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MULTI-OMICS AND NETWORK PHARMACOLOGY APPROACHES FOR DECODING INDIAN RICE-BASED TRADITIONAL FERMENTED FOODS

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Abstract

India's rice-based fermented preparations like Pazhaya Sadham (locally Neeragaram), Idli, Dosa, Uttapam, Appam and Kallappam, together with the millet-rice composites Koozh and Ambali are now being looked at seriously as functional foods, with documented probiotic, glycemic and gut-supportive properties. Their bioactive landscape and the mechanisms behind those reported effects, however, remain only partially worked out, because the older toolbox of culture isolation and single-target assays cannot do justice to either the compositional richness or the inherently multi-target pharmacology of a fermented matrix. This study sets out an integrated methodological framework that brings together five lines of evidence: amplicon sequencing of the 16S rRNA gene and shotgun metagenomics on the community side; untargeted and targeted metabolomics on GC-MS, LC-MS/MS and NMR platforms; a network-pharmacology pipeline running from compound retrieval through ADME and Lipinski-style filtering, target prediction, disease-target mining, protein–protein interaction analysis and pathway enrichment; molecular docking and dynamics simulations to validate binding modes; and the more recent multi-omics integration strategies that knit these layers together. Each step is laid out with the parameter thresholds in current use, the software ecosystem available, and the pitfalls that recur in published work, among them the routine misapplication of blind docking, the patchy reporting of MSI confidence levels in metabolomic studies, and the inflation of pseudo-targets in protein–protein interaction networks. The methodological literature on Indian rice ferments specifically remains thin, and so the present study leans on adjacent fermented systems such as Tualang honey, soy ferments and other Indian traditional foods as illustrative templates, while Indian rice ferments remain the central application context. The study closes by pointing to future directions, the construction of microbe–substrate–metabolite–target–disease networks, integration with strain-level genomics, and the development of defined microbial consortia for reproducible Indian rice ferment production.

Keywords: Indian fermented foods, metagenomics, metabolomics, network pharmacology, molecular docking.

I. Introduction

Traditional fermented foods sit at the meeting point of cultural heritage and contemporary functional-food science, and rice-based preparations occupy a particularly important place within that meeting point across South and Southeast Asia (Tamang et al., 2020). Across India, rice is a dominant fermentation substrate. It carries the entire weight of preparations like Pazhaya Sadham (Neeragaram) and Kanji; it forms the bulk of cereal–pulse batters such as Idli, Dosa, Uttapam, Appam and Kallappam; and it appears as a minor but consistent component in millet-based gruels including Koozh and Ambali (Antony & Chandra, 2020; Ilango & Antony, 2014; Kumar et al., 2013). For all the cultural depth of this tradition, the scientific characterisation of these foods has lagged the cultural and economic value they carry. The bulk of published work has rested on culture isolation, single-target enzymatic assays and proximate composition analysis,

none of which is well-suited to capture the multi-microbe, multi-metabolite, multi-target nature of a fermented matrix.

The past decade has, however, brought a methodological shift. High-throughput sequencing has redrawn what we know about the microbial diversity of fermented foods (Mandhania et al., 2019; Kavitake et al., 2022). Mass-spectrometry-based metabolomics has surfaced metabolite landscapes far richer than the older targeted approaches could ever show (Mannaa et al., 2021; Wen et al., 2023). Network pharmacology has emerged as a tool for mapping the polypharmacology of natural-product mixtures onto disease-relevant target networks (Hopkins, 2008; Shamsol Azman et al., 2023). Molecular docking and dynamics have provided structural validation for those predictions (Pinzi & Rastelli, 2019). Multi-omics integration is now increasingly seen as the natural endpoint of all this (Mannaa et al., 2021; Wen et al., 2023).

One thing has to be acknowledged upfront: the published methodological literature on Indian rice ferments specifically is still modest. The most rigorous applications of network pharmacology and multi-omics to fermented systems have so far come out of work on honey, soy ferments, kimchi and East Asian rice and dairy ferments (Elkhalifa et al., 2023; Shamsol Azman et al., 2023; Wen et al., 2023). The present study leans on those adjacent systems as methodological templates and applies the resulting framework to Indian rice ferments, using Pazhaya Sadham, Idli and Koozh as the primary application context.

The intent is not to recycle descriptive ethnobotany, that ground has been well covered elsewhere. Instead, we walk step by step through the workflow that takes a fermented food sample from collection through to a defensible multi-omics characterisation and a credible network-pharmacology hypothesis. The aim is a practical roadmap for researchers in food science, food microbiology, nutrition and ethnopharmacology working with Indian fermented foods who need to translate this methodological revolution into work that is reproducible, transparent and clinically relevant.

II. Rice-Based Fermented Foods of India

2.1 Pazhaya Sadham (Neeragaram)

Pazhaya Sadham — Neeragaram in colloquial Tamil is a household preparation in which cooked rice, set aside at the end of the day, is left covered with water overnight and consumed together with the soaking liquid the next morning (Anandharaj et al., 2015; Kumar et al., 2013). The dish has remained part of household nutrition across Tamil Nadu, Andhra Pradesh, Kerala, Karnataka, West Bengal and Odisha for generations, particularly in agricultural communities where it is valued for its cooling, hydrating and reportedly restorative qualities. Mineral composition work has shown that the preparation is a substantial source of calcium, potassium, sodium and iron, with leaching from the rice during overnight soaking presumed to drive part of this enrichment (Kumar et al., 2013). The microbial community of Pazhaya Sadham, by contrast, has been characterised only in fragments, and a thorough metagenomic profile remains an active research priority.

2.2 Idli, Dosa, Uttapam, Appam and Kallappam

Idli is the most thoroughly studied South Indian fermented food and the de facto reference system for cereal–pulse fermentation in India. Its overnight batter fermentation has been the subject of multiple high-throughput sequencing studies (Kavitake et al., 2022; Mandhania et al., 2019). Dosa, Uttapam, Appam and Kallappam are closely related preparations made from variants of the same cereal–pulse batter, differing in rice-to-pulse ratio, additional ingredients, and the final cooking step. From a methodological standpoint, the relative wealth of microbiological data on Idli makes it a useful benchmark against which less-studied Indian rice ferments can be compared.

2.3 Koozh and Ambali

Koozh and Ambali are millet-based fermented gruels that pair finger millet (*Eleusine coracana*) or pearl millet (*Pennisetum glaucum*) with a small fraction of rice (Antony & Chandra, 2020; Ilango & Antony, 2014). Although millets are the dominant substrate, the rice contribution carries both nutrients and microbial inoculum, and the community that emerges is distinct from that of pure cereal–pulse batters. With the global resurgence of interest in millets, these preparations are well placed to attract renewed scientific attention.

2.4 Indian rice cultivars and their metabolomic distinctiveness

The metabolomic background of Indian rice ferments cannot be discussed in isolation from the rice cultivars used. Indian rice landraces among them the South Indian Milagu Samba, Mappillai Samba, Seeraga Samba, Kichili Samba and Kullakar, plus pigmented and non-pigmented landraces from other regions differ markedly in pigmentation, secondary metabolite content and grain physicochemistry (Goufo & Trindade, 2014). Pigmented rice varieties are particularly noted sources of phenolic acids, flavonoids, anthocyanins and tocopherols, which collectively set the bioactive baseline of any fermented preparation built on them (Goufo & Trindade, 2014). This cultivar-level metabolomic specificity has direct

consequences for the bioactive landscape of the fermented preparations made from each. Table 1 summarises the major Indian rice-based fermented foods alongside their substrates, fermentation conditions, dominant microbiota and reported key metabolites.

Table 1. Indian rice-based traditional fermented foods, their substrates, fermentation parameters, dominant microbiota and reported key metabolites.

Food	Substrate	Fermentation conditions	Dominant microbiota	Reported key metabolites	References
Pazhaya Sadham (Neeragaram)	Cooked rice + water	Overnight (8-12 h), ambient	*Lactobacillus*, *Weissella*, *Pediococcus*, yeasts	Lactic acid; minerals (Ca, K, Na, Fe); B vitamins	Kumar et al. (2013); Anandharaj et al. (2015)
Kanji	Rice water	6-24 h, ambient	LAB and yeasts	Organic acids; B vitamins	Ray et al. (2016)
Idli	Black gram + rice (1:2 to 1:4)	8-16 h, ambient	*Lactiplantibacillus plantarum*, *Weissella confusa*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Saccharomyces cerevisiae*	GABA, EPS, bacteriocins, B vitamins	Mandhania et al. (2019); Kavitake et al. (2022)
Dosa / Uttapam	Black gram + rice	8-16 h, ambient	Similar to Idli	Similar to Idli	Soni et al. (1986)
Appam / Kallappam	Rice + coconut milk or palm sap	6-12 h, ambient	LAB and yeasts	Organic acids; coconut-derived volatiles	Kumar et al. (2010)
Koozh	Finger millet / pearl millet ± rice	12-18 h, ambient	*Enterococcus hirae*, *E. faecalis*, *L. plantarum*, *Bacillus amyloliquefaciens*	Phenolics, organic acids, bacteriocins	Ilango & Antony (2014); Ilango et al. (2016)
Ambali	Finger millet / pearl millet	12-18 h, ambient	LAB and yeasts	Phenolics, organic acids	Antony & Chandra (2020)

III. Conventional Versus Omics Approaches

Microbiological characterisation of fermented foods has historically rested on culture isolation on selective media, biochemical profiling, and (more recently) Sanger sequencing of the 16S rRNA gene on individual isolates. These approaches built much of the foundational knowledge in the field but suffer from well-known constraints: the cultivable fraction of any microbial community is typically a small slice of what is actually present, slow-growing or fastidious taxa are systematically passed over, and the dynamics of community succession during fermentation are hard to capture at any meaningful temporal resolution (De Filippis et al., 2018; Walsh et al., 2017). The metabolite side has historically depended on equally narrow tools, with single-compound HPLC or GC assays targeting lactic acid, a handful of organic acids, or selected volatiles. Useful, but nowhere near sufficient for a fermented matrix that may carry hundreds of metabolites of substrate, microbial and interaction origin.

Omics approaches tackle these constraints head-on. Metagenomics captures the entire microbial community, including unculturable taxa, in a single sequencing run. Metabolomics particularly when run on high-resolution mass spectrometers can profile hundreds to thousands of metabolites untargeted. The pairing of the two allows the obvious next question to be asked: are the metabolites observed consistent with the microbial functional potential present in the sample? The constraints have moved from those of biological access to those of computational interpretation, and the methodological choices made along the workflow now matter more than the raw availability of data (Mannaa et al., 2021; Wen et al., 2023).

IV. Metagenomics in Fermented Food Research

4.1 Amplicon sequencing of the 16S rRNA gene

Amplicon sequencing of the 16S rRNA gene continues to be the workhorse of fermented-food microbiome research, on grounds of low per sample cost, a well-established workflow, and straightforward downstream analysis. For bacterial communities in fermented foods, the V3–V4 hypervariable region is the most commonly targeted segment, typically run on the Illumina MiSeq with 2 × 300 bp paired-end chemistry (De Filippis et al., 2018). DNA extraction is the single most consequential step before sequencing even begins. Cereal–pulse batters and rice ferments combine PCR inhibitors with tough microbial cell walls, a combination that effectively forces the user into bead-beating protocols; the choice of extraction kit can reshape the recovered community profile to a surprising degree. Mock-community controls and extraction blanks, although still inconsistently included in published Indian work, are essential for credible analysis and ought to be treated as standard practice going forward.

The downstream bioinformatics workflow has moved decisively from the older operational taxonomic unit (OTU) clustering approach to amplicon sequence variants (ASVs) generated by DADA2 or Deblur (Callahan et al., 2017). ASVs deliver single-nucleotide resolution, are reproducible across studies, and now represent current best practice. QIIME2 (Bolyen et al., 2019) gives a comprehensive ecosystem for ASV-based analysis with taxonomy assignment against SILVA, Greengenes2 or GTDB. The analytical core is built around alpha-diversity metrics (Shannon, Simpson, Faith's phylogenetic diversity), beta-diversity ordinations (UniFrac, Bray-Curtis) and differential abundance testing (DESeq2, ANCOM, ALDEx2). Functional prediction from 16S data is available through PICRUSt2 (Douglas et al., 2020) with the explicit caveat that this is inference from gene-content prediction, not direct measurement of function, and that prediction accuracy varies with ecosystem type.

4.2 Shotgun metagenomics

Shotgun metagenomic sequencing addresses several constraints of amplicon-based work in one stroke. It delivers species and strain-level resolution where 16S rarely resolves below the genus level; it picks up fungi, viruses and archaea alongside bacteria; and it allows direct functional profiling through gene content rather than through inference (De Filippis et al., 2018; Mannaa et al., 2021). The bioinformatic pipeline typically opens with quality control and host-read removal, then moves to *de novo* assembly with MEGAHIT or metaSPAdes, gene calling with Prodigal, and binning into metagenome-assembled genomes (MAGs) using MetaBAT2, MaxBin2 or CONCOCT. Functional profiling can then be run either at the read level using HUMAnN3 against UniRef90 and MetaCyc, or at the assembly level by annotating predicted genes against KEGG, eggNOG and CAZy.

Recovery of high-quality MAGs from fermented food samples is now increasingly feasible and gives an unprecedented level of resolution. Recent shotgun studies on fermented foods have demonstrated that MAGs meeting MIMAG completeness and contamination thresholds can be reliably recovered (Walsh et al., 2017). For Indian rice ferments, shotgun metagenomics remains underused, and that represents a clear methodological opportunity for the field.

4.3 Case studies in Indian fermented food metagenomics

Mandhania and colleagues (2019) published the first comprehensive 16S rRNA-based survey of Idli batter fermentation, demonstrating that *Weissella* dominates the active fermentation phase between the 6th and 12th hour. Kavitake and co-workers (2022) extended that work and showed a clear shift from *Proteobacteria* dominance at time zero to *Firmicutes* dominance late in fermentation, with *Lactococcus*, *Weissella*, *Pediococcus*, *Lactobacillus*, *Enterococcus*, *Bacillus* and *Macrococcus* as principal genera. Comparable metagenomic work on other Indian fermented foods, particularly those of the North-East including kinema and gundruk, has progressed faster (Tamang et al., 2022) and offers useful methodological precedents for application to Indian rice ferments.

V. Metabolomics Applications in Fermented Foods

5.1 GC-MS-based metabolomics

Gas chromatography–mass spectrometry remains the default platform for analysing volatile and small polar metabolites in fermented foods. Headspace solid-phase microextraction (HS-SPME) coupled to GC-MS suits fermented matrices particularly well, since it captures the volatile fingerprint without elaborate sample preparation and without solvents that might interfere with downstream analysis. Sample preparation typically runs through equilibration at controlled temperature, fibre exposure to the headspace, thermal desorption into the GC inlet, separation on a polar or mid-polarity capillary column, and detection on a quadrupole or time-of-flight analyser (Wen et al., 2023).

Compound identification is conducted against spectral libraries - NIST, Wiley and the Golm Metabolome Database are the standard choices with retention indices on a homologous alkane series providing a second

dimension of confidence. Reporting of MSI confidence levels (Sumner et al., 2007) should be transparent for every compound called. Level 1 is identification confirmed against an authentic reference standard run on the same instrument under the same conditions; Level 2 is a putative annotation by spectral library match and retention index; Level 3 is a putative compound class; and Level 4 is an unknown spectrum. Most published HS-SPME-GC-MS work on Indian rice ferments sits at MSI Level 2, and that should be stated explicitly in any publication for methodological transparency.

The metabolomic specificity of Indian rice cultivars is itself a consideration that fermented-food researchers cannot afford to ignore. Pigmented and aromatic rice landraces differ substantially in phenolic, flavonoid and tocopherol content from non-pigmented mainstream cultivars (Goufo & Trindade, 2014), and those baseline differences propagate into the fermented preparations downstream. Cultivar level baseline metabolomics is therefore an important context for any subsequent metabolomic study on an Indian rice ferment.

5.2 LC-MS/MS-based metabolomics

Coupling liquid chromatography to high-resolution tandem mass spectrometry extends metabolic coverage to non-volatiles such as phenolics, flavonoids, peptides, polar lipids and glycosylated species. Reversed-phase chromatography on C18 columns covers the semi-polar to non-polar range, while hydrophilic interaction liquid chromatography (HILIC) picks up the polar metabolites that elute too early on reversed-phase systems. Orbitrap and Q-TOF mass analyzers deliver the resolving power and mass accuracy that confident annotation demands. For untargeted profiling, data-independent acquisition (DIA) is steadily replacing data-dependent acquisition (DDA), since DIA delivers better reproducibility and more comprehensive fragment coverage (Wen et al., 2023).

5.3 NMR-based metabolomics

Nuclear magnetic resonance spectroscopy contributes a complementary view. It is non-destructive, quantitative without compound-specific calibration, and excellent for the major-metabolite classes such as sugars, organic acids, amino acids, short-chain fatty acids. Its main limitation is sensitivity, which restricts its use for low-abundance metabolites. But for the major-metabolite landscape of a rice ferment, it remains highly informative (Wen et al., 2023). The complementarity of GC-MS, LC-MS/MS and NMR has driven a steady move toward platform-integrated approaches in fermented-food metabolomics.

5.4 Bioinformatics for metabolomics

The bioinformatics workflow for untargeted metabolomics typically opens with raw data conversion to mzML or mzXML format, then runs through peak picking with XCMS or MZmine, alignment, gap filling and annotation against community libraries like MoNA, MassBank, GNPS and HMDB are the standard set. MetaboAnalyst (Pang et al., 2024) is widely used for downstream univariate and multivariate statistics, including PCA, OPLS-DA, hierarchical clustering and pathway mapping. Reporting standards from the Metabolomics Standards Initiative should be followed throughout (Sumner et al., 2007).

VI. Network Pharmacology Workflow for Fermented Food Bioactives

6.1 Conceptual framework

Network pharmacology was developed to address the basic mismatch between the multi-target nature of natural products and the single-target paradigm of conventional pharmacology (Hopkins, 2008; Zhang et al., 2021). For fermented foods, where the bioactive payload includes substrate-derived metabolites, microbial metabolites and interaction products, that multi-target perspective is even more apt. The workflow runs through compound retrieval, ADME and drug-likeness filtering, target prediction, disease-target intersection, protein–protein interaction analysis, pathway enrichment, and validation through molecular docking and dynamics. Figure 1 presents a schematic overview as applied to rice-based fermented foods.

6.2 Compound retrieval

The first step in any network-pharmacology workflow is the assembly of a chemically defensible compound list. For fermented foods, the list should be drawn wherever possible from the metabolomic results of the same or comparable samples, supplemented by literature-based compounds known from the substrate and from the dominant microbial community. PubChem provides canonical SMILES, InChI and three-dimensional conformers; KNApSAcK is particularly useful for plant-derived metabolites; FoodB covers food chemicals at large; and the FooDB project includes microbial metabolites. Standardising compound IDs across databases through SMILES or InChIKey is essential without it, the duplication that creeps in inflates downstream networks.

6.3 ADME and drug-likeness filtering

ADME (absorption, distribution, metabolism, excretion) screening narrows the compound list down to species likely to be orally bioavailable and biologically meaningful. SwissADME (Daina et al., 2017) is the most widely used free tool. The standard filter is Lipinski's rule of five — molecular weight ≤ 500, LogP ≤ 5, no more than five hydrogen-bond donors and no more than ten acceptors which, as originally formulated, allows up to one violation. Veber's rules (rotatable bonds ≤ 10, polar surface area ≤ 140 Å²) are often layered on as a secondary filter. Several authors retain compounds that violate one of these rules in order to avoid losing bioactives that exhibit non-classical bioavailability behaviour. The blood-brain barrier (BBB) score that SwissADME outputs is informative when neuroactive effects are of interest, although for simple volatile compounds, the BBB prediction needs to be read with caution.

6.4 Target Prediction

Target prediction maps each compound onto the human protein space. SwissTargetPrediction (Daina et al., 2019) is the most widely used tool and applies a similarity-based approach against a curated database of known compound–protein interactions. A confidence threshold of 70% is conventional, and many studies retain only the top 100 predicted targets per compound to keep the downstream network size manageable. BindingDB and STITCH offer complementary prediction streams. The output is consolidated into a non-redundant list of human protein targets, typically annotated with UniProt and gene-symbol identifiers.

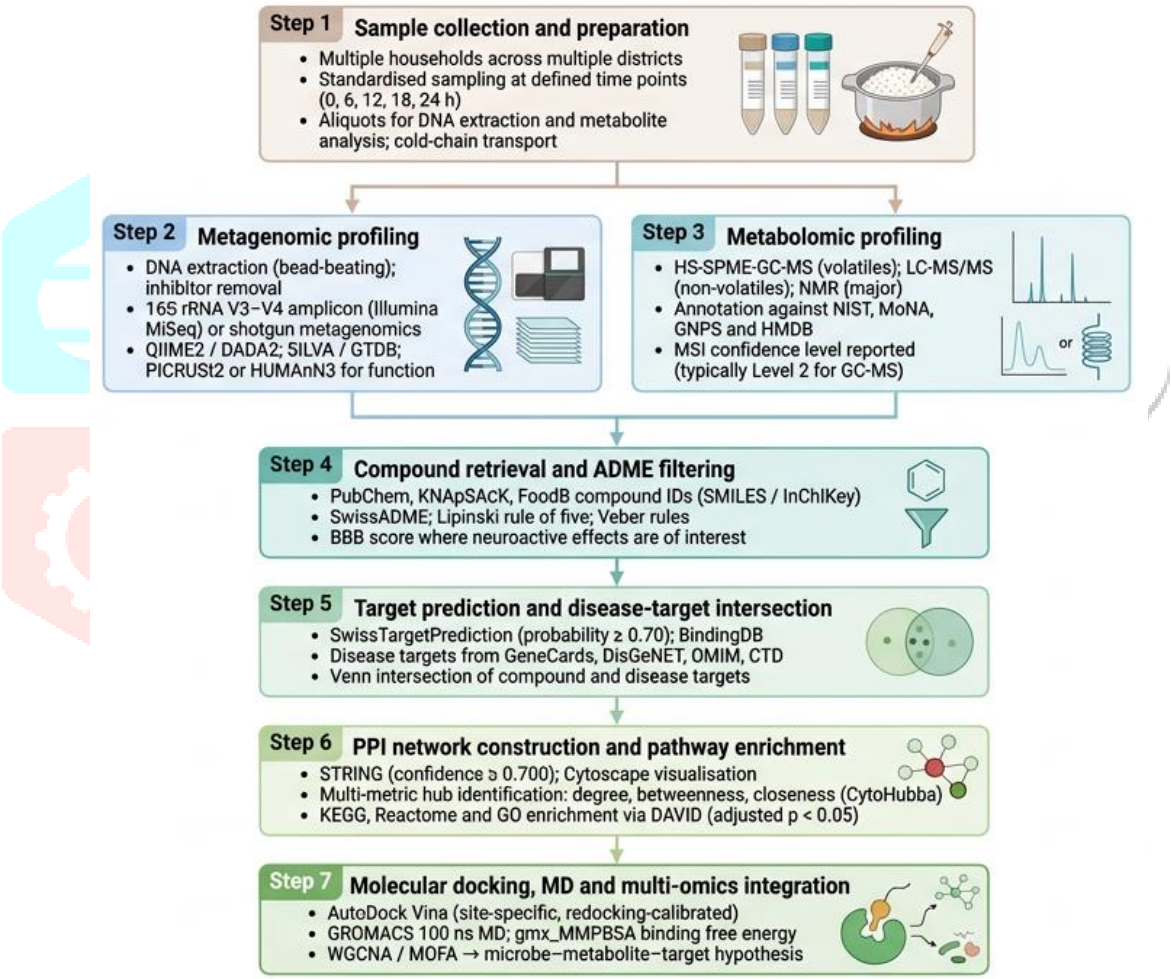

Figure 1. Integrated workflow for the multi-omics and network pharmacology characterisation of Indian rice-based traditional fermented foods. Sample collection (Step 1) feeds two parallel wet-laboratory arms — metagenomic profiling (Step 2) and metabolomic profiling (Step 3) — whose outputs converge into a sequential in silico pipeline (Steps 4–7) comprising compound retrieval and ADME filtering, target and disease-target prediction, protein–protein interaction (PPI) network construction with pathway enrichment, and molecular docking, molecular dynamics (MD) and multi-omics integration to generate a testable mechanistic hypothesis. ADME, absorption–distribution–metabolism–excretion; BBB, blood–brain barrier; CTD, Comparative Toxicogenomics Database; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MOFA, Multi-Omics Factor Analysis; MSI, Metabolomics Standards Initiative; WGCNA, weighted gene co-expression network analysis.

6.5 Disease-target mining

The disease-target list for the condition of interest is assembled from GeneCards, DisGeNET, OMIM and the Comparative Toxicogenomics Database (CTD). For inflammatory and gut-related conditions of

relevance to fermented foods, additional disease-specific resources may be brought in. The intersection between compound-predicted targets and disease-associated targets is usually visualised through a Venn diagram and supplies the working target set for the PPI analysis that follows.

6.6 Protein–protein interaction network analysis

The intersection target set is uploaded to STRING (Szklarczyk et al., 2023) for protein–protein interaction network construction. STRING offers four confidence cut-offs: low (≥ 0.150), medium (≥ 0.400), high (≥ 0.700) and highest (≥ 0.900). The medium cut-off is commonly used for exploratory work; high or highest is preferred when downstream interpretation depends critically on hub identification, because lower cut-offs raise the risk of pseudo-targets. The STRING network is exported into Cytoscape (Shannon et al., 2003) for visualisation and topological analysis.

Hub gene identification is the most consequential analytical step in this part of the workflow. Single-metric hub identification through degree centrality alone is widespread in the literature but methodologically weak; degree, betweenness and closeness centrality should be applied side by side to identify robust hub genes (Shamsol Azman et al., 2023). The CytoHubba plugin offers eleven topological algorithms — MCC, MNC and Degree among them and the intersection of top-ranked nodes across several algorithms is more reliable than any single metric. A recurring pitfall in published network-pharmacology work is the retention of an excessive number of hub genes (often 30 or more spread across multiple themes), which dilutes the signal. Reduction to the top five hubs per theme, with multiple centrality measures applied in parallel, is methodologically sounder.

6.7 Pathway enrichment analysis

Functional enrichment is conducted against KEGG pathways (Kanehisa et al., 2023), Reactome and Gene Ontology (GO) terms. DAVID, ShinyGO and Enrichr provide accessible interfaces. Benjamini-Hochberg-adjusted p-values below 0.05 are the conventional threshold. Table 2 summarises the principal databases and tools that constitute the network-pharmacology software ecosystem for fermented-food bioactives.

Table 2. Software and database ecosystem for the multi-omics and network-pharmacology analysis of fermented foods.

Workflow step	Tool / Database	Function	Reference
Amplicon sequencing analysis	QIIME2 / DADA2	ASV-based microbial community profiling	Bolyen et al. (2019); Callahan et al. (2017)
Taxonomic assignment	SILVA / Greengenes2 / GTDB	Reference databases for 16S taxonomy	De Filippis et al. (2018)
Functional inference	PICRUSt2 / HUMAnN3	Gene-content prediction from amplicon / shotgun data	Douglas et al. (2020)
Shotgun assembly	MEGAHIT / metaSPAdes	De novo metagenome assembly	Walsh et al. (2017)
MAG binning	MetaBAT2 / MaxBin2 / CONCOCT	Metagenome-assembled genome recovery	Mannaa et al. (2021)
Metabolomics peak picking	XCMS / MZmine	Untargeted metabolite peak detection	Pang et al. (2024)
Metabolite annotation	NIST / MoNA / GNPS / HMDB	Spectral library matching	Sumner et al. (2007)
Statistical analysis	MetaboAnalyst	Multivariate metabolomic statistics	Pang et al. (2024)
Compound retrieval	PubChem / KNApSAcK / FoodB	Canonical compound identifiers and structures	Daina et al. (2017)
ADME prediction	SwissADME	Drug-likeness and pharmacokinetic prediction	Daina et al. (2017)
Target prediction	SwissTargetPrediction	Compound-protein interaction prediction	Daina et al. (2019)
Disease-target mining	GeneCards / DisGeNET / OMIM / CTD	Disease-associated gene retrieval	Zhang et al. (2021)

PPI network analysis	STRING / Cytoscape / CytoHubba	Network construction and topology	Szklarczyk et al. (2023); Shannon et al. (2003)
Pathway enrichment	KEGG / Reactome / DAVID	Functional pathway annotation	Kanehisa et al. (2023)
Molecular docking	AutoDock Vina / Glide	Binding-mode prediction	Trott & Olson (2010); Eberhardt et al. (2021)
Molecular dynamics	GROMACS / AMBER	Trajectory simulation and binding stability	Abraham et al. (2015)
Binding free energy	gmx_MMPBSA	MM-PBSA / MM-GBSA calculation	Valdes-Tresanco et al. (2021)

VII. Molecular Docking and Dynamics Simulations

7.1 Receptor and ligand preparation

Molecular docking supplies the structural validation for network-pharmacology hub proposals. Receptor preparation begins with retrieval of crystal structures from the RCSB Protein Data Bank, with preference given to high-resolution structures co-crystallised with relevant ligands. Water molecules and existing ligands are stripped out in BIOVIA Discovery Studio or PyMOL, missing residues are repaired, hydrogens are added at physiological pH, and the structure is saved in PDBQT format for AutoDock Vina or MAE format for Glide. Ligand preparation starts with retrieval of canonical SMILES from PubChem, then three-dimensional structure generation, energy minimisation with the MMFF94 force field, and conversion to PDBQT.

7.2 Docking protocol

AutoDock Vina (Trott & Olson, 2010), AutoDock Vina 1.2 (Eberhardt et al., 2021) and Glide are the principal docking engines in use. Site-specific docking, where the search box is centred on the known active site or co-crystallised ligand, is strongly preferred over blind docking. Recent methodological commentary has been explicit that blind docking is routinely misapplied in network-pharmacology studies and yields unreliable results in many published works (Nguyen et al., 2020; Pinzi & Rastelli, 2019). Cross-validation by redocking the co-crystallised ligand and confirming RMSD below 2 Å against the experimental binding pose is essential, and the docking score for the redocked native ligand provides the appropriate calibration benchmark. A binding energy more negative than −5.0 kcal/mol is commonly cited as a working threshold in the published natural-product docking literature, although this is a heuristic rather than a formally established cut-off, the energy of the redocked native ligand offers the more relevant local benchmark.

7.3 Molecular dynamics simulation

Molecular dynamics extends docking from a static binding pose to a time-resolved view of binding stability. GROMACS (Abraham et al., 2015) and AMBER are the most widely used engines. OPLS-AA, CHARMM36 or AMBER ff14SB are appropriate force fields for protein, in combination with TIP3P or SPC water models, neutralising counter-ions, periodic boundary conditions and Particle Mesh Ewald electrostatics. A typical fermented-food bioactive simulation runs through energy minimisation, NVT and NPT equilibration, and a 100 ns production trajectory, although longer simulations are increasingly common. Trajectory analysis includes RMSD, RMSF, radius of gyration, hydrogen-bond occupancy and binding-pocket volume.

7.4 MM-PBSA / MM-GBSA binding free energy

Binding free energy calculation through MM-PBSA or MM-GBSA gives a quantitative metric beyond the docking score. MM-PBSA uses a Poisson-Boltzmann description of solvation, while MM-GBSA uses a Generalised Born approximation; the two methods produce broadly correlated rankings but can differ in absolute values, and stating which has been used is essential for reproducibility. The gmx_MMPBSA tool (Valdes-Tresanco et al., 2021) integrates with GROMACS trajectories and supports both. Decomposing the binding energy into van der Waals, electrostatic, polar solvation and non-polar solvation contributions gives mechanistic insight into the binding interaction (Genheden & Ryde, 2015; Valdes-Tresanco et al., 2021), and this kind of decomposition is increasingly considered standard practice in network-pharmacology validation work.

VIII. Multi-Omics Integration and Future Directions

8.1 Integration strategies

Bringing metagenomic and metabolomic data together is the natural endpoint of the methodological workflow set out above (Mannaa et al., 2021; Wen et al., 2023). The most informative integration strategy depends on the biological question being asked. For correlation-based discovery, weighted gene co-

expression network analysis (WGCNA) and canonical correlation analysis (CCA) identify covarying microbial taxa and metabolite features. Multi-Omics Factor Analysis (MOFA) provides a probabilistic framework for joint factorisation of multi-omics datasets. For mechanism-driven analysis, microbe–substrate–metabolite–target–disease networks can be assembled by linking metagenomic functional modules to specific metabolites and then to their predicted human targets.

8.2 Machine learning approaches

Machine learning is increasingly being applied to fermented-food multi-omics data for phenotype prediction, biomarker discovery and quality prediction. Random forests, support vector machines and gradient boosting see wide use on tabular multi-omics data, while deep-learning architectures - convolutional and graph neural networks are applied to spectral and network data respectively. The interpretability of these models requires explicit attention through approaches like SHAP analysis, to make sure that the predicted features are biologically meaningful and not artefacts of overfitting.

8.3 Defined microbial consortia for reproducible fermented foods

The downstream translational endpoint of multi-omics characterisation is the design of defined microbial consortia (DMC) capable of reproducing the desired functional profile of a traditional fermented food under standardised conditions. This calls for identification of the core microbial community responsible for the desired phenotype, isolation of the constituent strains, genome-level safety screening, formulation as a starter culture, and validation through pilot-scale fermentation against the traditional product. Recent work on cheeses, kimchi and Asian fermented foods has shown that DMC approaches can recapitulate traditional product characteristics with substantially improved reproducibility (Tamang et al., 2022). For Indian rice ferments, this remains an open and high-value research direction.

8.4 Selected network pharmacology applications to fermented foods

Network pharmacology has been applied to several fermented-food and food-bioactive systems with promising results. Table 3 summarises representative published applications drawn from adjacent systems that serve as methodological templates for Indian rice ferments.

Table 3. Applications of network pharmacology and molecular docking to fermented foods and food bioactives.

System	Bioactive sources	Disease focus	Methodological approach	Key findings	Reference
Tualang honey	Six flavonoids (catechin, fisetin, hesperetin, kaempferol, luteolin, ethyl oleate)	Atherosclerosis	Compound retrieval → ADME → SwissTargetPrediction → STRING → DAVID → AutoDock Vina	PIK3CA identified as primary hub; binding energies in the −10 kcal/mol range for several flavonoids	Shamsol Azman et al. (2023)
Soy fermented food products	Soy isoflavones	Lung cancer	Network pharmacology, molecular docking, molecular simulation and in vitro validation	Mechanism mapped to multiple kinase and matrix metalloprotease targets	Elkhalifa et al. (2023)
Indian fermented foods (general)	Multiple LAB metabolites	Gut and metabolic health	Multi-omics review and integration framework	Demonstrates the feasibility of omics-based functional characterisation across Indian fermented systems	Tamang et al. (2022)
General fermented foods	Multi-strain bioactives	Multiple phenotypes	Multi-omics review of fermentation flavour and function	Provides a methodological blueprint for fermented-food multi-omics	Wen et al. (2023)

IX. Conclusion

The methodological landscape for the characterisation of traditional fermented foods has shifted substantially over the past decade. Indian rice-based ferments - Pazhaya Sadham, Idli, Dosa, Uttapam, Appam, Kallappam, Koozh and Ambali are well placed to benefit from this shift. Targeted 16S rRNA gene amplicon sequencing and shotgun metagenomics together give a comprehensive view of the microbial community. HS-SPME-GC-MS, LC-MS/MS and NMR metabolomics deliver a multi-platform metabolite landscape. Network pharmacology supplies a systematic framework for translating compound lists into mechanistic hypotheses. Molecular docking, molecular dynamics and MM-PBSA or MM-GBSA binding-free-energy calculations provide structural and quantitative validation. Knitting all of this into a coherent multi-omics framework backed by transparent reporting of MSI confidence levels in metabolomics, multi-metric hub identification in network analysis, and site-specific docking with redocking calibration will be essential for credible work in this space. The translational endpoint, the design of defined microbial consortia capable of reproducing the functional profile of traditional rice ferments under standardised conditions is now within methodological reach. For Indian rice ferments, where the cultural depth of fermentation remains under-served by modern food science, this represents both an opportunity and a research responsibility.

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XI. References

1. Abraham, M. J., Murtola, T., Schulz, R., Pall, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, 1, 19-25.
2. Anandharaj, M., Sivasankari, B., Santhanakaruppu, R., Manimaran, M., Rani, R. P., & Sivakumar, S. (2015). Determining the probiotic potential of cholesterol-reducing *Lactobacillus* and *Weissella* strains isolated from gherkins (fermented cucumber) and South Indian fermented koozh. *Research in Microbiology*, 166(5), 428-439.
3. Antony, U., & Chandra, T. S. (2020). Ethnic fermented foods and beverages of Tamil Nadu. In J. P. Tamang (Ed.), *Ethnic fermented foods and beverages of India: Science history and culture* (pp. 539-560). Springer.
4. Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852-857.
5. Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, 11(12), 2639-2643.
6. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42717.
7. Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, 47(W1), W357-W364.
8. De Filippis, F., Parente, E., & Ercolini, D. (2018). Recent past, present, and future of the food microbiome. *Annual Review of Food Science and Technology*, 9, 589-608.
9. Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, 38(6), 685-688.
10. Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. *Journal of Chemical Information and Modeling*, 61(8), 3891-3898.
11. Elkhalifa, A. E. O., Banu, H., Khan, M. I., & Ashraf, S. A. (2023). Integrated network pharmacology, molecular docking, molecular simulation, and in vitro validation revealed the bioactive components in

soy-fermented food products and the underlying mechanistic pathways in lung cancer. *Nutrients*, 15(18), 3949.
12. Genheden, S., & Ryde, U. (2015). The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, 10(5), 449-461.
13. Goufo, P., & Trindade, H. (2014). Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Science & Nutrition*, 2(2), 75-104.
14. Hopkins, A. L. (2008). Network pharmacology: The next paradigm in drug discovery. *Nature Chemical Biology*, 4(11), 682-690.
15. Ilango, S., & Antony, U. (2014). Studies on the microbiological composition and quality characteristics of Koozh, an Indian fermented food. *African Journal of Microbiology Research*, 8(3), 308-312.
16. Ilango, S., Pandey, R., & Antony, U. (2016). Functional characterization and microencapsulation of probiotic bacteria from koozh. *Journal of Food Science and Technology*, 53(2), 977-989.
17. Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research*, 51(D1), D587-D592.
18. Kavitake, D., Suryavanshi, M. V., Kandasamy, S., Devi, P. B., Shouche, Y., & Shetty, P. H. (2022). Bacterial diversity of traditional fermented food, idli by high through-put sequencing. *Journal of Food Science and Technology*, 59(10), 3918-3927.
19. Kumar, R. S., Varman, D. R., Kanmani, P., Yuvaraj, N., Paari, K. A., Pattukumar, V., & Arul, V. (2010). Isolation, characterization and identification of a potential probiont from South Indian fermented foods (Kallappam, Koozh and Mor Kuzhambu) and its use as a starter culture. *Probiotics and Antimicrobial Proteins*, 2(3), 145-151.
20. Kumar, P. P., Begum, H. V., & Kumaravel, S. (2013). Mineral nutrient compositions in pazhaiya sadham (overnight cooked rice), an unexplored traditional fermented food of South India. *International Journal of Nutrition and Metabolism*, 4(11), 144-146.
21. Mandhania, M. H., Paul, D., Suryavanshi, M. V., Sharma, L., Chowdhury, S., Diwanay, S. S., Shouche, Y. S., & Patole, M. S. (2019). Diversity and succession of microbiota during fermentation of the traditional Indian food idli. *Applied and Environmental Microbiology*, 85(13), e00368-19.
22. Mannaa, M., Han, G., Seo, Y. S., & Park, I. (2021). Evolution of food fermentation processes and the use of multi-omics in deciphering the roles of the microbiota. *Foods*, 10(11), 2861.
23. Nguyen, N. T., Nguyen, T. H., Pham, T. N. H., Huy, N. T., Bay, M. V., Pham, M. Q., Nam, P. C., Vu, V. V., & Ngo, S. T. (2020). Autodock Vina adopts more accurate binding poses but Autodock4 forms better binding affinity. *Journal of Chemical Information and Modeling*, 60(1), 204-211.
24. Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A. F., MacDonald, P. E., Wishart, D. S., Li, S., & Xia, J. (2024). MetaboAnalyst 6.0: Towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Research*, 52(W1), W398-W406.
25. Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, 20(18), 4331.
26. Ray, M., Ghosh, K., Singh, S., & Mondal, K. C. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *Journal of Ethnic Foods*, 3(1), 5-18.
27. Shamsol Azman, A. N. S., Tan, J. J., Abdullah, M. N. H., Bahari, H., Lim, V., & Yong, Y. K. (2023). Network pharmacology and molecular docking analysis of active compounds in Tualang honey against atherosclerosis. *Foods*, 12(9), 1779.
28. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research*, 13(11), 2498-2504.
29. Soni, S. K., Sandhu, D. K., Vilkhu, K. S., & Kamra, N. (1986). Microbiological studies on dosa fermentation. *Food Microbiology*, 3(1), 45-53.
30. Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., et al. (2007). Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics*, 3(3), 211-221.
31. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., et al. (2023). The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1), D638-D646.
32. Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., Mayo, B., Westerik, N., & Hutkins, R. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety*, 19(1), 184-217.

33. Tamang, J. P., Anupma, A., & Shangpliang, H. N. J. (2022). Ethno-microbiology of Tempe, an Indonesian fungal-fermented soybean food and Koji, a Japanese fungal starter culture. *Current Opinion in Food Science*, 48, 100912.
34. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455-461.
35. Valdes-Tresanco, M. S., Valdes-Tresanco, M. E., Valiente, P. A., & Moreno, E. (2021). gmx_MMPBSA: A new tool to perform end-state free energy calculations with GROMACS. *Journal of Chemical Theory and Computation*, 17(10), 6281-6291.
36. Walsh, A. M., Crispie, F., Claesson, M. J., & Cotter, P. D. (2017). Translating omics to food microbiology. *Annual Review of Food Science and Technology*, 8, 113-134.
37. Wen, P., Sun, Y., Huang, C., Pan, Y., Ge, W., Zhao, Y., & Sun, Z. (2023). Applications of multi-omics techniques to unravel the fermentation process and the flavor formation mechanism in fermented foods. *Critical Reviews in Food Science and Nutrition*, 64(23), 8367-8383.
38. Zhang, R., Zhu, X., Bai, H., & Ning, K. (2021). Network pharmacology databases for traditional Chinese medicine: Review and assessment. *Frontiers in Pharmacology*, 12, 722887.