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Metagenomic and Molecular Docking Analysis of Probiotic-Rich Fermented Foods with Potential Anti-SARS-CoV-2 Activity

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ABSTRACT

Probiotics and fermented foods have gained considerable attention for their ability to modulate immune responses and exhibit antiviral activity, particularly against SARS-CoV-2. However, integrated evidence linking their nutritional composition, microbial profile, and molecular antiviral potential remains limited. Selected traditional fermented foods (ogi, garri, fufu, iru oworo, and iru pete) were analyzed for vitamin composition, physicochemical characteristics, and antioxidant activity using standard analytical methods. Microbial profiling was conducted using full-length 16S rRNA sequencing. Bioactive metabolites associated with probiotics were identified and evaluated through molecular docking against the SARS-CoV-2 main protease (Mpro). Significant variations were observed among the samples. Iru pete exhibited the highest levels of vitamins B3, B6, B12, vitamin C, and antioxidant capacity. Metagenomic analysis revealed a predominance of probiotic-associated species, including Lactobacillus delbrueckii and Limosilactobacillus fermentum. Molecular docking results showed that violacein and sitagliptin exhibited more favorable predicted binding affinities (−8.0 and −7.5 kcal/mol, respectively), than nirmatrelvir (−6.6 kcal/mol), under the docking conditions employed.. In Conclusion, traditional fermented foods represent valuable sources of probiotics and bioactive compounds with nutritional and potential antiviral benefits. The combined biochemical, microbial, and computational findings highlight their promise as adjunct functional foods in antiviral strategies against SARS-CoV-2. Further in vivo and clinical studies are required to confirm their therapeutic relevance.

INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, was identified as a global pandemic and public health emergency from 2019 to 2022, garnering substantial international focus. COVID-19 exhibits a wide range of clinical manifestations, from mild symptoms to severe respiratory distress, potentially advancing to sepsis, multi-organ failure, and mortality (Kandeel *et al.*,2022). As of 31 May 2023, the World Health Organization (WHO) documented more than 766 million confirmed cases and an estimated 6.9 million deaths worldwide. On 11 May 2023, the World Health Organization announced the cessation of COVID-19 as a global health emergency; however, the virus persists in circulation, continues to evolve, and remains a global health concern, albeit with diminished intensity (Rahman, *et al.*,2023). There is a significant necessity to investigate new therapeutic agents with antiviral efficacy against SARS-CoV-2.

SARS-CoV-2 is an enveloped virus characterized by a positive-sense single-stranded RNA (+ssRNA) genome, which is associated with membrane (M) and envelope (E) proteins. The spike (S) glycoprotein facilitates viral entry through its interaction with the angiotensin-converting enzyme 2 (ACE2) receptor present on host cells. The spike protein is composed of two subunits, S1 and S2, with the S1 subunit facilitating receptor binding. This interaction leads to conformational changes that promote viral entry into host cells (Rahman *et al.*,2023; García *et al.*, 2021; Gao *et al.*, 2022). Research indicates that SARS-CoV-2 has a greater binding affinity for ACE2 than SARS-CoV-1, which contributes to its increased transmissibility (Harrison *et al.*,2020; Hu *et al.*,2021). The findings highlight the significance of identifying pharmacologically active compounds, including natural products, capable of inhibiting viral entry or replication.

Probiotics are characterized as live microorganisms that, when provided in sufficient quantities (generally $\geq 10^6$ CFU/g), offer health advantages to the host (Mack D, 2011; Bezirtzoglou, E. and Stavropoulou, 2011; Marinova *et al.*,2019). Probiotic genera such as Lactobacillus and Bifidobacterium have been thoroughly investigated for their capacity to influence gut microbiota and inhibit pathogenic organisms (Salminen *et al.*,2005; Mousavi Khaneghah *et al.*, 2020). In addition to gastrointestinal effects, probiotics play a role in host health by modulating the immune system, preserving the integrity of the epithelial barrier, and regulating various signaling pathways (Wan *et al.*, 2016).

Recent studies suggest that individuals recovering from COVID-19 may exhibit persistent dysbiosis of gut microbiota for a duration of up to six months following infection (Chen *et al.*, 2021). This imbalance, typically marked by diminished levels of beneficial microbes like Lactobacillus and Bifidobacterium, may hinder recovery and immune function (Xu *et al.*,

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2020). Restoring homeostasis in the gut–lung axis through probiotic intervention may provide therapeutic advantages. Probiotics and their metabolites, supported by evidence of anti-inflammatory (Yahfoufi *et al.*, 2018; Plaza-Díaz *et al.*, 2017), antioxidant (Wang *et al.*, 2017), and antiviral (Al Kassaa ,2017) properties, are promising candidates for the mitigation of SARS-CoV-2 infection. A more comprehensive understanding of their molecular mechanisms of action is necessary

In recent years, computational methods have become essential in biomedical research, especially in drug discovery and microbiome investigations. During the COVID-19 pandemic, limitations on laboratory operations expedited the implementation of in silico methods, such as computer-aided drug design and machine learning-based predictive modeling. These methodologies facilitate the efficient evaluation of bioactive compounds and yield significant insights into protein-ligand interactions and mechanisms associated with the microbiome. This study examines the potential role of probiotics in managing COVID-19, emphasizing computational strategies that may enhance our understanding of probiotic functionality and aid in the development of new antiviral agents.

LITERATURE REVIEW

Probiotic-Rich Fermented Foods and Human Health

Traditional diets include fermented foods for their nutritional, sensory, and health benefits. Microorganisms include lactic acid bacteria (LAB), yeasts, and certain fungi ferment food to preserve it, improve nutritional bioavailability, and produce bioactive chemicals with health benefits. Fermented foods like ogi, fufu, garri, and fermented locust beans are consumed across Africa and contain beneficial microbes and metabolites.

Live probiotic bacteria help the host when given in sufficient proportions (Mack, 2005; Bezirtzoglou & Stavropoulou, 2011). Lactobacillus, Limosilactobacillus, Lactiplantibacillus, Bifidobacterium, and Pediococcus are common probiotic genera. These microorganisms maintain gut microbial balance, exclude harmful bacteria, and create antimicrobials including organic acids and bacteriocins (Salminen *et al.*, 2005; Mousavi Khaneghah, 2020).

Besides intestinal health, probiotics regulate the immune system. Studies show that probiotic bacteria boost innate and adaptive immune responses, intestinal barrier integrity, and inflammatory pathways (Wan *et al.*, 2016). Fermentation also boosts vitamin, antioxidant, and bioactive peptide levels in foods, boosting their nutritional value and functionality. Thus, fermented foods are being studied as functional foods that can improve health beyond nutrition.

Metagenomic Characterization of Fermented Food Microbiota

Comprehending the microbial makeup of fermented foods is crucial for assessing their safety, quality, and functional characteristics. Historically, the identification of microorganisms has depended on culture-based techniques. Nevertheless, these approaches have inherent limitations, as numerous microorganisms do not easily grow in laboratory settings. Recent developments in molecular biology and sequencing technologies have facilitated culture-independent methods for thorough microbial characterization.

The use of 16S rRNA gene sequencing has transformed the field of microbial ecology by enabling precise identification of bacterial communities found within intricate food matrices. Full-length 16S rRNA sequencing platforms, such as PacBio, offer enhanced taxonomic resolution in comparison to short-read sequencing technologies, facilitating species-level classification of microorganisms. This method has been extensively utilized in research on fermented foods to explore microbial diversity, fermentation processes, and the presence of beneficial microorganisms.

Numerous research efforts have indicated that fermented foods are primarily characterized by the presence of lactic acid bacteria, especially the species belonging to Lactobacillus, Limosilactobacillus, Lactiplantibacillus, and Leuconostoc. These microorganisms play a significant role in enhancing sensory attributes and generate metabolites that can have beneficial effects, including antioxidant, antimicrobial, and immunomodulatory properties. Microbial community profiling offers valuable insights into the biological mechanisms that contribute to the health benefits linked to fermented foods.

Probiotics, the Gut–Lung Axis, and Antiviral Activity

Recent studies have underscored the significance of the gut–lung axis in modulating immune responses in the context of respiratory infections. The gut microbiota plays a significant role in shaping systemic immunity by producing metabolites, modulating inflammatory signaling pathways, and interacting with immune cells. As a result, alterations in the composition of gut microbes could impact an individual's vulnerability to respiratory diseases and subsequently affect the outcomes of these conditions.

Patients with COVID-19 often show notable changes in the composition of their gut microbiota, which is marked by a decrease in beneficial bacteria and a rise in opportunistic pathogens (Chen *et al.*, 2021). These changes can continue for several months following recovery and are linked to extended inflammatory responses and compromised immune function. The restoration of microbial balance using probiotic supplementation has been suggested as a beneficial therapeutic approach.

Probiotics and their metabolites exhibit various biological properties that could play a role in enhancing antiviral defense. These encompass the improvement of mucosal immunity, the stimulation of antiviral cytokines, the regulation of inflammatory responses, and the production of metabolites that exhibit direct antimicrobial activity (Yahfoufi *et al.*, 2018). Moreover, compounds derived

from probiotics have shown significant antioxidant and anti-inflammatory properties, which could potentially mitigate tissue damage linked to viral infections (Plaza-Díaz *et al.*, 2017; Wang *et al.*, 2017). While direct evidence opposing SARS-CoV-2 is still scarce, these observations indicate that probiotics could play a role in enhancing host defense mechanisms during viral infections.

Molecular Docking in SARS-CoV-2 Drug Discovery

The swift worldwide dissemination of SARS-CoV-2 prompted significant initiatives aimed at discovering new therapeutic agents that can effectively inhibit viral replication. Computational methods have emerged as essential instruments in contemporary drug discovery, facilitating the swift evaluation of numerous compounds and minimizing both the time and expenses linked to laboratory experiments.

Molecular docking is a commonly employed computational method that forecasts the binding orientation and interaction energy between a ligand and its target protein. One of the most thoroughly studied targets in SARS-CoV-2 is the main protease (Mpro), an enzyme that is essential for the processes of viral replication and maturation. Inhibiting Mpro can interfere with the processing of viral polyproteins, thereby reducing the spread of the virus.

Many research efforts have employed molecular docking techniques to discover natural products, phytochemicals, microbial metabolites, and synthetic compounds that may exhibit inhibitory effects on SARS-CoV-2 proteins (Rauf *et al.*, 2020; Kandeel *et al.*, 2022). Although molecular docking does not yield direct proof of biological activity, it functions as a valuable initial screening method for pinpointing compounds with favorable binding properties that can later be assessed through in vitro and in vivo studies.

Research Gap and Study Rationale

While considerable research has explored the nutritional advantages of fermented foods, the role of probiotic microorganisms, and the discovery of antiviral drugs, there remains a scarcity of studies that have combined these methodologies within a unified framework. Prior research has predominantly concentrated on the nutritional makeup of fermented foods, the characterization of microbial communities, or the computational assessment of specific bioactive compounds. As a result, there is a scarcity of information concerning the integrated nutritional, microbial, and molecular antiviral properties of traditional African fermented foods.

Furthermore, although various metabolites derived from probiotics have shown antimicrobial and immunomodulatory properties, their potential interactions with therapeutic targets related to SARS-CoV-2 are still not well understood. Consequently, it is essential to conduct research that concurrently assesses the nutritional quality of fermented foods, characterizes their probiotic microbiota through advanced sequencing technologies, and explores the antiviral potential of related bioactive metabolites utilizing molecular docking methods.

This research aims to fill the existing knowledge gap by combining vitamin analysis, antioxidant assessment, physicochemical characterization, comprehensive 16S rRNA microbial profiling, and molecular docking analysis of metabolites associated with probiotics in relation to the SARS-CoV-2 main protease. The findings enhance the existing understanding of functional fermented foods and their possible roles in promoting antiviral strategies and overall human health.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of Ogi (fermented maize), Iru (fermented locust bean), Fufu (fermented cassava mash), and Garri (fermented and roasted cassava granules) were collected in sterile food-grade containers from multiple artisanal producers Both raw and fermented variants were collected for each food type. Samples were homogenized, labeled, and stored at −4 °C until analysis.

Vitamin Content Analysis

Analysis of Vitamin A and B-Complex

The vitamin B-complex content of the samples was assessed utilizing the methodology outlined by Achikanu, Nwosu, and Ogu (2013). One gram of the sample was accurately measured and subsequently macerated with 20 milliliters of n-hexane in a test tube for a duration of 10 minutes. The solution was subsequently subjected to centrifugation for an additional 10 minutes. Following centrifugation, the solution underwent filtration, and 3 ml of the filtrate was aliquoted into a clean, dry test tube in duplicate, subsequently evaporating to dryness utilizing a boiling water bath. Following this, 2 ml of 0.5 N alcoholic potassium hydroxide was introduced to the residue, and the resulting mixture was subjected to boiling in a water bath for a duration of 30 minutes. Subsequent to the saponification process, 3 ml of n-hexane was introduced, and the solution was subjected to vigorous shaking. The n-hexane layer was meticulously transferred into a separate set of dry test tubes and subsequently evaporated to dryness. The resultant residue was reconstituted using 2 mL of ethanol. Subsequently, 1 ml of 0.2% ferric chloride in ethanol was introduced, followed by the incorporation of 1 ml of 0.5% 1,1-dipyridyl in ethanol. An extra 1 ml of ethanol was incorporated to achieve a final volume of 5 ml. The solution was meticulously mixed, and the absorbance was recorded at 520 nm utilizing a spectrophotometer, with a reagent blank serving as the reference.

Assessment of Vitamin C Concentration

The vitamin C (ascorbic acid) concentration in the samples was assessed utilizing the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method as outlined by the Association of Official Analytical Chemists (AOAC, 2005). A precisely

measured 5 g of the fresh or adequately homogenized sample was weighed and combined with 100 ml of 1% metaphosphoric acid to facilitate the extraction of ascorbic acid. The mixture was subjected to filtration using Whatman No. 1 filter paper, and the resulting clear filtrate was collected for subsequent analysis. A 10 ml aliquot of the filtrate was subjected to titration using a standardized solution of 2,6-dichlorophenolindophenol until a stable light pink hue was noted, enduring for a minimum of 15 seconds. The vitamin C content was determined by analyzing the volume of dye utilized, with results expressed in milligrams of ascorbic acid per 100 grams of sample (mg/100g). The dye solution was standardized utilizing pure ascorbic acid, and the resulting data were employed to ascertain the vitamin C concentration in the samples.

Physicochemical Properties

Determination of pH

The pH of the food samples was assessed utilizing the methodology outlined by AOAC (2005). A 10 g aliquot of the homogenized sample was accurately measured and subsequently dispersed in 100 ml of distilled water within a beaker. The mixture was stirred comprehensively to ensure a uniform suspension and permitted to stand for 30 minutes at room temperature. Following the equilibration process, the pH of the suspension was assessed utilizing a calibrated digital pH meter. The pH meter underwent standardization using buffer solutions with pH values of 4.0 and 7.0 before conducting measurements. The electrode was thoroughly rinsed with distilled water between measurements and carefully immersed in the sample solution. The pH of the sample was recorded as stable.All measurements were performed in triplicate to guarantee the precision and consistency of the results.

Antioxidant Analysis

Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) of the samples was determined using the method described by Benzie and Strain (1996), with slight modifications. The FRAP assay is based on the reduction of the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to its ferrous (Fe^{2+}) form at low pH, which produces a blue color with an absorption maximum at 593 nm.

Preparation of FRAP Reagent:

The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1 (v/v/v). The reagent was incubated at 37°C before use.

Sample Preparation and Analysis:

0.2 ml aliquot of the sample extract was mixed with 1.8 ml of the FRAP reagent and incubated in the dark at 37°C for 30 minutes. The absorbance of the resulting blue-colored complex was read at 593 nm using a UV-Visible spectrophotometer.

Quantification:

A standard curve was prepared using different concentrations of ferrous sulphate ($FeSO_4 \cdot 7H_2O$). The FRAP value of each sample was expressed in micromoles of Fe^{2+} equivalents per gram of sample (μmol Fe^{2+}/g). All analyses were conducted in triplicate, and the mean values were recorded.

DPPH Radical Scavenging Assay

The antioxidant activity of the samples was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, as described by Brand-Williams, Cuvelier, and Berset (1995), with slight modifications.

Principle of the Method:

The DPPH assay measures the ability of antioxidants in the sample to donate hydrogen or electrons to the stable free radical DPPH, reducing it to a non-radical form. This reduction causes a color change from deep violet to pale yellow, which can be measured spectrophotometrically at 517 nm.

Preparation of Reagents and Samples:

A 0.1 mM solution of DPPH was prepared in methanol. Exactly 1.0 ml of this DPPH solution was added to 1.0 ml of the sample extract in a test tube. The mixture was vortexed and then incubated in the dark at room temperature for 30 minutes.

Measurement of Absorbance:

After incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV-Visible spectrophotometer. Methanol was used as the blank, and a control was prepared by replacing the sample with methanol.

Calculation of Radical Scavenging Activity:

The percentage of DPPH radical scavenging activity was calculated using the following formula:

Reduction of absorbance (%) = [(AB - AA) / AB] x 100

AB = Absorbance of blank sample (t = 0 min)

AA = Absorbance of tested extract solution (t = 30 min)

DNA Extraction Protocol for Bacteria Samples

Genomic DNA was extracted from the samples received using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Catalogue No. D4300).

The protocol followed is highlighted below:

1. 250ul of the sample was added to a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm). Afterwards, 750ul of ZymoBIOMICS™ Lysis Solution was added into the tube.

2. The tube was secured in a bead beater (Disruptor Genie) and processed for 20 minutes.

3. The ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) was thereafter centrifuged at 10000 x g for 1 minute.

4. 400ul of the resulting supernatant was transferred

into a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8000 x g for 1 minute. Afterwards, the Zymo-Spin™ III-F Filter was discarded.

5. 1200ul of ZymoBIOMICS™ DNA Binding Buffer was added to the filtrate obtained at Step 4 and mixed well.

6. 800ul of the mixture from Step 5 was transferred to a Zymo-Spin™ IC Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute.

7. The flow-through was discarded and Step 6 was repeated.

8. The Zymo-Spin™ IC Column was transferred to a new collection tube and 400ul of ZymoBIOMICS™ DNA Wash Buffer 1 was added to it. This was thereafter centrifuged at 10,000 x g for 1 minute.

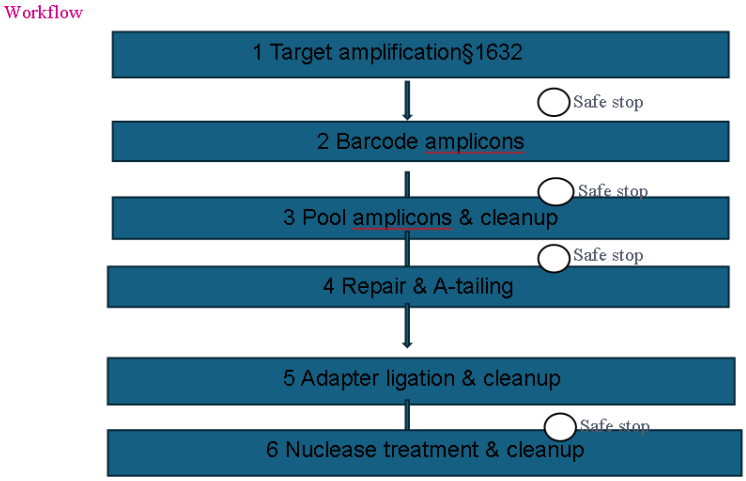
9. The flow-through from Step 8 was discarded and 700 µl of ZymoBIOMICS™ DNA Wash Buffer 2 was added to the ZymoSpin™ IC Column in a collection tube and centrifuged at 10,000 x g for 1 minute.

10. The flow-through from Step 9 was discarded and 200 µl of ZymoBIOMICS™ DNA Wash Buffer 2 was added to the ZymoSpin™ IC Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute.

11. The Zymo-Spin™ IC Column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of ZymoBIOMICS™ DNase/RNase Free Water was added directly to the column matrix and incubated for 1 minute. The assembly was thereafter ccentrifuged at 10,000 x g for 1 minute to elute the DNA.

16S bacterial metagenomics workflow

Genomic DNA samples were PCR amplified using a universal primer pair 27F and 1492R - targeting the V1 -V9 region of the bacterial 16S rRNA gene. Resulting amplicons were barcoded with Pacbio M13 barcodes for multiplexing through limited cycle PCR. The resulting barcoded amplicons were quantified and pooled equimolar and AMPure PB bead-based purification



step was performed. The PacBio SMRTbell library was prepared from the pooled amplicons following manufacture protocol (attached). Sequencing primer annealing and Polymerase binding were done following SMRTlink Link software protocol (online) to prepare the library for sequencing on PacBio Sequel IIe system. The work flow is shown below:

Molecular docking

A flexible docking approach, as outlined by Trott and Olson (Trott O, Olson AJ. AutoDock Vina, 2010), was utilized to perform the molecular docking with minor modifications. The molecular docking analysis of the selected ligands with the target protein was performed utilizing PyRx (version 0.8), which integrates Auto Dock Vina. The PDBQT files for the proteins were produced utilizing their previously established PDB files as inputs. The receptor was made rigid by allowing all the ligand's linkages to rotate freely and by modifying the grid box to align with the active sites of the protein molecule. Text files containing score results were generated to enable manual comparative analysis following the completion of the molecular docking studies, with ten configurations established for each protein-ligand interaction across all phytocompounds. Minimum binding energy (BE, kcal/mol).

Protein Optimization

The crystal structure of COVID-19 main protease was obtained from RCSB using PDB ID 6LU7 and a resolution of 2.90 Å. located at (https://www.rcsb.org/structure/6LU7). The homology modeling was done using the Swiss Model Server (https://swissmodel.expasy.org/interactive/K84PtA/). The 3D structure of COVID-19 main protease was modeled using the coordinate file of a template from the protein data bank (PDB ID: 6LU7). Water and ligand coordinates were removed prior to molecular docking. In this experiment, Discovery Studio 2024 was used to remove the native ligand so the tested compounds could access the target protein's active sites later in the docking procedure. Subsequently, we determined the coordinates (X =

-25.2571882, Y = 184536746, and Z = 55.2194902) of the active site, and ultimately, we integrated the polar hydrogen into this structure.

Starting coordinates: Probiotics secondary metabolites

From the metagenomic analysis of full length 16s gene amplicons of probiotics found in fufu, ogi and iru, twelve bioactive compounds where identified through pubchem. The three-dimensional (3D) structures of the ligands were obtained in simple data format (SDF) from the PubChem server utilizing Open Babel within PyRx (version 0.8). This procedure effectively optimized the ligands to achieve their most stable conformations utilizing the Merck Molecular Force Field 94 (MMFF94).

RESULTS AND DISCUSSION

vitamin content:

The vitamin A concentration in the fermented food samples ranged from 10.16 to 28.16 mg per 100 grams. The ogi sample demonstrated a notably elevated vitamin A concentration (28.16 mg/100 g) in comparison to the minimum value recorded in iru oworo (10.16 mg/100 g) ($p < .05$). No significant differences ($p > .05$) were observed among garri, fufu, iru oworo, and iru pete. The values obtained in this study surpass the previously reported range of 2.19–987.5 µg/100 g for fermented vegetables (Kiczorowski *et al.*, 2022) and 0.291–0.548 mg/100 g in fermented sorghum–soybean weaning diets (Abimbola *et al.*, 2024). Their values exceed the 7.23–9.28 mg/100 g documented for lafun enriched with soy curd (Capulet A.U, 2023), while being comparable to those reported for traditional condiments such as ogiri, okpei, and dawadawa (12.59–16.03 mg/100 g) (Frances and Nwakuche, 2024). The significant prevalence of vitamin A deficiency in sub-Saharan Africa, especially among women and children (Oluba *et al.*, 2018), underscores the nutritional importance of fermented foods.

The content of Vitamin B1 (thiamine) varied between 0.08 and 0.79 mg/100 g, with iru pete exhibiting the highest concentration and fufu the lowest. Notable differences ($p < .05$) were identified among the samples. The observed values align with the range documented for garri derived from yam–cassava blends (0.18–0.19 mg/100 g) (Ibukun *et al.*, 2022) and are in agreement with thiamine concentrations found in masa (0.20–0.24 mg/100 g) (Lawal *et al.*, 2019). Nevertheless, the observed values are lower than those documented for soybean-fortified ogi (0.59–0.88 mg/100 g) (Omah *et al.*, 2021) and enriched lafun flour (1.21–8.85 mg/100 g) (Capulet *et al.*, 2023), indicating that fortification markedly improves thiamine content.

Vitamin B2 (riboflavin) concentrations varied between 0.04 and 0.40 mg/100 g, with fufu exhibiting the highest level and ogi the lowest. No significant difference ($p > .05$) was detected between fufu and ogi; however, other samples exhibited significant variation ($p < .05$). The observed riboflavin contents align with those documented in fermented vegetables (0.03–0.06 mg/100 g) (Knez *et al.*, 2023) and maize–pigeon pea ogi (0.14–1.40 mg/100 g) (Okafor *et al.*, 2017). Riboflavin is essential for iron and folate metabolism and contributes to the maintenance of mucosal integrity within the digestive system (Abimbola *et al.*, 2024; Oyarekua *et al.*,2022).

The content of Vitamin B3 (niacin) varied between 0.38 and 5.11 mg/100 g, with iru pete exhibiting the highest concentration and ogi the lowest ($p < .05$). The values for iru-based condiments align with those documented for enriched lafun, ranging from 1.12 to 9.70 mg/100 g (Capulet, A.U., 2023). In contrast, foods based on cassava and maize (garri, fufu, ogi) exhibited lower values, aligning with prior reports (0.27–0.63 mg/100 g) (Ibukun *et al.*,2022). Only iru oworo and iru pete met the recommended daily intake levels for vulnerable populations (2–18 mg/day) (Lawal *et al.*, 2019), suggesting that these condiments serve as superior dietary sources of niacin. Niacin plays a critical role in energy metabolism and is vital for the maintenance of healthy skin, eyes, and liver function (Oyarekua *et al.*, 2022).

Vitamin B6 (pyridoxine) concentrations varied between 0.16 and 4.18 mg/100 g, with iru pete exhibiting the highest level and garri the lowest. No significant difference ($p > .05$) was observed between garri and fufu; however, other samples exhibited significant differences ($p < .05$). The values are partially consistent with those documented for fortified ogi (1.11–2.52 mg/100 g) (Adelekan *et al.*,2021) and enriched lafun (1.35–2.23 mg/100 g) (Capulet, A.U., (2023), suggesting that fermentation and enrichment may affect pyridoxine concentrations.

The Vitamin B12 content exhibited significant variability, ranging from 0.00 to 465.11 mg per 100 g. Iru pete exhibited the highest concentration, while ogi showed no detectable levels of vitamin B12. Statistically significant differences ($p < .05$) were identified among the samples, except for the comparison between fufu and ogi ($p > .05$). The minimal concentrations in cassava- and maize-based foods indicate their plant origin, which is devoid of vitamin B12 (Abiodun *et al.*,2024). The elevated values detected in iru samples may be ascribed to microbial synthesis occurring during fermentation. These values significantly exceed previously reported ranges, including 5.61–6.32 mg/100 g in enriched lafun (Capulet, A.U., (2023) and 0.09–0.39 µg/100 g in cereal–legume blends (Adebayo *et al.*, 2024) , indicating a requirement for further validation. Vitamin B12 is essential for neurological function and the preservation of the myelin sheath (Rizzo, G. and Laganà, A. S. 2020).

The content of Vitamin C (ascorbic acid) varied from 0.12 to 135.62 mg/100 g, with iru pete exhibiting the highest concentration and ogi the lowest ($p < .05$). The low value of ogi indicates the restricted vitamin C content present in maize. The values for garri (10.16 mg/100 g) and pupuru (11.04 mg/100 g) align with earlier research on fermented products, which reported a range of 11.10–21.10 mg/100 g (Esther *et al.*, 2022). The vitamin C levels in iru samples (128.91–135.62 mg/100 g) were

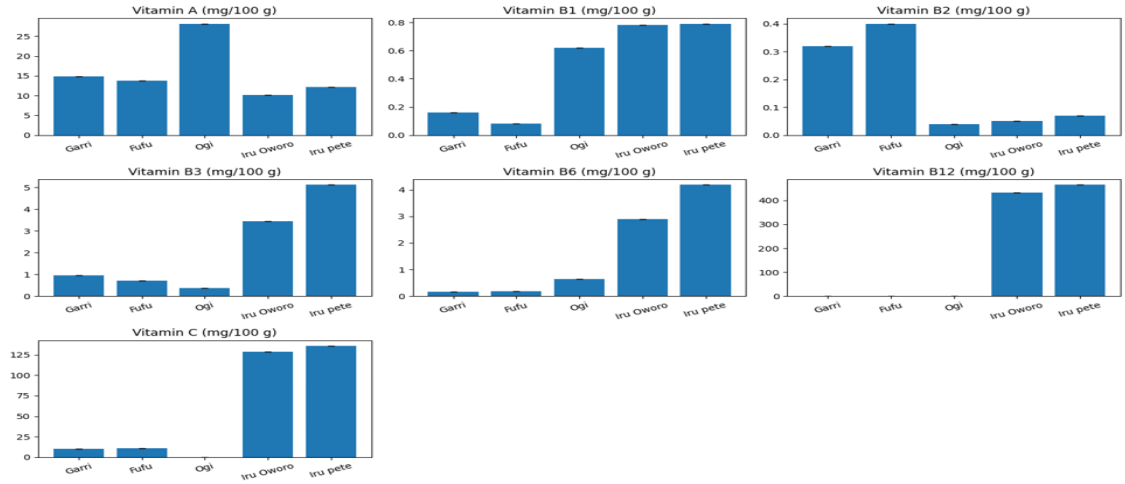


Figure 1: Vitamin composition of selected fermented food

significantly greater than previously reported values (0.56–19.31 mg/100 g) (Termote *et al.*,2022), potentially attributable to variations in processing, microbial activity, or the composition of raw materials.

The vitamin C (ascorbic acid) content in the fermented food samples ranged from 0.12 to 135.62 mg/100 g, with the highest level found in iru pete (135.62 mg/100 g) and the lowest in the ogi sample (0.12 mg/100 g). Significant differences ($p < .05$) were noted in the vitamin C contents of the fermented food samples. The lowest vitamin C content in the ogi sample indicates the relatively low vitamin C present in raw maize, measured at 0.12 mg (Shah *et al.*, 2016).

Antioxidant

DPPH Scavenging Activity (%Inhibition)

The DPPH radical scavenging activities of the fermented food samples exhibited a range of 51.24 to 87.93% inhibition per mg of dry matter. Iru pete demonstrated the highest activity with an inhibition rate of 87.93 % per mg DM, whereas fufu exhibited the lowest rate at 51.24 % per mg DM. Notable differences ($p < .05$) were detected among the samples. The findings align with the values presented by Okobia et al (Okobia *et al.*,2024), who recorded DPPH radical scavenging activities of 51.57–84.19 % inhibition/mg DM and 50.52–80.16 % inhibition/mg DM for pre-heated and non-preheated fufu, respectively, available in Ozoro, Nigeria. Ruth *et al.*(Ruth *et al.*,2018) documented DPPH scavenging activities between 37.0 and 59.0 % inhibition/mg DM for dawadawa derived from Mucuna pruriens and Parkia biglobosa, aligning with the results of the current study.

FERRIC Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the fermented food samples varied between 0.11 and 5.12 mmol/100 g, with significant differences identified at the 95% confidence level ($p < .05$). Iru pete demonstrated the highest reducing power at 5.12 mmol/100 g, whereas ogi exhibited the lowest value at 0.11 mmol/100 g.The measured antioxidant capacity aligns with values documented for processed garri derived from cassava roots, which varied from 3.76 to 12.37 µg TE/g dry weight (Laya, A., (2023), while acknowledging the need to consider variations in units and assay expression. In this study, the FRAP values measured are significantly lower than those documented for fermented moringa seeds (37.16–42.12 mmol/100 g) (James *et al.*, 2022) and quinoa

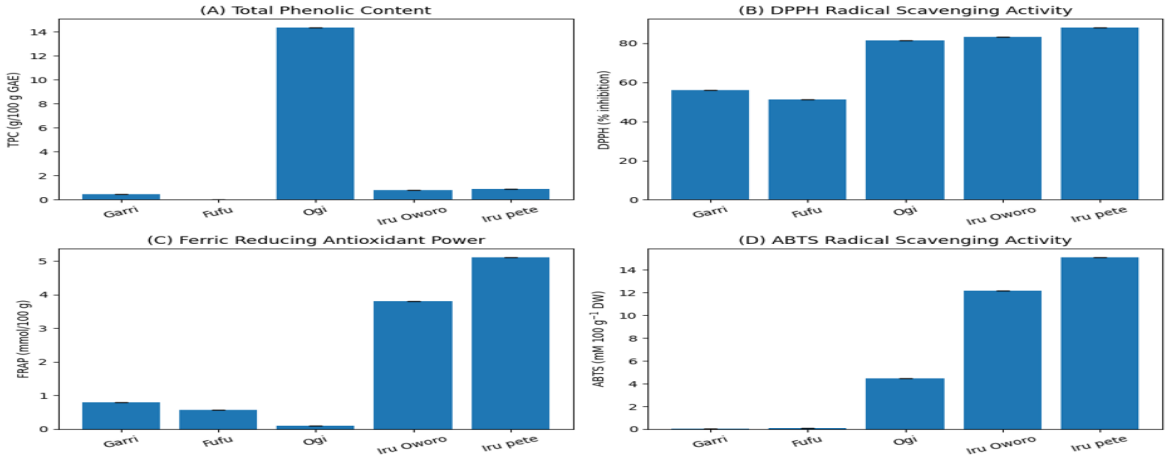


Figure 2: Antioxidant analysis of selected fermented food

dough fermented with Saccharomyces cerevisiae and lactic acid bacteria (19.02–91.73 mmol/100 g) (Arjmand *et al.*, 2023). The observed variations can be ascribed to discrepancies in raw material composition, fermentation processes, and the levels of bioactive compounds, including phenolics and flavonoids, which are recognized for their impact on reducing power.

Physiochemistry

The pH levels of the fermented food samples varied between 3.43 and 8.34, with iru pete showing the highest pH at 8.34 and ogi the lowest at 3.43 ($p < .05$). The elevated pH values detected in garri, iru oworo, and iru pete suggest a decrease in acidity, potentially affecting product safety, shelf life, and overall quality. Reduced acidity may diminish the inhibitory action of fermentation on spoilage microorganisms and pathogens, consequently elevating the risk of contamination. The low pH values observed for ogi and fufu indicate increased acidity, which is beneficial for preservation, as acidic environments inhibit microbial proliferation and improve food safety. The pH values recorded in this study align with previously documented ranges for fermented foods, such as garri (5.31–5.50) (Ozoh *et al.*, 2024), lafun (4.10–5.25) (Oyeyinka *et al.*, 2024), fermented sandbox condiments (5.4–5.7), and fermented locust bean and soybean condiments (8.70–8.90). In a similar manner, pupuru flour and other cassava-derived products in Nigeria have been documented to display pH ranges of 4.01–7.14 (Abass *et al.*,2019), corroborating the variability noted in the current study. The total titratable acidity (TTA) of the fermented food samples varied from 0.04% to 0.95%, with iru oworo exhibiting the highest value at 0.95% and fufu the lowest at 0.04%. Notable differences ($p < .05$) were identified among the samples. The values obtained exceed the 0.006–0.011% range reported for garri (Ozoh *et al.*, 2024) but are marginally lower than the 0.99–1.02% range documented for cassava roots subjected to fertilizer treatments. Variations in TTA may be associated with the availability of fermentable sugars and the metabolic activity of microorganisms, as these factors directly affect organic acid production during fermentation.

The total soluble solids (TSS) in the fermented food samples varied between 5.30% and 16.10%, with ogi presenting the highest value and fufu the lowest ($p < .05$). Excluding ogi, the total soluble solids (TSS) values of the other samples were predominantly lower than those documented for fermented green asparagus roots (11.73–19.83 °Brix) (Giang *et al.*, 2024), yet comparable to the values reported for Mahewu (4.57–5.43 °Brix) (Daji *et al.*, 2022) and naturally fermented obiolor (9.00 °Brix) (Ogundeji *et al.*, 2020).

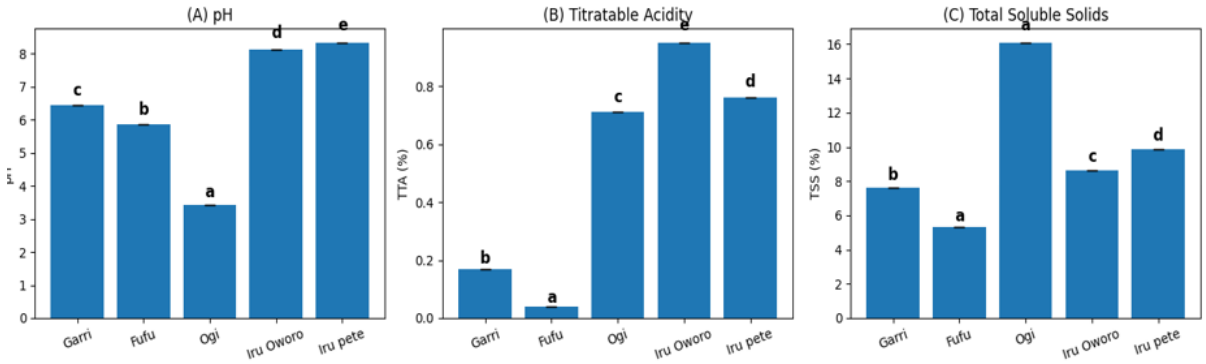


Figure 3: Physiochemistry composition of selected fermented food

The variations in total soluble solids (TSS) can be attributed to differences in the composition of raw materials, initial sugar content, microbial activity, fermentation duration, temperature, and processing methods. The aforementioned factors collectively impact the degradation of carbohydrates and the accumulation of soluble metabolites throughout the fermentation process.

Microbial Community Profiling by Full-Length 16S rRNA Sequencing

This report contains the summarized metagenomic analysis of full length 16s gene amplicons. Samples were sequenced on the Revio system by PacBio (www.pacb.com). Raw sub-reads were processed through the SMRTlink (v25.1.0.257715) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were processed through DADA2 (https://benjjneb.github.io/dada2/index.html) and QIIME2 (https://docs.qiime2.org/2021.11/) for quality control assessment and taxonomic classification, respectively

Molecular Docking

Computational methods are acknowledged as critical instruments in the design and identification of new therapeutic agents (Chirravuri *et al.*, 2025). The COVID-19 pandemic remains a significant global health challenge, leading to heightened interest in bioactive compounds for the development of antiviral drugs and the enhancement of the immune system. Prior coronavirus outbreaks, such as SARS and MERS, have highlighted the therapeutic potential of natural products, indicating their significance in the management of current and emerging viral

Table 3: 4a Species Level(FUFU-16S)

Species	Abundance	Percentage
Lactobacillus delbrueckii	3187	49.50%
Janthinobacterium tructae	2004	31.12%
Janthinobacterium lividum	403	6.26%
Janthinobacterium psychrotolerans	287	4.46%
Clostridium saccharobutylicum	139	2.16%
Janthinobacterium sp030944405	139	2.16%
Pseudomonas E sp002874965	128	1.99%
Pseudomonas E sp000242655	66	1.03%
Pseudomonas E paracarnis	54	0.84%
Limosilactobacillus fermentum	12	0.19%
Ureibacillus thermosphaericus	10	0.16%
Flavobacterium piscis	6	0.09%
Sphingobacterium sp000938735	4	0.06%

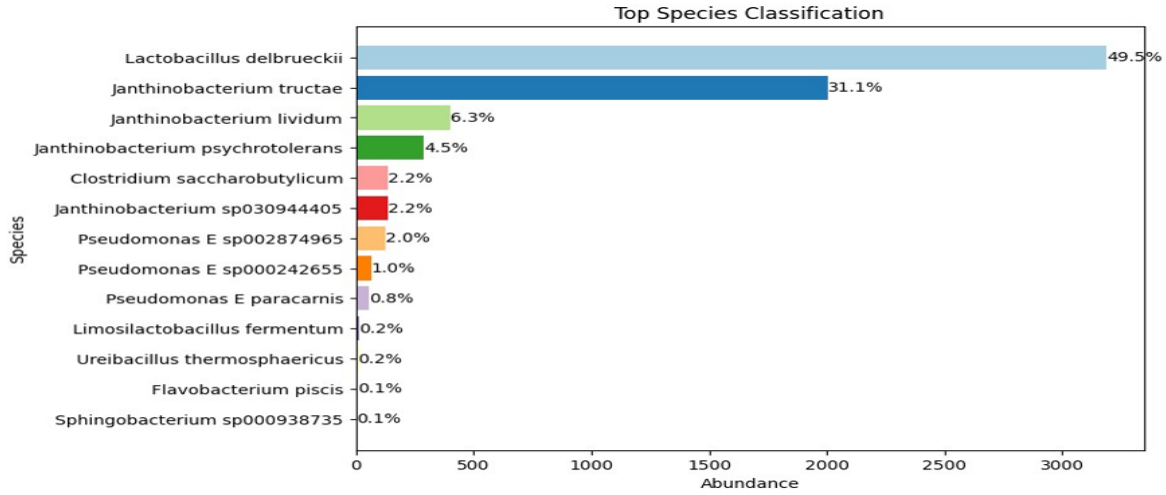


Figure 4a: Top species classification of Species Level(FUFU-16S)

Table 3: 4b Species Level (L0CUST-BEANS-16S)

Species	Abundance	Percentage
Staphylococcus arlettae	3840	54.16%
Staphylococcus saprophyticus	2304	32.50%
Staphylococcus piscifermentans	724	10.21%
Staphylococcus hominis	222	3.13%

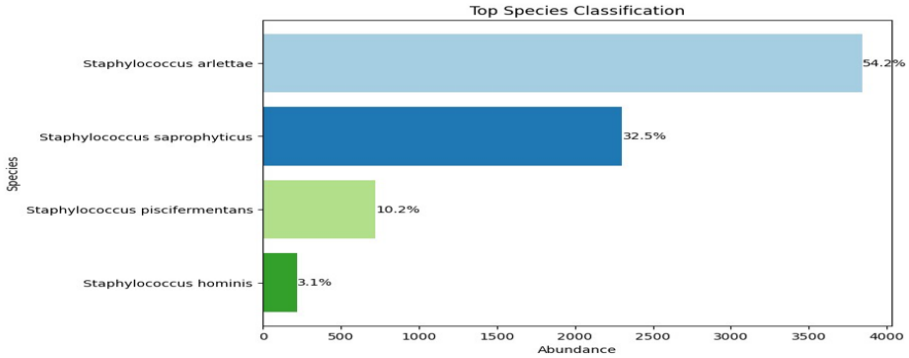


Figure 4b: Top species classification of Species Level(L0CUST-BEANS-16S)

Table 4c: Species Level(PAP-16S)

Species	Abundance	Percentage
Lactobacillus delbrueckii	8907	98.73%
Limosilactobacillus fermentum	73	0.81%
Lactobacillus helveticus	40	0.44%
Lactiplantibacillus plantarum	2	0.02%

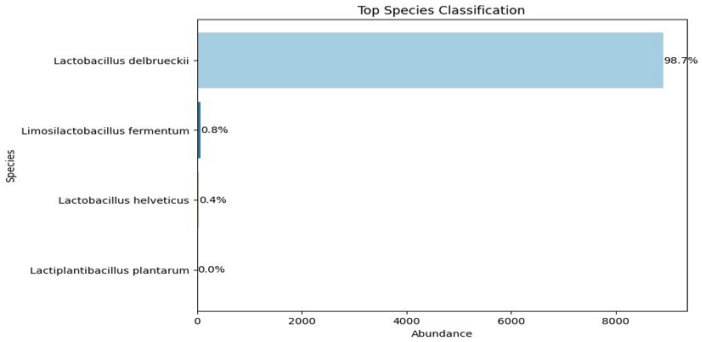


Figure 4b: Top species classification of Species Level(PAP-16S)

infections. Of the thirteen bioactive compounds assessed (Table 1), violacein and sitagliptin exhibited the highest binding affinity for the crystal structure of the SARS-CoV-2 main protease (Mpro). Violacein demonstrated the highest docking score of −8.0 kcal/mol, followed by sitagliptin at −7.5 kcal/mol, both surpassing the reference drug nirmatrelvir, which scored −6.6 kcal/mol, and is an FDA-approved antiviral agent. The results suggest potential interactions with the viral protease and warrant further experimental validation. Interaction analysis indicated that violacein established stable hydrogen bond interactions with critical active site residues, specifically

Table 4a: Bioactive compound in Lactobacillus delbrueckii (tripeptide)

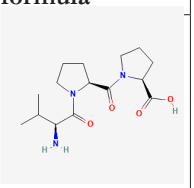
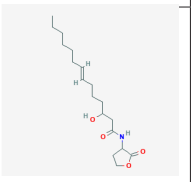
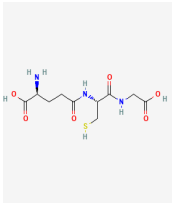
S/N	Compound	Molecular formula	Molecular mass	PubChem CID	Molecular formula
1.	Val-pro-pro	$C_{15}H_{25}N_3O_4$	311.38 g/mol	9818200	
2.	bacteriocin	$C_{18}H31NO_4$	325.4 g/mol	17933559	

Table 4b: Bioactive compound in Limosilactobacillus fermentum

3.	Glutathione	$C_{10}H_{17}N_3O_6S$	307.33 g/mol	124886	

THR23, HIS41, CYS145, HIS164, and THR26. Sitagliptin formed multiple hydrogen bonds with residues including ALA285, LEU286, LEU28, LYS137, THR199, TYR239, GLY275, LEU271, GLU290, and ASP289. The interactions demonstrate significant binding stability within the ligand-binding domain of the protease. Figures 2 and 3 illustrate the two-dimensional interaction profiles of the ligand–protein complexes.

Table 4c: Bioactive compound in Lactobacillus helveticus

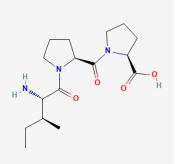
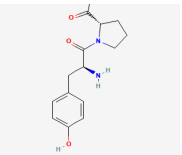
4.	Isoleucyl-prolyl-proline	$C_{16}H_{27}N3O_4$	325.40 g/mol	9949212	
5.	Tyrosyl-proline	$C_{14}H_{18}N_2O_4$	278.30 g/mol	9795637	

Table 4d: Bioactive compound in Lactobacillus helveticus

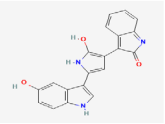
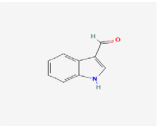
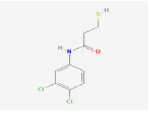
6.	Violacein	$C_{20}H_{13}N_3O_3$	343.3 g/mol	11053	
7.	Indole-3-Carboxaldehyde	C_9H_7NO	145.16 g/mol	10256	
8.	Metallo-|A-lactamase-IN-2	$C_9H_9C_{l2}NOS$	250.14 g/mol	83773718	

Table 4e: Bioactive compound in Pseudomonas E

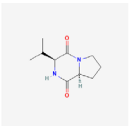
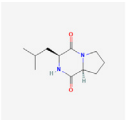
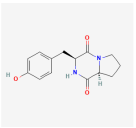
9.	cyclo(L-Pro-L-Val)	$C_{10}H_{16}N_2O_2$	196.25 g/mol	6992261	
10.	Gancidin W	$C_{11}H_{18}N_2O_2$	210.27 g/mol	7074739	
11.	cyclo(L-Pro-L-Tyr)	$C_{14}H_{16}N_2O_3$	260.29 g/mol	119404	

Table 4f: Bioactive compound in Limosilactobacillus fermentum

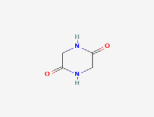
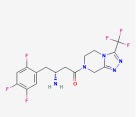
12.	2,5-Piperazinedione	$C_4H_6N_2O_2$	114.10 g/mol	7817	
13.	Sitagliptin	$C_{16}H_{15}F_6N_5O$	407.31 g/mol	4369359	

Table 5: Molecular docking results

S/N	Compound	Docking score(Kcal/mol)
1.	Val-pro-pro	-6.2
2.	bacteriocin	-5.2
3.	Glutathione	-4.8
4.	Isoleucyl-prolyl-proline	-5.6
5.	Tyrosyl-proline	-6.3
6.	Violacein	-8.0
7.	Indole-3-Carboxaldehyde	-5.0
8.	Metallo-|A-lactamase-IN-2	-4.2
9.	Gancidin W	-5.5
10.	cyclo(L-Pro-L-Tyr)	-6.5
11.	2,5-Piperazinedione	-4.5
12.	Sitagliptin	-7.5
13.	cyclo(L-Pro-L-Val)	-5.6
14	Nirmatrelvir(Standard)	-6.6

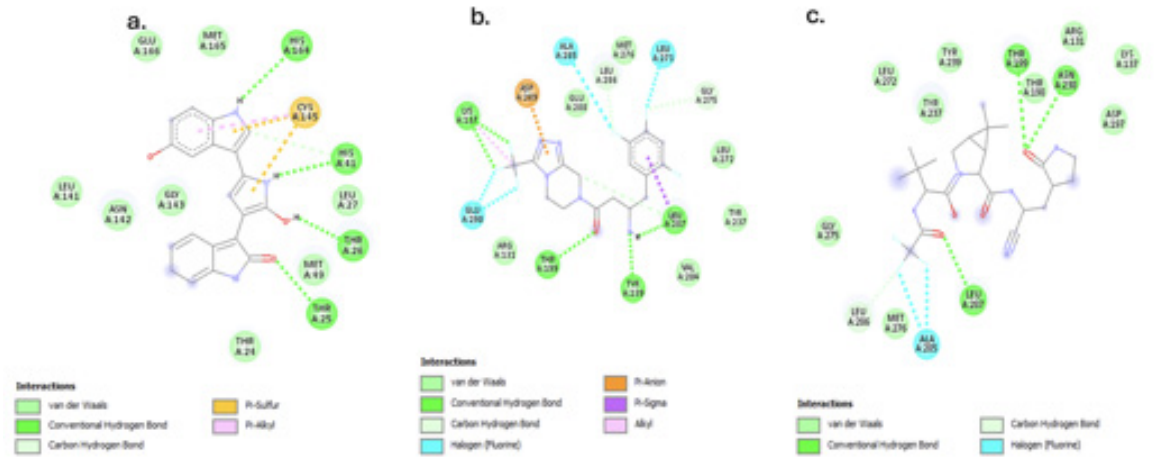


Figure 5: Two-Dimensional (2D) Molecular Interaction Analysis of the Lead Compounds with the Target Protein: (a) Violacein, (b) Sitagliptin, and (c) Nirmatrelvir (Reference Standard).

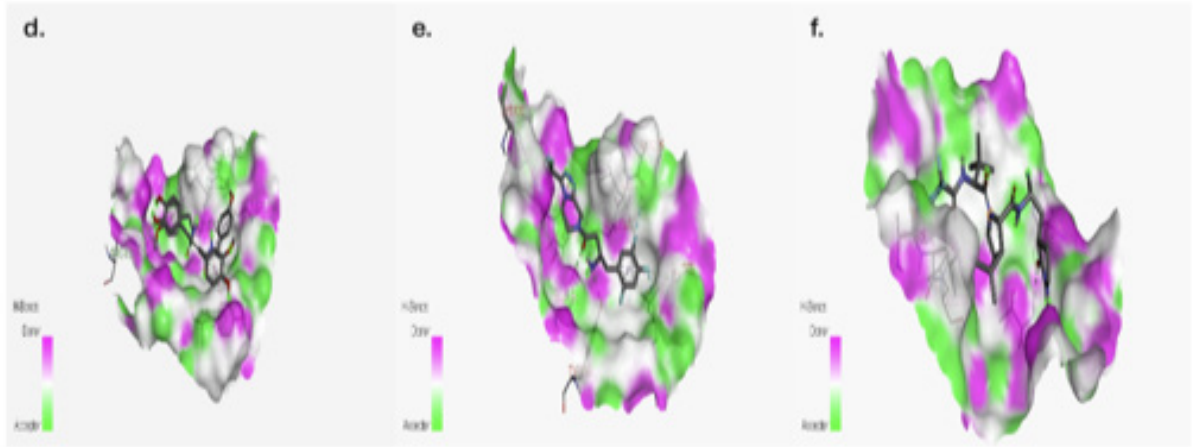


Figure 6: Three-Dimensional (3D) Binding Pose and Surface Interaction of the Lead Compounds within the Target Protein Binding Pocket: (d) Violacein, (e) Sitagliptin, and (f) Nirmatrelvir (Reference Standard).

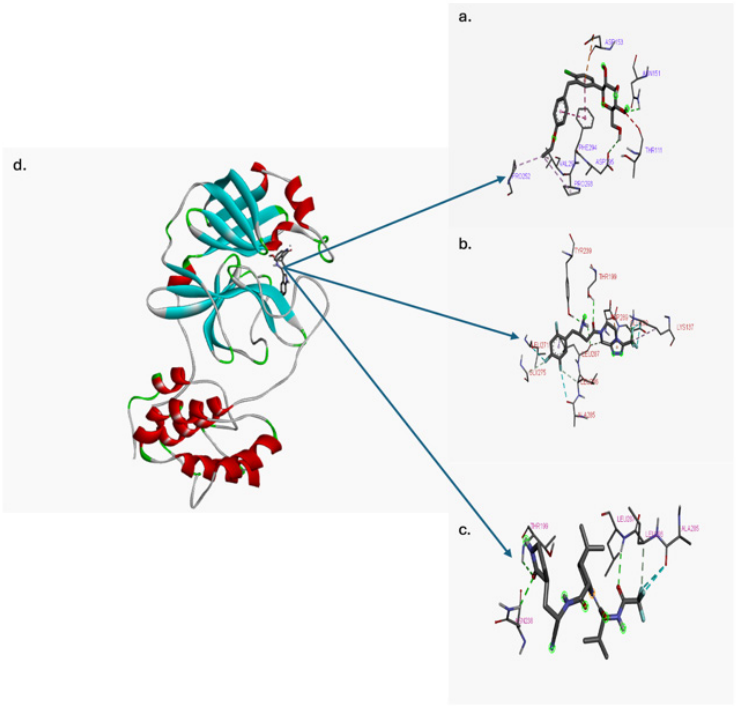


Figure 7: 3D view of the molecular interaction of the lead compounds with the receptor: (a.) Violacein (b.) Sitagliptin (c.) Nirmatrelvir(Standard) (d.) COVID-19 main protease

CONCLUSION

This research illustrates that traditional fermented foods serve as significant sources of essential micronutrients, antioxidant compounds, and probiotic microorganisms, which collectively play a role in immune modulation and may exhibit antiviral activity. Among the evaluated samples, iru pete demonstrated a superior nutritional composition, enhanced antioxidant capacity, and significant probiotic-associated bioactive potential. Metagenomic profiling confirmed the dominance of beneficial lactic acid bacteria, underscoring the significance of fermentation in improving microbial quality and functional characteristics. Molecular docking analysis revealed that the selected bioactive compounds, specifically violacein and sitagliptin, demonstrate a strong binding affinity for the SARS-CoV-2 main protease, suggesting potential interactions with SARS-CoV-2 Mpro that require further biological validation. The results, corroborated by in vitro assays and in silico predictions, offer mechanistic insights into the potential role of fermented foods and probiotics in functional nutrition and as adjuncts to antiviral strategies. These results must be interpreted with caution due to their reliance on experimental and computational models. Subsequent research should prioritize in vivo validation, evaluation of bioavailability, and the execution of clinical trials to determine their translational significance in the prevention and management of COVID-19.

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