus capsici* strain NEB 659	100	CP154860.1

Key: ASC-Almond seeds condiment

DISCUSSION

The present study demonstrates that back-slopping fermentation significantly reduces mycotoxin concentrations and biogenic amines (BAs) in spontaneously fermented almond seed condiments (ASC). These findings corroborate earlier reports describing fermentation as an effective strategy for mitigating toxic metabolites in fermented foods. Previous studies have shown that controlled fermentation techniques, including inoculation and

back-slopping, can substantially reduce mycotoxin levels in soybean paste, nuts, and cereal-based products (Ademola *et al.,* 2021; Woo *et al*., 2025). The observed reductions in fumonisin B1, aflatoxins, and fumonisin B2 in ASC1 are consistent with evidence that microbial activity during fermentation suppresses fungal growth and promotes enzymatic degradation of mycotoxins (Ndiaye *et al.,* 2022; Nahle *et al.,* 2022; Guan *et al*., 2023; Sun *et al.*, 2023). The absence of certain aflatoxins in ASC1 indicates a strong detoxification effect, which is particularly relevant considering the health risks associated with these toxins, including hepatocellular carcinoma and immunosuppression (Balan *et al.*, 2024; Gamede *et al.,* 2025; Kipkoech *et al.,* 2025).

Regulatory limits for mycotoxins vary across regions. For example, the European Union permits a maximum of 2 µg/kg aflatoxin B1 and 4 µg/kg total aflatoxins in foods (Kayola *et al.*, 2024). In contrast, the Ethiopian Standards Agency specifies a maximum of 10 µg/kg total aflatoxins in maize. The concentrations of most aflatoxins detected in the condiments exceeded these limits, with the exception of aflatoxin G1 in ASC, which remained below 2 µg/kg. Although this threshold primarily applies to aflatoxin B1, aflatoxin G1 is considered less toxic than AFB1 (Benkerroum *et al.,* 2020).

Fermentation also resulted in a marked reduction in BAs, particularly cadaverine, tyramine, and histamine, consistent with previous studies demonstrating the capacity of microbial fermentation to reduce harmful amines in fermented foods (Banicod *et al.,* 2025; Costa-Catala *et al.,* 2026). Although no established acceptable daily intake exists for cadaverine, tolerable concentrations ranging from 430 mg/kg in sauerkraut to 1540 mg/kg in seasonings have been proposed (Turna *et al.,* 2024). The values recorded in this study fall below these suggested limits, indicating that the condiments are within acceptable safety margins. Elevated cadaverine levels, particularly in combination with other BAs, often indicate food spoilage and may contribute to undesirable flavour or potential toxic effects, especially when interacting with nitrates or in sensitive individuals (Tsafack & Tsopmo, 2022; Turna *et al.,* 2024). Because BAs are associated with food-borne intoxication, their reduction during fermentation enhances both safety and nutritional quality of fermented products (Xie *et al.,* 2015; Hernández-Macías *et al.*, 2022; Świder *et al.*, 2024). The significant decrease in cadaverine and histamine in the 10th mother culture (ASC1) therefore suggests improved consumer safety, particularly for individuals sensitive to these compounds. Nevertheless, histamine concentrations in both condiments remained above typical regulatory limits of 200–400 mg/kg, while doses exceeding 500 mg/kg have been associated with acute intoxication. Such levels may pose risks to individuals with histamine intolerance resulting from reduced capacity for histamine degradation (DeBeer *et al.,* 2021). No significant difference was observed between histamine and putrescine concentrations in ASC.

The identification of five bacterial isolates (ASC1, ASC2, ASC3, ASC4, and ASC5) from the 10th mother culture further highlights the role of microbial communities in improving fermented food safety. The isolates—*Bacillus subtilis*, *Lysinibacillus capsici*, *Enterococcus faecium*, *Bacillus subtilis* subsp. *subtilis*, and *Lysinibacillus macrolides* represent taxa commonly associated with fermentation processes. The substantial reduction in mycotoxins and BAs observed in the 10th mother culture likely resulted from the synergistic metabolic activities of these microorganisms, enriched through serial propagation during back-slopping. This approach has been widely reported to enhance the performance of beneficial microorganisms as biological control agents (Achi *et al.,* 2019; Alireza *et al.,* 2024; Liao *et al.,* 2024). In particular, *Bacillus subtilis* has been previously associated with degradation of mycotoxins and biogenic amines in various food matrices (Eom *et al.,* 2015; Nguyen *et al.,* 2024), supporting its probable contribution to detoxification in ASC.

Evaluation of virulence factors revealed that three isolates (ASC1, ASC2, and ASC3) obtained from the 20th mother culture differed markedly in safety characteristics. ASC1 and ASC2 were considered suitable starter cultures because they did not produce haemolysin or DNase, both of which are associated with pathogenicity (Sharma *et al.,* 2019; Yasmin *et al.,* 2020; Piame *et al.*, 2022; Perez *et al.,* 2023). Their inability to produce gelatinase further suggests a reduced likelihood of spoilage or development of undesirable flavours (Chukwuma *et al.*, 2025). Although both strains produced protease, this activity likely reflects metabolic adaptation for nutrient acquisition rather than virulence. In contrast, ASC3 produced haemolysin, DNase, gelatinase, and protease, indicating high virulence potential and making it unsuitable for use as a fermentation starter due to possible contamination risks (Kieliszek *et al.,* 2021; Chen *et al.*, 2021; Erken, 2022).

The nutritional findings of this study also align with previous research on starter-culture fermentation of traditional condiments. Controlled fermentation

using defined starter cultures has been shown to enhance protein content in fermented foods (Teniola *et al.,* 2023; Knez *et al.*, 2023; Madilo *et al.,* 2025; Asefa *et al.,* 2025). Similarly, ASC1 and ASC1 + ASC2 exhibited higher protein levels compared with spontaneously fermented ASC, suggesting that selected microbial cultures may improve protein bioavailability. Studies have also demonstrated that starter cultures influence carbohydrate profiles through enzymatic modification of polysaccharides (Wang *et al.*, 2021; Kulathunga *et al.,* 2023). The higher carbohydrate content observed in ASC2 relative to the spontaneously fermented variant supports this observation and may indicate enhanced production of fermentable sugars by specific microbial strains (Sawant *et al.,* 2025). Fat content remained relatively stable across samples, consistent with previous reports indicating minimal lipid variation during fermentation (Wang *et al.,* 2025). Conversely, the higher crude fibre and ash contents recorded in spontaneously fermented samples align with studies showing that natural fermentation can favour fibre and mineral retention (N'zi *et al.,* 2021; Mustafa *et al.,* 2022; Islam et al., 2024). These findings suggest that while controlled fermentation optimizes certain macronutrients, spontaneous fermentation may retain higher fibre and mineral levels. In addition, spontaneously fermented ASC showed significantly greater moisture content than controlled variants, consistent with observations in other fermented condiments where natural fermentation enhances water retention and influences texture and shelf stability (Chen *et al*., 2018; Hernández-Figueroa *et al.,* 2023).

The starter cultures used for controlled fermentation were prepared from ASC1 and ASC2 and applied to almond seed condiments that had been frozen for 24 h to reduce microbial load prior to fermentation. Molecular identification confirmed these strains as *Bacillus subtilis* and *Lysinibacillus capsici*. *Bacillus subtilis* has been widely reported as a dominant fermentative organism in legume-based condiments, including African locust bean products, where it contributes to protein hydrolysis and improved nutritional quality (Adesanya *et al*., 2021; N'zi *et al.,* 2024). Similar increases in protein content have been documented during soybean fermentation with *B. subtilis* starter cultures. The enhanced crude protein content observed in *B. subtilis*-fermented ASC in the present study therefore supports previous findings, including reports that fermentation of black and red lima bean (*Phaseolus lunatus*) with *B. subtilis* ATCC 6051 increased protein levels while reducing carbohydrates (Onwusu-Kateng *et al.*, 2022). However, some studies have reported contrasting outcomes, such as decreased protein or increased crude fibre during *B. subtilis* fermentation of locust beans (Liu *et al.,* 2024; Onwusu-Kateng *et al.*, 2022), highlighting the influence of substrate type and fermentation conditions.

The higher crude fibre and carbohydrate contents observed in *Lysinibacillus capsici*-fermented samples compared with those fermented by *Bacillus subtilis* may reflect differences in metabolic pathways and enzymatic capabilities between the two organisms. *L. capsici* may possess enzymes that promote synthesis or partial hydrolysis of polysaccharides, leading to accumulation of certain carbohydrates, whereas *B. subtilis* may preferentially metabolize these compounds through pathways favouring amino acid and lipid metabolism (Liu *et al*., 2024; Gao *et al.,* 2025). Although limited information exists regarding the role of *L. capsici* in improving proximate composition of fermented foods, its potential probiotic properties have been documented (Zeng *et al.*, 2023; Chen *et al.,* 2023), suggesting promising applications in functional food fermentation.

CONCLUSION

The reduction in mycotoxins and BAs through back slopping, as demonstrated in this study, has important implications for consumer health. Controlled, fermented almond-condiments could offer a safer alternative to spontaneously fermented products, which may carry higher levels of harmful substances. The findings suggest that back slopping can serve as an effective and low-cost method for reducing contamination, enhancing the safety, and improving the quality of fermented foods, thereby benefiting consumers who rely on these products for their daily diets. This study contributes to the growing body of knowledge on bio-control strategies in food safety. It also supports the growing body of literature that suggests controlled fermentation with starter cultures can improve the nutritional profile of fermented condiments, while also reinforcing the idea that spontaneous fermentation may still offer certain benefits, particularly in terms of fiber and minerals contents.

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