



Research Article

Bacterial Diversity and Metabolomic Profile of Castor Oil Bean Seed (*Ricinus communis* L.) Condiment

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ABSTRACT

This study provides a comprehensive characterization of spontaneously fermented castor oil bean condiment (COBC) by integrating metagenomic and metabolomic analyses. High-throughput 16S rRNA sequencing revealed a highly diverse bacterial community (Shannon index = 3.8), with rarefaction analysis confirming adequate sequencing depth. The microbiota was predominantly bacterial (99.0%), largely composed of Bacillota (83.4%) and Bacillota A (16.5%), with minor contributions from Actinomycetota (0.3%). At finer taxonomic resolution, Lactobacillales dominated, particularly *Atopostipes* (51.2%) and *Vagococcus* A (28.5%), while genera such as *Peptostreptococcus* and *Bacillus* A occurred at moderate abundance. This provides a detailed account of bacterial taxa present and their relative proportions, including previously underreported groups. Metabolites were analyzed using gas chromatography–mass spectrometry, revealing 18 volatile organic compounds across nine chemical classes, with esters (28.0%) and amines (22.0%) predominating. The most abundant compound was 9,12-octadecadienoic acid methyl ester (18.86%). Non-volatile compounds were primarily fatty acids, with monounsaturated fatty acids (50%) being most abundant, followed by saturated (37.5%) and polyunsaturated (12.5%) forms. Amino acid analysis identified 17 compounds, with non-essential amino acids predominating; glutamic acid (12.52 mg/100 g) and aspartic acid (7.60 mg/100 g) were highest, while leucine (5.60 mg/100 g) and phenylalanine (4.21 mg/100 g) were the leading essential amino acids. COBC exhibits a distinct microbial and biochemical profile, highlighting its potential nutritional and functional relevance, while providing high-resolution account of the bacterial community structure offering a more complete understanding than previously reported using culture-dependent techniques.

Keywords: Abundance; Amino acids; Bacteria; Community; Metabolites

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INTRODUCTION

The castor oil bean (*Ricinus communis* L.) seed is widely utilized in the preparation of traditional fermented condiments across several regions of Africa and Asia. These condiments are produced through controlled or spontaneous fermentation

processes that convert the seeds into products characterized by distinctive aroma, flavour, and enhanced nutritional attributes. Importantly, fermentation plays a critical role in detoxifying the seeds, as raw *R. communis* contains toxic constituents, notably ricin. Microbial activity during fermentation facilitates the degradation of these

toxic compounds, thereby rendering the product safe for consumption while simultaneously improving its organoleptic and nutritional qualities (Ikenna, 2023; Okwunodulu *et al.*, 2021; Adesemoye *et al.*, 2025).

The fermentation process is inherently dynamic, involving continuous succession and interaction of diverse microbial communities (Wang *et al.*, 2022; Zhao *et al.*, 2023). Despite this complexity, product quality is often governed by a relatively small number of dominant microbial taxa whose metabolic activities shape the physicochemical and sensory properties of the final product. Consequently, the identification and functional characterization of these key microorganisms are essential for understanding and optimizing fermentation processes (Wei *et al.*, 2021).

To date, investigations into the microbiology of fermented *R. communis* seed condiments have largely relied on culture-dependent techniques, which are limited in their ability to capture the full extent of microbial diversity. Although temporal changes in microbial populations during fermentation have been documented, there remains a paucity of information regarding the associated metabolomic transformations, particularly in castor oil bean seed fermentations. Addressing these limitations requires the application of culture-independent, high-resolution approaches. In this context, metagenomic sequencing offers a powerful tool for comprehensive profiling of microbial communities, enabling deeper insights into the structure and function of the fermentation microbiome (Zhu *et al.*, 2024; Fonseca *et al.*, 2024; Wei and Huang, 2025; Liu *et al.*, 2025).

The integration of metagenomics with metabolomic analyses provides a holistic framework for elucidating the biochemical and microbial processes underpinning fermentation. Such an approach enables the identification of metabolic pathways associated with flavor development, antimicrobial activity, and the synthesis of bioactive compounds in fermented castor oil bean seed condiments (Liu *et al.*, 2023; Liu *et al.*, 2025). Furthermore, linking specific microbial taxa to metabolite profiles can enhance understanding of how fermentation influences product safety, quality, and potential health benefits. This study therefore employs high-throughput sequencing and advanced bioinformatics tools to characterize the bacterial diversity and metabolomic profile of spontaneously fermented *R. communis* seed condiments. The outcomes are expected to advance current knowledge of the fermentation microbiome and provide a scientific basis for the

optimization and standardization of production processes for improved commercial application.

MATERIALS AND METHODS

Production of the Castor Oil Bean Seeds Condiment

Castor oil seeds were sorted, winnowed, and boiled for six hours, the hull is removed and boiled for an additional two hours (Saheed *et al.*, 2016). In some regions, the seeds for *Ogiri* production are wrapped in leaves after the initial few hours of boiling to facilitate softening or hull removal (Obboh, 2006; Ibeabuchi *et al.*, 2014; Saheed *et al.*, 2016).

Extraction of Volatile Compounds

The volatile compounds in the condiment were extracted through hydro distillation using a Clevenger apparatus for 3 hours. A 0.5 ml sample of the extract was analysed by gas chromatography-mass spectrometry (GC-MS) (Oladimeji, 2018; Fagbemi *et al.*, 2021; Xu *et al.*, 2022).

The GC-MS Analysis of the Volatile Organic Compounds

The GC-MS analysis was carried out using a modified version of method Abiona *et al.* (2025). A solid-phase micro-extraction (SPME) fiber (75 μ m, carboxen/polydimethylsiloxane) was employed to extract volatile compounds, which were analyzed using a Varian 3800/4000 gas chromatograph-mass spectrometer. 0.5 ml of the extract was placed in a 15 ml glass vial, sealed with a PTFE/BYTL septum, and incubated at 55°C for 30 minutes. During this time, the SPME fiber was exposed to the headspace for volatile equilibration. After the incubation, the sample was injected in splitless mode at 250°C for 3 minutes. Volatile compounds were separated on a DB-WAX capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent), with a carrier gas flow rate of 1.8 mL/min using nitrogen. The column temperature was programmed as follows: 40°C for 3 minutes ramped to 80°C at 5°C/min, further ramped to 160°C at 10°C/min for 0.5 minutes, to 175°C at 2°C/min, to 230°C at 10°C/min, and held at 230°C for 7 minutes. The mass spectrometric detector operated in electron impact mode at 70 eV and 35 μ A, scanning from 40 to 800 m/z at 4.45 scans/s. Identification of volatile organic compounds was performed by matching the sample's mass spectra with those in the NIST 08 and Wiley 6.0 libraries, as well as available literature data.

Analysis of Non-Volatile Metabolites

Sample Pre-treatment

The sample was pulverized using a mixer mill (MM 400) with a zirconia bead at 30 Hz for 1.5 minutes. 100 mg of the ground sample was mixed with 0.6 ml of

70% methanol and stored in the refrigerator at 4 °C for 12 hours. During this period, the sample was vortexed six times. Afterward, the sample was centrifuged at 4 °C for 10 minutes at 12,000 rpm. The resulting extract was filtered using a 0.22 µm SCAA-104 filter (ANPEL, Shanghai, China) and stored in an injection vial for further GC/MS analysis.

The GC/MS Analysis of Non-Volatile Organic Compounds

The samples were analyzed using a Varian 3800/4000 GC-MS system with an Agilent splitter (split/splitless) and a BP5 capillary column (30 m × 0.25 mm × 0.25 µm). Nitrogen was used as the carrier gas. A 1.0 µl sample was injected in splitless mode with the injector set to 270°C. The oven temperature was programmed to increase from 80°C to 200°C (1-minute hold) at 5°C/min, then raised to 280°C and held for 6 minutes. The total analysis time was approximately 45 minutes, with the GC-MS interface at 280°C. Mass spectrometry scanning covered 40–800 amu (Abiona *et al.*, 2025; Lambe *et al.*, 2025). The blank was injected first, followed by the sample. Organic compounds were identified using the NIST 08 and Wiley Mass Spectral Libraries, with a match score above 95%. For lower match scores, fragmentation patterns were analyzed to confirm compound identification. Compound concentrations were expressed as percentages of the total sample. The chromatograms were integrated without peak correction for co-elution, and the results were given as total abundance. Only compounds with ≥ 90% spectral matching were reported, without the calculation of response factors (Premnath and Zubair, 2025).

De-fattening of sample and Derivatization of Amino Acids

The sample was defatted by using a chloroform/methanol mixture (2:1 ratio). A 1.0 g portion of the sample was placed in an extraction thimble and extracted for 1 hour in a Soxhlet apparatus. Next, 100 mg of the defatted sample was combined with 1 g of quartz sand and ground in a ceramic dish, then homogenized with 5 ml of distilled water. After centrifugation for 5 minutes, 0.5 ml of the supernatant was slowly passed through a separating funnel filled with wool and eluted with 4M ammonium hydroxide (NH₄OH). For derivatization, the carboxyl group was esterified by adding 200 ml of butanol/HCl (3M) and heating at 110°C for 1 hour. The amine group was then acetylated with 100 ml of trifluoroacetic anhydride for 20 minutes at 60°C. After the reactions, excess solvents were evaporated under nitrogen. The dried derivatives were dissolved in 500

ml of ethyl acetate and analyzed by GC-MS (Schwardorf and Muller, 1991).

Extraction and Injection of Sample

Amino acid extraction was performed on 100 mg of powdered sample using ultrasound with 1 ml of 6% trichloroacetic acid. The extract was centrifuged at 6000 rpm for 5 minutes and purified on a Dowex 50W-W8 ion exchange column (Dowex®, Merck Millipore). For quantitative analysis, 50 µg of [15N]-glycine was used as the internal standard. The amino acids were eluted with 4 M ammonium hydroxide. Derivatization involved esterification of the carboxylic acid group by treating the dried sample with 200 µl of butanol: acetyl chloride (4:1 v/v) for 1 hour at 110°C. This was followed by acetylation of the amine group using 100 µl of trifluoroacetic anhydride for 20 minutes at 80°C. GC-MS analysis was conducted using a Varian 3800/4000 gas chromatograph-mass spectrometer. The samples were injected onto an Agilent 30 m x 0.25 mm capillary column with a 0.25 µm film thickness. The temperature program started at 70°C (2 min), increased to 110°C at 50°C/min, to 290°C at 100°C/min, and finally to 300°C at 160°C/min. Nitrogen (1 ml/min) was used as the carrier gas. The transfer line, injector, and ion source temperatures were 250°C, 200°C, and 250°C, respectively. The electron energy was 70 eV with a 100-mA emission current, and a 10:1 splitter was used. For method validation, amino acid standards were injected following the extraction and derivatization procedures. A statistically significant linear regression curve was obtained with standards ranging from 0 to 100 µg/ml and an internal standard concentration of 50 µg/ml. The precision and accuracy for glycine were assessed with standards of 20 and 30 µg/ml (n=7), showing values below 6% and 11%, respectively. The glycine isotopic abundances were determined through matrix calculations and regression curve analysis (Adeniji *et al.*, 2025).

Metagenomic Extraction of Bacterial DNA from Castor Oil Bean Seeds Condiments

Genomic DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Catalog No. D4300). To start, 250 µl of the samples were placed in separate 0.1 and 0.5 mm ZR BashingBead™ Lysis Tubes, followed by the addition of 750 µl ZymoBIOMICS™ lysis solution. The mixture was processed in a bead beater (Disruptor Genie) for 20 minutes, then centrifuged at 1000 x g for 1 minute. Next, 400 µl of the supernatant was transferred to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8000 x g for 1 minute. The filter was discarded, and 1200 µl of ZymoBIOMICS™ DNA

Binding Buffer was added to the filtrate. This mixture was transferred to a Zymo-Spin™ IC Column and centrifuged at 10,000 x g for 1 minute. The flow-through was discarded, and the process was repeated. The column was then moved to a new collection tube, and 400 µl of ZymoBIOMICS™ DNA Wash Buffer 1 was added. After centrifuging at 10,000 x g for 1 minute, the flow-through was discarded. Subsequently, 700 µl of ZymoBIOMICS™ DNA Wash Buffer 2 was added to the column, and it was centrifuged at 10,000 x g for 1 minute. Finally, the column was transferred to a clean 1.5 ml micro-centrifuge tube, 50 µl of DNase/RNase Free Water was added, and the solution was incubated for 1 minute. DNA was eluted by centrifuging at 10,000 x g for 1 minute (Adewale *et al.*, 2024).

Amplification of the Extracted DNA

The genomic DNA from both samples was sent to Inqaba Biotechnical Industries for next-generation sequencing (NGS). PCR amplification was performed using universal primers 27F (/5AmMC6/GTAAAACGACGGCCAGT(N)n) and 1492R (/5AmMC6/CAGGAAACAGCTATGAC(N)n), targeting the V1-V9 region of the 16S rRNA gene. The generated amplicons were pooled (300-1000 ng) to optimize sequencing yield. Barcoding was done using Pacbio M13 barcodes for multiplexing via limited cycle PCR. Afterward, the barcoded amplicons were quantified and pooled equimolarly (Senthilraj *et al.*, 2016; Karin *et al.*, 2023).

Purification of the Barcoded Amplicons

The barcoded amplicons were purified using AMPure PB bead-based purification. An equal volume of each barcoded amplicon was pooled in a 2.0 ml DNA LoBind tube, bringing the total volume to 800 µl. To the pool, 1.3X v/v of room temperature SMRTbell cleanup beads were added, mixed thoroughly, and the tube was centrifuged to collect the liquid. The mixture was left at room temperature for 10 minutes to allow DNA binding to the beads. The beads were separated using a magnetic rack, and the cleared supernatant was discarded. Next, 500 µl of 80% ethanol was added to the beads, left for 30 seconds, then discarded. This step was repeated, and 47 µl of low TE buffer was added to the beads, which were then re-suspended by pipetting 10 times. The solution was micro-centrifuged and left for 5 minutes to elute the DNA. The tube was placed on the magnetic rack to separate the beads, and the clear supernatant was carefully transferred to a 0.2 ml strip tube. The old tube with beads was discarded. The DNA concentration was measured using a Qubit fluorometer with the 1x dsDNA HS Kit, ensuring at

least 300-1000 ng of DNA was available for SMRT library preparation (Costa, 2023).

Sequencing of the DNA

DNA sequencing was performed using the PacBio Sequel IIe system, following the SMRTlink protocol for primer annealing and polymerase binding. The sequencing was carried out on the PacBio Revio system. Raw sub-reads were processed using the SMRTlink Circular Consensus Sequences (CCS) algorithm (v25.1.0.257715) to generate high-accuracy reads (>QV40). These reads were further analyzed using the DADA2 denoising algorithm for quality control, and taxonomic classification was performed with QIIME2 (Duckworth *et al.*, 2024).

Data Analysis

The result was analysed using Statistical Package for Social Sciences (SPSS) version 2.0 and Analysis of Variance (ANOVA) was used to test the the groups' means differences while reflecting the level of significance. Also post hoc test of comparison was conducted with Duncan tests

RESULTS

The bacterial diversity of castor oil bean seeds condiment (COBC) was evaluated using 16S rRNA gene metagenomic sequencing. Alpha diversity analysis based on the Shannon diversity index derived from rarefaction analysis revealed substantial microbial diversity within the sample (Shannon index = 3.8) (Fig. 1). The rarefaction curve approached an asymptote at approximately 5,500 sequencing reads, beyond which the Shannon index remained stable. This trend indicates that the sequencing depth was sufficient to capture the majority of bacterial taxa present, suggesting adequate sampling coverage of the microbial community.

The taxonomic composition of the COBC microbiota is presented in Fig. 2. Bacterial sequences constituted 99.0% of the total classified reads, whereas 0.97% remained unassigned. The microbial community was distributed across three phyla: Bacilliota, Bacilliota_A, and Actinomycetota. Bacilliota was the dominant phylum, accounting for 83.4% of the total relative abundance, followed by Bacilliota_A (16.5%), while Actinomycetota occurred at a very low proportion (0.3%).

At the class level, the community was primarily composed of Bacilli (83.4%), with Clostridia representing 16.5% of the sequences. Actinomycetes contributed only a minor fraction (0.2%) to the overall community structure. Consistent with these findings, Lactobacillales emerged as the predominant order, comprising 79.8% of the sequences. Other notable

orders included Peptostreptococcales (9.1%) and Bacillales (7.5%), while Tissierellales (2.1%), Paenibacillales (0.8%), Staphylococcales (0.7%), and Mycobacteriales (0.2%) were present at relatively low abundances.

At the family level, Carnobacteriaceae (51.2%) and Vagococcaceae (28.6%) dominated the microbial community. Peptostreptococcaceae (9.0%) and Bacillaceae_G (7.5%) were moderately represented, whereas Tissierellaceae (2.1%), Paenibacillaceae (0.8%), Staphylococcaceae (0.7%), and Mycobacteriaceae (0.2%) occurred at comparatively low frequencies.

Nine bacterial genera were identified in the COBC microbiota. The community was largely dominated by *Atopostipes* (51.2%) and *Vagococcus_A* (28.5%). Other genera detected included *Peptostreptococcus*

(9.0%), *Bacillus_A* (7.5%), *JACRTK01* (2.1%), *Paenibacillus_B* (0.8%), *Staphylococcus* (0.7%), *Corynebacterium* (0.2%), and *Vagococcus* (0.1%).

Species-level classification further resolved several taxa (Fig. 3), including *Atopostipes suicloacalis*, *Vagococcus_A lutrae*, *Peptostreptococcus russelli*, *Bacillus_A cytotoxicus*, *JACRTK01 sp. 014385225*, *Bacillus_A pacificus*, *Paenibacillus_B sp. 900539405*, *Staphylococcus simulans*, *Corynebacterium sp. 001815985*, and *Vagococcus fluvialis*. Overall, the microbial community of COBC was strongly dominated by members of the order *Lactobacillales*, particularly *Atopostipes* and *Vagococcus*-related taxa, while taxa affiliated with Clostridia and Actinomycetota contributed only marginally to the community structure.

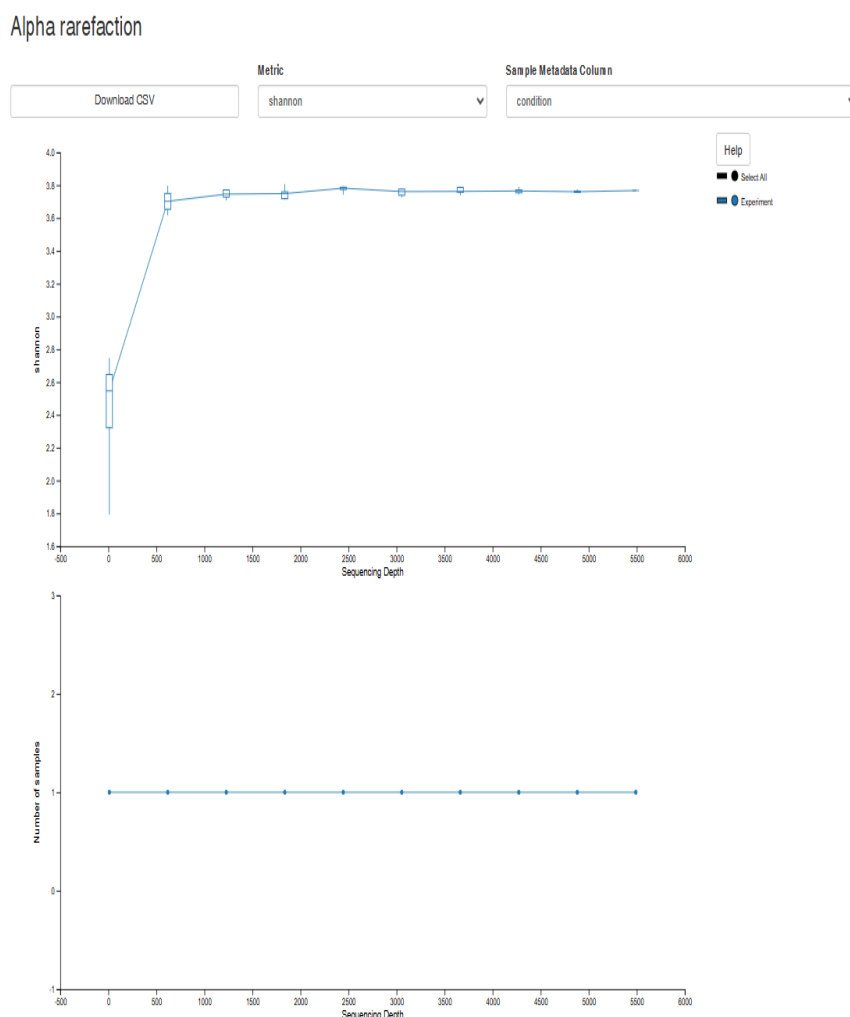


Fig 1: Castor oil Bean Seeds Condiment (COBC)-Alpha Rarefaction Curve



Fig 2: Relative Frequency of Bacterial species in Castor oil Bean Seeds Condiment COBC

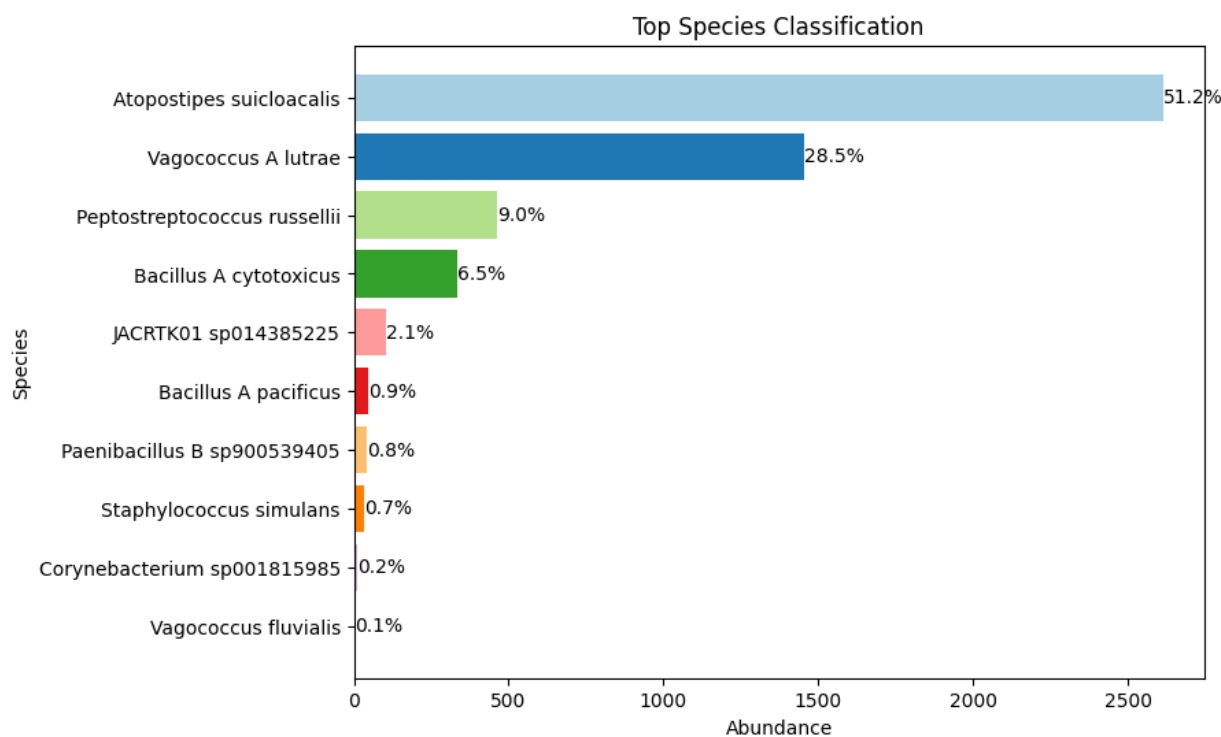


Fig 3: Bacterial Species Abundance in Castor Oil Bean Seeds Community

GC-MS analysis of castor oil bean seeds condiment (COBC) revealed nine major groups of volatile organic

compounds (VOCs), including esters, acids, phenolics, amines, cyclic amides, heterocyclic aromatics,

aromatic compounds, cyclic alkynes, and ketones (Table 1). Esters were the most prevalent group, accounting for 28.0% of total VOCs, followed by amines (22.0%). Phenolics, cyclic amides, heterocyclic aromatics, cyclic alkynes, and ketones each contributed minimally (5.6%), while acids and aromatic compounds occurred at moderate levels (11.0%).

A total of 18 individual VOCs were identified in COBC (Table 2). The most abundant compound was 9,12-octadecadienoic acid (Z, Z) methyl ester, representing 18.86% of the total VOCs, followed by phenol (8.93%) and 10,13-octadecadienoic acid (8.62%). Compounds detected at the lowest concentrations included benzene propanoic acid methyl ester (0.36%), cyclododecyne (0.49%), diphenyl amine (0.50%), and indole (0.59%). Several compounds exhibited identical concentrations, including 9,12-octadecadienoyl chloride (Z, Z) and cyclododecyne (0.68%), indole and benzyl nitrile (0.59%), and 1-butanamine,2-methyl-N-(2-methylbutylidene) and benzen-d5-amine (3.67%). The ketone 2-oxecanone, 10-methyl-, was detected at 2.57%.

Analysis of non-volatile organic compounds (NVOCs) in COBC indicated that fatty acids were the predominant class, categorized as saturated, monounsaturated, or polyunsaturated (Table 3). Monounsaturated fatty acids constituted the largest proportion (50%) of total fatty acids, followed by saturated (37.5%) and polyunsaturated fatty acids (12.5%). Among individual NVOCs, the polyunsaturated fatty acid 9,12-octadecadienoic acid (cis isomer) was most abundant (72.45%), while n-hexadecanoic acid, a saturated fatty acid, was present at 41.04%. Monounsaturated fatty acids such as 9-

hexadecenoic acid occurred in both cis (Z) and trans (E) configurations (36.85%), whereas oleic acid was detected exclusively in the cis configuration at 36.85%. The lowest concentrations were observed for octadecenoic (Z) methyl oleate and 8-octadecenoic acid (0.35%). Additionally, the trans isomer of 9,12-octadecadienoic acid methyl ester (E, E) was detected at 12.01% (table 4).

Collectively, these findings indicate that COBC is characterized by a complex mixture of both volatile and non-volatile compounds, with esters and monounsaturated fatty acids dominating the VOC and NVOC profiles, respectively.

Seventeen amino acids were identified in spontaneously fermented castor oil bean seed condiments (COBCAA) (Table 3). Non-essential amino acids predominated over essential amino acids. Glutamic acid (12.52 mg/100 g) and aspartic acid (7.60 mg/100 g) occurred at the highest levels, with arginine recorded at 7.06 mg/100 g. Within this group, glycine (3.92 mg/100 g) and alanine (3.66 mg/100 g) showed comparable concentrations, whereas serine (3.08 mg/100 g), proline (2.42 mg/100 g), tyrosine (2.28 mg/100 g), and histidine (2.80 mg/100 g) did not differ significantly ($p < 0.05$) and represented the lowest values among the non-essential amino acids.

However, for the essential amino acids, leucine (5.60 mg/100 g) and phenylalanine (4.21 mg/100 g) were most abundant, while valine (3.54 mg/100 g) and isoleucine (3.07 mg/100 g) occurred at moderate levels. Methionine (1.31 mg/100 g) and tryptophan (1.08 mg/100 g) exhibited the lowest concentrations within this category.

Table 1: Percentage Occurrence of the Volatile Organic Groups of Castor oil Seeds Condiments

Organic compound groups	Castor oil seeds condiment (%)
Ester	28.0
Acids	11.0
Phenolic	5.6
Amines	22.0
Cyclic amide	5.6
Heterocyclic aromatic	5.6
Aromatic compound	11.0
Cyclic alkyne	5.6
Ketone	5.6

Table 2: Volatile Organic Compounds of Castor Oil Seeds Condiments (%)

VOC	VOCCOBC	Peak area	Conc.
Ester			
1. Cis 9,12-octadecadienoic acid (z,z), methyl ester	45.93		18.86
2. Methyl 2-oxo-1-pyrrolidine acetate	1.58		3.67
3. Benzene propanoic acid methyl ester	0.28		0.36
4. Dichloroacetic acid, 3-tridecyl ester	0.28		0.36
5. 11,14-octadecadienoic acid methyl ester	1.44		2.57
Acids			
6. 10,13-octadecadienoic acid	1.07		8.62
7. 9,12-octadecadienoyl chloride (z,z)-	0.46		0.68
Phenolic			
8. Phenol	3.57		8.93
Amines			
9. 1-butanamine,2-methyl-N-(2-methyl butylidene)	1.58		3.67
10. Benzen-d5-amine	1.58		3.67
11. Diphenyl amine	0.33		0.50
12. Piperidine, 1-hydroxyl-	1.44		3.35
Cyclic amide			
13. 2-piperidinone	0.82		0.83
2-piperidinone	0.82		0.83
Heterocyclic aromatic			
14. Indole	0.70		0.59
Indole	0.70		0.59
Aromatic compounds			
15. Benzyl nitrile	0.70		0.59
16. Pyridine, 3- (phenyl methyl-	0.33		0.49
Cyclic alkyne			
17. Cyclododecyne	0.46		0.68
Ketone			
18. 2-oxecanone, 10-methyl- (+/-)	1.44		2.57

Keys: VOC; Volatile organic compound, VOCCOBC; Volatile organic compound of castor oil Bean Seeds
condiment

Table 3: Percentage Occurrence of Non-Volatile Organic Compounds in Castor Oil Bean Seeds Condiment

NVOC	NVOCOBC (%)
Saturated fatty acids	37.5
Mono-unsaturated fatty acids	50.0
Poly-unsaturated fatty acid	12.5

Key: NVOBC; Non-volatile organic compounds of castor oil bean seeds condiment

Table 4: Non-Volatile Organic Compounds Castor Oil Bean Condiments (%)

NVOC	Peak area of NVOC	Conc.
Saturated fatty acids		
1. n-hexadecanoic acid (Palmitic acid)	36.87	41.04
n-hexadecanoic acid	36.87	41.04
n-hexadecanoic acid	36.87	41.04
2. Hydrocinnamic acid	1.25	2.20
Hydrocinnamic acid	1.25	2.20
3. Methyl stearate	0.48	0.34
Methyl stearate	0.48	0.34
Methyl stearate	0.48	0.34
Methyl stearate	0.48	0.34
Monounsaturated fatty acids		
4. 9-octadecenoic acid-E- (elaidic acid)	50.11	36.85
9-octadecenoic acid (z)	50.11	36.85
5. Oleic acid-z	50.11	36.85
6. 9-octadecenoic acid (z) methyl oleate	3.17	2.33
7. 8-octadecenoic acid	3.17	2.33
Poly unsaturated omega-6-fatty acids		
8. 9, 12-octadecadienoic acid methyl ester (E, E)	1.07	12.01

Key: NVOC; Non-volatile organic compounds of castor oil seeds condiment

Table 3: Amino Acids Profile of Castor Oil Seeds Condiment

Amino acids	Peak area (%)	Castor Oil Bean Seeds Condiment Amino Acids Concentration (mg/100 g)
Aspartic acid	7.64	7.60 ^b
Threonine	2.49	2.43 ^{ij}
Serine	3.11	3.08 ^{gh}
Glycine	4.01	3.92 ^{ef}
Alanine	3.72	3.66 ^{ef}
Phenylalanine	2.25	4.21 ^e
Proline	2.90	2.42 ^{ij}
Methionine	3.68	1.31 ^k
Leucine	3.13	5.60 ^{de}
Isoleucine	1.31	3.07 ^{gh}
Tyrosine	5.81	2.208 ^j
Valine	1.29	3.54 ^{fg}
Glutamic acid	12.93	12.52 ^{ab}
Histidine	3.02	2.80 ^{hij}
Arginine	8.04	7.06 ^c
Lysine	3.49	2.95 ^{hi}
Tryptophan	3.72	1.08 ^k

DISCUSSION

The present study provides a comprehensive evaluation of the microbial ecology, metabolite composition, and nutritional attributes of castor oil bean seed condiment (COBC), specifically produced as *ogiri* from *Ricinus communis*. Although *Ricinus communis* seeds have long been used in condiment production, most previous studies investigating their microbiology have relied on culture-dependent

methods, which restrict the detection of the full diversity of microbial communities (Barber *et al.*, 1988; Jideani and Okeke, 1991; Ukaoma *et al.*, 2021; Nkeiruka *et al.*, 2021). To date, no 16S rRNA amplicon-based metagenomic studies have been reported for the characterization of the bacterial community in *ogiri*. In this study, we employed 16S rRNA gene amplicon sequencing to address this gap, thereby providing a comprehensive and high-resolution

insight into the bacterial diversity associated with castor seed fermentation and significantly advancing current understanding of its microbial ecology.

The observed Shannon diversity index indicates a moderately diverse microbial ecosystem, suggesting that the fermentation process supports a selective but stable consortium of microorganisms (Xun *et al.*, 2019; Ye *et al.*, 2023; Atuna *et al.*, 2025; Lee *et al.*, 2026). Compared to earlier culture-based studies that emphasized a narrow range of dominant taxa—primarily *Bacillus* species (Odunfa, 1985; Ibe and Orabuike, 2009; Ojinnaka and Ojimelukwe 2013; Dike-Ndudim *et al.*, 2022), the present findings reveal a broader and more complex microbial structure. This underscores the importance of metagenomic approaches in overcoming methodological biases and capturing previously unreported taxa, thereby refining current understanding of the microbial ecology of castor seed-based *ogiri*.

A key finding of this study is the dominance of members of the order Lactobacillales, particularly the genera *Atopostipes* and *Vagococcus*. This contrasts with earlier reports that described *ogiri* fermentation as predominantly driven by *Bacillus* spp (Bamidele *et al.*, 2017; Olabiwoninu and Odunfa, 2018). The discrepancy can be explained by the dynamic nature of fermentation. It is well established that early stages of fermentation are typically dominated by lactic acid bacteria (LAB), which thrive under relatively acidic conditions and initiate fermentation through carbohydrate metabolism and acid production. As fermentation progresses, the environment gradually shifts toward alkalinity due to protein degradation and ammonia production, thereby favoring the proliferation of *Bacillus* and other alkaline-tolerant organisms (Papadimitriou *et al.*, 2016; Siddiqui *et al.*, 2023; Aguirre-Garcia *et al.*, 2024).

In this context, the predominance of LAB-related genera observed in the present study suggests that the microbial profile captured may reflect either the early or mid-stage of fermentation, or a fermentation system in which acidogenic processes remain significant throughout. The relatively low abundance of *Bacillus* spp. further supports this interpretation and highlights a possible deviation from the classical alkaline fermentation pathway commonly associated with traditional *ogiri* (Oyedemi *et al.*, 2013; Meena *et al.*, 2022; Bezim *et al.*, 2023). This represents an important observation, as it demonstrates that castor seed fermentation is not strictly limited to a single microbial succession pattern but may vary depending

on processing conditions, fermentation duration, and environmental factors (Ukaoma *et al.*, 2019).

The prominence of *Atopostipes*, a genus rarely reported in fermented condiments, is particularly noteworthy. Its high abundance suggests a strong ecological adaptation to the castor seed fermentation environment and indicates a potential functional role in acidification, detoxification, and metabolite production. Similarly, the substantial presence of *Vagococcus* reinforces the importance of LAB-related organisms in shaping both the safety and sensory attributes of the product (Ishiwu *et al.*, 2015; Guangyu *et al.*, 2018; Hairong *et al.*, 2022; Jensen *et al.*, 2023). These findings expand the known diversity of microorganisms associated with *ogiri* and challenge the traditional emphasis on *Bacillus*-dominated systems.

The metabolite profile obtained through GC-MS analysis further reflects the interplay between microbial diversity and biochemical transformations. The predominance of esters suggests active esterification processes typically associated with LAB metabolism and early-stage fermentation, contributing to desirable fruity and mild aroma characteristics. In contrast, the presence of amines indicates ongoing protein degradation, which becomes more pronounced during later stages of fermentation when alkaline conditions favor *Bacillus* activity (Sharma *et al.*, 2020; Zhang *et al.*, 2023; Wei *et al.*, 2026). The coexistence of these compounds in castor oil bean seeds condiment (COBC) suggests a transitional or balanced fermentation state, where both acidogenic and proteolytic processes contribute to the overall flavor profile. This balance may result in improved sensory acceptability compared to traditional *ogiri*, which is often characterized by strong ammonia-like odours associated with advanced alkaline fermentation (Zeng *et al.*, 2022; Xu *et al.*, 2023; Zhang *et al.*, 2023).

Beyond their sensory roles, several volatile organic compounds identified in this study may confer health benefits. Fatty acid methyl esters have been associated with antimicrobial and anti-inflammatory activities, while phenolic compounds exhibit antioxidant properties that may help reduce oxidative stress (Sun & Shahrajabian, 2023). The presence of indole in low concentrations may also contribute to gut health by supporting intestinal homeostasis. These findings suggest that the VOC profile of COBC is not only important for flavor but may also enhance its functional value as a food condiment (Kumar and Goel, 2019; Rahman *et al.*, 2021; Matsumura *et al.*, 2023; Sun *et al.*, 2023; Boaru *et al.*, 2024).

The non-volatile fatty acid composition further supports the nutritional relevance of COBC. The predominance of monounsaturated fatty acids, along with the presence of polyunsaturated fatty acids such as 9,12-octadecadienoic acid, is associated with cardiovascular health benefits, including improved lipid profiles and anti-inflammatory effects (Patel *et al.*, 2016; Shramko *et al.*, 2020; Yeboah *et al.*, 2021; Ojha *et al.*, 2024; Pedersen *et al.*, 2025). The preservation of these fatty acids during fermentation indicates that the process maintains their nutritional integrity. The detection of cis and trans isomers suggests that fermentation induces structural modifications, reflecting the influence of microbial and processing factors on lipid chemistry. This contributes to existing knowledge by highlighting an often-overlooked aspect of lipid transformation in fermented condiments (Patel *et al.*, 2016; Yeboah *et al.*, 2021; Ojha *et al.*, 2024; Nevigato *et al.*, 2025).

Proteolysis during fermentation was evident from the amino acid profile, with non-essential amino acids predominating. The high levels of glutamic acid and aspartic acid are particularly important for imparting umami taste, thereby enhancing the palatability of the condiment. Additionally, these amino acids play roles in metabolic and neurological functions (Elango, 2023; Yoshida & Ninomiya, 2024; Matsumoto *et al.*, 2025; Wang *et al.*, 2025). The relatively high arginine content is noteworthy due to its involvement in nitric oxide synthesis, which supports vascular health and immune function. This indicates that COBC may offer functional health benefits beyond basic nutrition (Ishiwu *et al.*, 2015; Okwunodulu *et al.*, 2020; Urhaluk, 2023).

Among essential amino acids, leucine and phenylalanine were the most abundant. Leucine is critical for muscle protein synthesis and metabolic regulation, while phenylalanine serves as a precursor for neurotransmitters. Although methionine and tryptophan were present at lower levels, their inclusion contributes to the overall protein quality of the condiment. Tryptophan, in particular, is associated with serotonin production and may influence mood and cognitive function. The amino acid profile therefore supports both the nutritional and functional potential of COBC (Ananieva *et al.*, 2016; Chandel, 2021; Rajavel, 2023; Fayomi *et al.*, 2025).

Overall, this study bridges the gap between traditional knowledge and modern molecular analysis by providing a detailed metagenomic and biochemical characterization of castor oil bean seed condiment. By demonstrating the coexistence of LAB-

driven early fermentation processes and the potential for later-stage alkaline fermentation, the study offers a more dynamic understanding of microbial succession in castor seed condiments. The combined evidence from microbial profiling, VOCs, NVOCs, and amino acid composition highlights COBC as a complex fermented product with significant sensory, nutritional, and functional attributes. These findings not only refine existing knowledge but also open new avenues for optimizing fermentation strategies to enhance product quality, safety, and health benefits.

CONCLUSION

Overall, this study bridges the gap between traditional knowledge and modern molecular analysis by providing a detailed metagenomic and biochemical characterization of castor oil bean seed condiment (*ogiri*). By demonstrating the coexistence of lactic acid bacteria driven early fermentation processes and the potential for subsequent alkaline fermentation, the findings present a more dynamic and condition dependent model of microbial succession than previously described for castor seed condiments. The dominance of previously underreported genera, alongside a broader microbial diversity revealed through high throughput sequencing, further emphasizes the limitations of earlier culture-based studies and the importance of molecular approaches in accurately defining fermentation ecology. The integration of microbial profiling with volatile and non-volatile compound analysis, as well as amino acid composition, highlights the complexity of castor oil bean seed condiment (COBC) as a fermented food system. The coexistence of esters and amines reflects simultaneous acid forming and protein degrading activities, contributing to a more balanced and potentially improved sensory profile. In addition, the presence of bioactive metabolites, beneficial fatty acids, and nutritionally relevant amino acids emphasizes the product's functional and health promoting potential beyond its traditional role as a condiment. Taken together, these findings not only refine current understanding of castor seed fermentation but also provide a scientific basis for improving fermentation practices, process control, and product standardization. This work establishes a foundation for enhancing the safety, consistency, and nutritional quality of COBC-*ogiri*, while supporting further research into its microbial dynamics and overall value in food systems.

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